

OR01-1

The Farnesoid X Receptor (FXR) Stimulates Adrenal Steroidogenesis in Mice.

M Hoekstra PhD¹, Z Li MSc¹, RJ van der Sluis Bsc¹ and TJC Van Berkel PhD¹.

¹Leiden/Amsterdam Ctr for Drug Res Leiden, Netherlands.

The nuclear bile acid receptor FXR (NR1H4) is primarily expressed in the gastrointestinal tract where it inhibits hepatic bile acid synthesis and stimulates intestinal bile acid cycling. Interestingly, high protein expression of FXR has also been detected in murine adrenocortical cells of the zona fasciculata that produce glucocorticoids. In the current study we have therefore evaluated the potential role of FXR in adrenal steroidogenesis.

Treatment of C57BL/6 mice with the synthetic FXR ligand GW4064 stimulated the adrenal mRNA expression of the established FXR target gene OSTb 15-fold ($P < 0.001$), indicative of optimal FXR activation. Importantly, the fasting stress-induced increase in plasma corticosterone levels was 63% higher ($P = 0.013$) in GW4064-treated mice as compared to controls, resulting in an overall 45% higher ($P = 0.009$) plasma corticosterone level under fasting conditions. Plasma ACTH levels, the adrenal weight, and adrenal cortex neutral lipid levels were identical in GW4064-treated mice and controls. The mRNA expression level of the steroidogenic enzymes CYP11A1, HSD3B2, CYP21A1, and CYP11B1 could also not explain the increased steroidogenesis rate. Furthermore, no change was observed in the expression level of key cholesterol transport and metabolism genes StAR, HSL, ACAT1/2 and ABCA1. In contrast, marked increases in the adrenal mRNA expression of ACTH-regulated gene targets involved in cholesterol synthesis and uptake, HMG-CoA reductase (+235%; $P = 0.001$), LDL receptor (+180%; $P < 0.001$), and SR-BI (+90%; $P = 0.002$) were observed. Strikingly, the expression of MRAP, an activator of ACTH-induced signalling via the MC2 receptor, was stimulated 1.6-fold ($P = 0.006$) by GW4064, while the expression of the MC2 receptor itself was unaffected. In silico analysis revealed a putative FXR IR-1 response element in the murine promoter of MRAP. Combined, these findings suggest that FXR enhances ACTH action locally in the adrenals which is associated with an increased synthesis and receptor-mediated uptake of cholesterol and a higher steroidogenesis rate. In conclusion, we have identified a novel role for FXR in the modulation of adrenal glucocorticoid synthesis in mice. Our studies suggest that FXR ligands may possibly also affect the adrenal steroidogenesis rate in the human situation, which can limit their recent therapeutical application for the treatment of dyslipidemia, cholestasis, and other metabolic diseases.

Sources of Research Support: Netherlands Heart Foundation (NHS) Grant 2008T070 awarded to MH; TIPharma T2-110.

Nothing to Disclose: MH, ZL, RjvdS, TJCvB

OR01-2

DHT Promotes Muscle and Prostate Growth through a Positive Feedback Loop Involving Androgen Receptor, microRNA and Corepressors.

R Narayanan Ph.D.¹, J Jiang Ph.D.², YD Gusev Ph.D.³, A Jones BS¹, DD Miller Ph.D.¹, TD Schmittgen Ph.D.² and JT Dalton Ph.D.¹.

¹GTx Inc Memphis, TN ; ²The Ohio State Univ Columbus, OH and ³Georgetown Univ Washington, DC.

Androgen receptor (AR) function is critical for the development of male reproductive organs, muscle, bone and other tissues. Functionally impaired AR results in androgen insensitivity syndrome, muscle loss, osteoporosis and even cognitive disabilities. Though several groups have demonstrated that AR depends on coactivators and corepressors for its functions, knockout of these proteins only partially impairs the androgenic response indicating that multiple pathways impinge on AR signaling. Recent studies suggest that disturbances in microRNA (miR) expression contribute to prostate cancer and muscle disorders. We performed studies to examine the interactions between AR and miR signaling pathways. Castration of Sprague Dawley rats inhibited the expression of a large set of miRs in prostate and levator ani muscle, while treatment of castrated rats with 3 mg/day DHT prevented the loss of expression of these miRs, demonstrating that the expression of miRs is regulated by androgens and AR. To understand the consequence of this regulation, PSA gene expression was examined in LNCaP prostate cancer cells treated with vehicle or DHT in the presence of DICER or control siRNA. Inhibition of miR synthesis by DICER siRNA completely inhibited DHT-dependent PSA gene expression, indicating that miR expression is critical for AR function. Since the only function of miRs is to bind to 3' UTR and inhibit the translation of target genes, we postulated that androgens induce miRs to inhibit repressors of AR function. In concordance with the hypothesis, DICER siRNA induced the expression of NCoR and SMRT. Also, chromatin immunoprecipitation assays demonstrated that while DHT failed to recruit NCoR and SMRT to PSA enhancer, a selective androgen receptor modulator (SARM), which minimally altered miR expression, recruited SMRT and NCoR. To confirm the importance of miRs for AR function, tissue specific DICER knockout mice were generated by crossing DICER flox and MMTV cre mice. Six week old DICER knockout mice demonstrated a phenotype characterized by reduced prostate and levator ani muscle. In addition, these tissues failed to respond to DHT treatment, indicating an androgen insensitivity syndrome phenotype. These studies, for the first time, demonstrate a feedback loop between miRs, corepressors and AR and the imperative role of miRs in AR function in non-cancerous androgen-responsive tissues.

Nothing to Disclose: RN, JJ, YDG, AJ, DDM, TDS, JTD

OR01-3

Binding of the SARM LGD-4033 Induces Specific Conformational Changes in the AR-LBD and Exhibits a Unique Protein-Protein Interaction Profile Distinct from Steroidal Androgen Agonists and Other SARMS.

KB Marschke¹, A van Oeveren¹, MH Hong¹, J Sanders¹, EG Vajda¹ and L Zhi¹.

¹Ligand Pharmaceuticals, Inc La Jolla, CA.

Selective androgen receptor modulators (SARMS) are androgen receptor (AR) ligands that bind AR and display tissue-selective activation of androgenic signaling. SARMS are being developed as a new class of drugs to treat aging-associated sarcopenia, cancer cachexia, and other musculoskeletal wasting or muscle loss conditions. LGD-4033 is a novel non-steroidal, orally active SARM currently in Phase I multiple ascending dose studies that include biomarkers for muscle growth and strength. We have previously reported that in rodent models, LGD-4033 demonstrates antiresorptive and anabolic selective activity in bones and muscles with reduced impact on prostate growth in comparison to the non-selective steroidal androgen testosterone(1). In vitro, LGD-4033 selectively binds AR with high affinity, and is a potent, full agonist of AR-mediated gene expression in cell models of bone and muscle. To investigate mechanisms that contribute to the tissue-selective biologic effects of this SARM, we studied ligand-induced protein-protein interactions by measuring the interaction of the amino and carboxyl terminal ends of AR (i.e., N/C interaction) and recruitment of four co-activator peptides containing LxxLL- or FxxLF-like motifs using peptide-based two-hybrid assays. We also determined the co-crystal structure of wild-type AR ligand binding domain (LBD) with LGD-4033 at a 1.9 Å resolution to examine ligand-specific conformational changes. AR bound to LGD-4033 induced the AR N/C interaction and recruitment of the co-activator peptides with high affinity. However, LGD-4033 was selective in the degree to which it facilitated these interactions, exhibiting a unique pattern compared with steroidal androgen agonists (dihydrotestosterone, testosterone, oxandrolone, fluoxymesterone and R1881), and a selection of SARMS (andarine, ostarine, BMS 5564929 and LGD-2226). In the AR-LBD co-crystal structure, LGD-4033 induced a different change in orientation of Trp741 and Met894 from those reported for DHT, R1881 or other SARMS. Trp741 lies beneath the co-activator binding site on the surface of AR, whereas Met894, in helix 12, is part of the binding site. The data suggest that these subtle ligand-specific conformational changes modulate receptor surface topology and subsequent protein-protein interactions between AR and other co-regulators resulting in the tissue-specific effects seen with LGD-4033.

(1) Vajda EG et al., 62nd Annual Meeting of the Gerontology Society of America 2009

Nothing to Disclose: KBM, AvO, MHH, JS, EGV, LZ

OR01-4

Methylation of Androgen Receptor by Set9 Histone Methyltransferase and Its Functional Impact.

J Ahn MS¹, S Ko PhD¹, S Kim MS¹, K Knapczyk-Stwora PhD¹, CS Song PhD^{1,2} and B Chatterjee PhD^{1,2}.

¹Univ of Texas Hlth Sci Ctr at San Antonio San Antonio, TX and ²South Texas Veterans Hlth Care Syst San Antonio, TX.

Methylation of histones and non-histone transcription factors is a post-translational modification that is known to have important influences on mammalian gene transcription. We report that Set9, a histone-H3 methyltransferase, methylates androgen receptor (AR) at a single lysine residue *in vitro* and *in vivo*, and methylation enhances androgen-induced AR transcriptional activity in multiple cell lines. Recombinant Set9 catalyzed methyl group transfer from the cofactor SAM to GST-AR at lysine-630, and Set9 methylated AR in transfected 293A kidney cells. A pan-methyl-lysine antibody recognized AR from LNCaP prostate cancer cells and from AR-transfected 293A cells. K630→A substitution prevented AR methylation *in vitro* and *in vivo*. Ectopically introduced Set9 enhanced AR-mediated transactivation, whereas activity of the methylation-site-mutant AR was unaltered by Set9 over expression. Set9 depletion reduced endogenous expression of AR target genes in androgen-treated kidney and prostate cell lines. Set9 also enhanced AR N-C interaction in a K630-dependent manner, since K630→A substitution or Set9 depletion reduced androgen-dependent N-C interaction. Surprisingly, the K630A mutant AR failed to induce the endogenous target genes ODC1 and SGK1. Transiently transfected probasin promoter was still activated by the K630A mutant. These results indicate a direct role of Set9 in the modulation of AR activity through changes at the receptor amino acid level. Set9 depletion did not alter LNCaP cell proliferation rate. Given the dependence of methyltransferase activity on the intracellular level of the cofactor SAM, we speculate that AR methylation may be important in fine tuning AR activity to meet androgen-dependent specific metabolic or nutritional need of cells.

Sources of Research Support: NIH, VA , CTSA-Translational Science Training pre-doctoral fellowship (to JA) and DOD pre-doctoral fellowship(to SK).

Nothing to Disclose: JA, SK, SK, KK-S, CSS, BC

OR01-5

Skeletal Muscle Specific ER α Deletion Is Causal for the Metabolic Syndrome.

Vicent Ribas PhD¹, Brian G Drew PhD¹, Teo Soleymani¹, Pedram Daraei¹ and Andrea L Hevener PhD¹.

¹Univ of California Los Angeles Los Angeles, CA.

Impaired estrogen action is associated with aspects of the metabolic syndrome in human subjects⁽¹⁻⁴⁾ however little is known regarding the role of the estrogen receptor alpha (ER α) in glucoregulatory tissues. Recently we found that skeletal muscle ER α protein is diminished in postmenopausal women and this is associated with impaired glucose tolerance. As skeletal muscle is a primary tissue contributing to insulin-mediated glucose disposal and fatty acid oxidation, we experimentally tested the impact of skeletal muscle ER α expression on these metabolic processes in male and female mice. We find that selective deletion of ER α in skeletal muscle (MERKO) causes glucose intolerance, whole body insulin resistance, increased adipose tissue mass and impaired oxidative metabolism in mice of both genders. Furthermore, circulating insulin, PAI-1, and leptin levels were all significantly elevated, while adiponectin levels were markedly diminished in MERKO animals. These changes were concomitant with increased bioactive lipid accumulation in skeletal muscle and tissue inflammation. Consistent with findings from *in vivo* glucose clamp studies, we observed impaired insulin-mediated glucose disposal and insulin signaling in isolated soleus muscle from MERKO mice *ex vivo*. In addition, lentiviral-mediated knockdown of ER α in C2C12 myotubes led to impaired insulin action, reduced oxidative metabolism, and increased rates of fatty acid esterification *in vitro*. These findings demonstrate that loss of skeletal muscle ER α can recapitulate many features of the metabolic syndrome in rodents of both genders and suggests that reduced ER α action in muscle may underlie increased disease risk seen in women following menopause.

1. Nilsson M et al., Int J of Obesity 2007; 31:900-907.
2. Carr MC, J Clin Endo and Metab 2003; 88(6):2404-2411.
3. Okura T et al., Int J Obesity 2003; 27:1020-1027.
4. Smith EP et al., NEJM 1994; 331:1056-1061.

Sources of Research Support: Iris Cantor-UCLA Women's Health Center Foundation Grant; NIDDK 78760, 063491, 073227 awarded to ALH.

Nothing to Disclose: VR, BGD, TS, PD, ALH

OR01-6

Perturbation of Estrogen Receptor Signaling in the Hypothalamo-Pituitary-Ovarian Axis Leads to Ovarian Tumorigenesis in Mice.

MJ Laws MS¹, A Kannan PhD¹, MK Bagchi PhD¹ and IC Bagchi PhD¹.

¹Univ of Illinois at Urbana-champaign Urbana, IL.

Ovarian cancer is the most lethal malignancy of the female reproductive system. Due to the absence of specific symptoms and the lack of strategies for early detection of ovarian malignancies, the majority of women with ovarian cancer are diagnosed at a late stage when the cancer has spread beyond the confines of the ovary. The etiology of ovarian cancer is poorly understood, mainly due to the lack of an appropriate experimental model for studying the onset and progression of this disease.

We have recently developed a mouse model in which aberrant estrogen receptor alpha (ER α) signaling in the hypothalamo-pituitary-ovarian axis leads to ovarian tumorigenesis. In this mouse model, termed ER α d/d, a conditional deletion of ER α gene occurred in the anterior pituitary, but ER α expression remained intact in the hypothalamus and the ovary. Selective ablation of ER α in the pituitary created a systemic hormonal imbalance in these mice. Loss of the negative-feedback regulation of estrogen (E) in the pituitary led to elevated levels of luteinizing hormone (LH). LH hyperstimulation of ovarian cells resulted in elevated steroidogenic activities, leading to high circulating levels of E (70pg/ml), progesterone (9ng/ml), LH (2.5ng/ml), and testosterone (400ng/ml). The ER α d/d mice exhibited formation of ovarian tumors with 100% penetrance, starting at 5 months of age. These tumors grew up to 11 mm in size by 8 months of age and most mice died by 11-12 months.

The ER α d/d mice displayed an elevated ER α signaling in the surface epithelial cells of the ovary, which is apparently linked to ovarian tumorigenesis. We observed a marked elevation in the expression of the transcriptionally active, phosphorylated ER α (at Ser-118) in the epithelial cells of ER α d/d compared to its wild-type counterparts. The phosphoinositide-3 kinase/AKT pathway was also activated, as indicated by increased expression of phospho-AKT in the ovaries of mutant mice. Immunohistochemical analyses revealed that the cells within the ovarian tumors of ER α d/d mice are characterized by intense expression of cytokeratin 8 and 19 as well as nuclear staining of Wilms tumor 1, a well known marker of ovarian tumorigenesis. Further characterization of the nature of these tumors using additional biochemical markers is underway.

In summary, we have developed an animal model, which will serve as a powerful tool for exploring the involvement of ER α -dependent signaling pathways in the etiology of ovarian cancer.

Nothing to Disclose: MJL, AK, MKB, ICB

OR02-1

Identification and Characterisation of Ligand-Specific Coactivators of the Mineralocorticoid Receptor.

PJ Fuller MD, PhD¹, Y Yao MD¹, MJ Young PhD¹ and FM Rogerson PhD¹.

¹Prince Henry's Inst of Med Res Clayton, Australia.

The mineralocorticoid receptor (MR) is unique in responding to two physiological ligands, aldosterone and cortisol. In epithelial tissues aldosterone selectivity is determined by the activity of 11 β -hydroxysteroid dehydrogenase type II. In other tissues, including the heart and regions of the CNS, cortisol is the primary ligand for the MR; and in some tissues it may act as an antagonist. To identify mechanisms of tissue and ligand-specific MR activation, we sought to characterise ligand-specific co-regulatory molecules that interact with the ligand-binding domain (LBD) of the MR. A yeast 2-hybrid kidney cDNA library was screened with the human MR-LBD in the presence of either aldosterone or cortisol. Clones which exhibited an interaction the presence of one but not both ligands were identified and examined in a mammalian-2-hybrid (M-2-H) assay with the MR LBD. The full-length molecules encoded by 3 of these clones have now been examined. Two of these, tesmin and ZNF136, coactivate MR- mediated transactivation in the presence of aldosterone but not cortisol. One, coactivates MR- mediated transactivation in the presence of cortisol but not aldosterone. Only tesmin contains the classical LxxLL motifs although in all cases the interaction in the M-2-H assay is AF-2 dependent. That the LxxLL motifs mediate the interaction of tesmin with the MR LBD has been confirmed in both the M-2-H and transactivation assays using site-directed mutagenesis. The nature of the motif mediating the interaction in the other two coactivators is currently being sought. The ligand specificity is retained across several promoters and cell lines. Co-immunoprecipitation of the MR by tesmin and by ZNF136 supports a direct interaction with the MR. This differential interaction between aldosterone and cortisol provides evidence of the adoption of ligand-dependent conformations by the MR LBD. It may provide the mechanism by which the reported difference in the responses to aldosterone and cortisol in the heart and central nervous system may be mediated. The successful identification of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue- and or ligand-specificity.

Sources of Research Support: National Health and Medical Research Council of Australia and the National Heart Foundation of Australia.

Nothing to Disclose: PJF, YY, MJY, FMR

OR02-2

Deficiency of 11 β -Hydroxysteroid Dehydrogenase Type 1 Reduces Systemic Inflammation and Inflammatory Cell Infiltration in Atherosclerotic Lesions of ApoE $^{-/-}$ Mice.

TMJ Kipari PhD¹, PWF Hadoke PhD¹, T Man BSc Hons¹, C White BSc Hons¹, BR Walker MD, PhD¹, JS Savill MD, PhD¹, KE Chapman PhD¹ and JR Seckl MD, PhD¹.

¹Univ of Edinburgh Edinburgh, UK.

High plasma levels of glucocorticoids cause cardio-metabolic disease (central obesity, hypertension, diabetes, atherosclerosis). 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) regenerates glucocorticoids in intact cells, converting inert cortisone (11-dehydrocorticosterone in rodents) into active cortisol (corticosterone). Recent work has shown the pathological importance of elevated adipose tissue 11 β -HSD1 expression in development of obesity, driving metabolic disease. 11 β -HSD1 deficiency ameliorates type II diabetes and obesity-related disease in humans and animal models. Importantly, 11 β -HSD1 inhibitors reduce atherosclerotic plaque size in atherosclerosis-prone ApoE $^{-/-}$ mice fed a "western" diet (WD), though the specificity of such new drugs is not fully defined.

Addressing this, we found reduced (27%) atherosclerotic lesion development in 11 β -HSD1 $^{-/-}$ ApoE $^{-/-}$ double knock-out (DKO) mice fed WD for 12-16 wks, compared with ApoE $^{-/-}$ controls. The reduction in atherosclerotic lesions was accompanied by reduced (49%) macrophage infiltration and T cell infiltration in lesions of DKO mice (CD3 $^{+}$ T cells/mm²; DKO, 275.8 \pm 44.9 ; ApoE $^{-/-}$, 432.1 \pm 22.4; p<0.01). WD-fed DKO mice also have more monocyte and neutrophil progenitors in their bone marrow (monocytes/femur; DKO, 9.36 \times 1.32 \times 10⁵ ; ApoE $^{-/-}$, 4.41 \pm 0.46 \times 10⁵; p<0.01; neutrophils/femur; DKO, 5.50 \pm 1.11 \times 10⁶ ; ApoE $^{-/-}$ 2.80 \pm 0.21 \times 10⁶; p<0.05) but fewer circulating monocytes in blood (DKO, 8.30 \pm 0.51 \times 10⁵ ; ApoE $^{-/-}$, 1.16 \pm 0.11 \times 10⁶ monocytes/ml; p<0.01). However circulating blood neutrophils and T lymphocyte numbers were unaffected by 11 β -HSD1 deficiency in WD-fed mice. Circulating levels of monocyte chemotactic protein-1 (MCP-1) were not different in ApoE $^{-/-}$ and DKO mice fed chow diet (DKO 42.5 \pm 7.4 pg/ml ; ApoE $^{-/-}$ 30.1 \pm 3.9 pg/ml). Following WD, plasma MCP-1 levels increased in ApoE $^{-/-}$ mice but not in DKO mice (DKO 31.6 \pm 4.0 pg/ml ; ApoE $^{-/-}$ 50.6 \pm 9.3 pg/ml), so DKO mice had significantly lower MCP-1 on WD. Finally WD-fed DKO mice exhibited increased numbers of regulatory T cells (Tregs) in the spleen (CD4 $^{+}$ FoxP3 $^{+}$ CD25 $^{+}$ Tregs; DKO, 8.47 \pm 1.30 \times 10⁵ ; ApoE $^{-/-}$, 5.15 \pm 0.85 \times 10⁵; p<0.05).

These data indicate that 11 β -HSD1 deficiency reduces atherosclerotic lesion formation in ApoE $^{-/-}$ mice associated with less recruitment of inflammatory cells, potentially due to increased Treg content.

Nothing to Disclose: TMJK, PWFH, TM, CW, BRW, JSS, KEC, JRS

OR02-3

Salivary Cortisone Is a Reliable Biomarker for Free Serum Cortisol.

I Perogamvros¹, L J Owen², B G Keevil², DW Ray¹ and PJ Trainer¹.

¹Univ of Manchester, Manchester Academic Hlth Sci Ctr Manchester, UK and ²Univ Hosp of South Manchester, Manchester Academic Hlth Sci Ctr Manchester, UK.

Salivary cortisol measurement has been used as a surrogate for serum free cortisol, as a filtrate without corticosteroid-binding globulin (CBG). However, parotid tissue harbors 11 β -HSD2 activity converting cortisol to cortisone. Therefore, we have studied the relationship between serum and salivary cortisol and cortisone. We studied groups of volunteers with differences in circulating CBG (+/- oral contraceptive, genetic CBG deficiency) and controls, with either Synacthen stimulation or IV/oral hydrocortisone administration post dexamethasone suppression. Serum and saliva samples were collected for the measurement of total serum cortisol (SerF) (electrochemiluminescent immunoassay, Roche Diagnostics GmbH), salivary cortisol (SalF) and cortisone (SalE) (previously published liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay) and free serum cortisol (FreeF) and cortisone (FreeE) by an ultrafiltration/ LC-MS/MS method.

ACTH stimulation increased SalE (t=0: 30.7 \pm 8.9 vs t=60: 79.3 \pm 17.5 nmol/l, P<0.0001) but there was no change in FreeE. SerF significantly decreased after stopping OCP but FreeF, SalF and SalE remained unchanged. In the hydrocortisone administration study, SalE AUC closely reflected the FreeF AUC (nmol.hr/L) (IV: 382.1 \pm 138.6 vs 365.6 \pm 155.3 nmol.hr/L, P=0.35, oral: 359.6 \pm 107 vs 326.4 \pm 160.2, P=0.21) and individual FreeF and SalE curves were nearly identical. When data from both protocols were combined (n=505 matched serum-saliva samples) the highest correlation was between SalE-FreeF (r=0.91, P<0.0001) and there was no evidence of 11 β -HSD2 saturation in the wide range of concentrations studied.

A major issue in the accurate assessment of the HPA axis is the variability of CBG, including its elevation by estrogens and degradation under conditions of inflammatory stress. Salivary sampling offers a non invasive, stress-free means to monitor HPA activity. However, salivary cortisol concentration is determined both by serum free cortisol, and parotid metabolism to cortisone. We now show that parotid 11 β -HSD2 activity produces a linear response between serum free cortisol and salivary cortisone, and therefore our novel data suggest that salivary cortisone, easily measured by the highly specific LC-MS/MS, is the preferred biomarker to accurately assess serum free cortisol. As such, its use both as a research tool and in clinical assessment is advocated. Further analysis under conditions of altered 11 β -HSD2 activity is now warranted.

Disclosures: PJT: Lecturer, Advisory Board Member, Research Funding, Pfizer, Inc., Ipsen, Novartis Pharmaceuticals.

Nothing to Disclose: IP, LJO, BGK, DWR

OR02-4

Angiotensin II-Dependent and Independent Mechanisms of Prorenin Effects on the Proliferation of Human Vascular Smooth Muscle Cells In Vitro.

M Naruse MD¹, K Sawai¹, T Usui MD¹, T Tagami MD¹, M Tsuiki MD², A Shimazu MD¹ and A Tanabe MD².

¹NHO Kyoto Med Ctr Kyoto, Japan and ²Tokyo Women's Med Univ Tokyo, Japan.

Background: Recent studies demonstrated that prorenin, a precursor of renin, is activated via a conformational change by binding to its novel receptors. Although activated prorenin has been implied to have roles in the end-organ damage of hypertension and diabetes mellitus, details of the effects of prorenin on the cardiovascular tissues remain to be elucidated. **Objective:** Aim of the study was to investigate direct effects of prorenin on the proliferation of vascular smooth muscle cells (VSMC) and signaling mechanism in vitro. **Methods:** Cultured VSMC from human coronary arteries (HCASMC) was used. After incubation for 2 days, cells were cultured for 1 day with the medium containing 0.2% FBS and subjected to the experiments in a serum-free medium. Cells were treated with recombinant human prorenin (10^{-13} M- 10^{-9} M) (Cayman Chemical Co.) over a period of 4 days. Cell proliferation (BrdU, labeling), cell cycle (flow cytometry), phosphorylation of MAP kinase-related protein (Western blot analysis), and mRNA expression of COX-2, type 1 collagen, and fibronectin (real-time quantitative RT-PCR) were determined. Specificity of the effects was determined by using decapeptide inhibitor (handle region peptide), knockdown of prorenin receptor with a siRNA. Involvement of renin-angiotensin II was determined by addition of AT1 receptor antagonist candesartan (Takeda Chemical Industry, Co.Ltd) and by knockdown of AT1 receptor and angiotensinogen with siRNAs. **Results:** Prorenin significantly stimulated cell proliferation of HCASMC (2 days), percentage of cells at S and G2/M phases, phosphorylation of the ERK1/2 and stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) (10 min), type 1 collagen, and fibronectin (20 min), COX-2 (90 min). The effects of prorenin were dose-dependent from the dose of 10^{-11} M. The effects of prorenin on MAP kinase phosphorylation were substantially attenuated by handle region peptide and by transfection of siRNA against human prorenin receptor. In addition, effects of prorenin on COX-2 mRNA expression but not on pERK were completely abolished by AT1 receptor antagonist or by knockdown of the AT1 receptor and angiotensinogen mRNA by siRNA. **Conclusions:** The present study clearly demonstrated that prorenin directly stimulates proliferation of VSMC through prorenin receptor-mediated MAPK activation. Both Ang II-dependent and -independent mechanisms are involved in the novel prorenin action on the cardiovascular system.

Sources of Research Support: Takeda Chemical Industry, Co.Ltd for providing candesartan.

Nothing to Disclose: MN, KS, TU, TT, MT, AS, AT

OR02-5

Extra-Adrenal Cortisol Production in Obese Men with Type Two Diabetes Mellitus - How Big Is the Therapeutic Target for 11 β HSD1 Inhibitors?.

RH Stimson PhD¹, R Andrew PhD¹, NC McAvoy MBChB², D Tripathi MD², PC Hayes PhD² and BR Walker MD¹.

¹Univ of Edinburgh Edinburgh, UK and ²Royal Infirmary of Edinburgh Edinburgh, UK.

11 β Hydroxysteroid dehydrogenase type 1 (11 β HSD1) inhibitors are being developed to prevent cortisol regeneration from cortisone in liver and adipose tissue in type 2 diabetes (T2DM). Early results with a prototype inhibitor, carbenoxolone, suggested this was more effective in lean T2DM than in obese non-diabetic men, potentially reflecting adaptive down-regulation of hepatic 11 β HSD1 in obesity by hyperinsulinaemia. To quantify in vivo 11 β HSD1 activity in obese T2DM patients (the proposed target group for enzyme inhibitors) and lean controls, we cannulated the hepatic vein during 9,11,12,12-[²H]₄-cortisol (d4-cortisol) tracer infusion and measured first pass hepatic conversion of cortisone to cortisol.

10 obese men with diet- or tablet-controlled T2DM and 7 lean healthy controls (age 52.3 \pm 2.9 vs 47.5 \pm 6.0 y, BMI 35.0 \pm 1.0 vs 23.5 \pm 1.1 kg/m²) were given 1mg oral dexamethasone the night prior to infusion of cortisol (60%, to suppress adrenal cortisol release) and 9,11,12,12-[²H]₄-cortisol (40%) at 1.74mg/h. Blood was obtained from the hepatic vein and an arterialised hand vein at steady state. Cortisone (5 mg) was then administered orally and appearance of cortisol in hepatic vein quantified by tracer dilution. Indocyanine green was infused to measure hepatic blood flow. Data are mean \pm SEM.

Whole body d3-cortisol appearance (a specific measure of 11 β HSD1 activity) was increased in obese T2DM (35.0 \pm 2.2 vs 28.9 \pm 1.4 nmol/min, p<0.05). Although basal splanchnic d3-cortisol release was similar (28.8 \pm 0.9 vs 29.5 \pm 5.9 nmol/min), cortisol appearance in the hepatic vein after oral cortisone was increased in obese T2DM (275 \pm 13 vs 203 \pm 25 nmol/min, p<0.05). Extra-splanchnic cortisol release was not significant in lean controls (10 \pm 12 nmol/min) but was measurable in obese T2DM (25 \pm 12 nmol/min); there were similar trends for extra-splanchnic d3-cortisol regeneration.

In contrast with down-regulation of liver 11 β HSD1 and lack of change in whole body cortisol regeneration which occurs in euglycaemic obesity, in obese men with T2DM liver 11 β HSD1 is sustained and whole body cortisol regeneration is increased. High extra-splanchnic cortisol and d3-cortisol release in obese T2DM most likely reflects increased 11 β HSD1 in subcutaneous adipose tissue. These findings further implicate insulin-dependent mechanisms in the regulation of liver 11 β HSD1 in humans, and highlight the potential therapeutic benefit of inhibiting both hepatic and adipose tissue 11 β HSD1 in obesity-associated diabetes.

Sources of Research Support: British Heart Foundation.

Nothing to Disclose: RHS, RA, NCM, DT, PCH, BRW

OR02-6

GILZ1 Regulates SGK1 Function, Expression and Subcellular Localization.

D Pearce MD¹, J Wang MD¹, D Melters MSc², I-C Shih PhD¹ and R Soundararajan PhD¹.

¹Univ of California San Francisco San Francisco, CA and ²Univ of California Davis Davis, CA.

Aldosterone plays a key role in the regulation of sodium (Na⁺) balance, and hence is central to the maintenance of extracellular fluid volume and blood pressure. Aldosterone acts in part by controlling the membrane trafficking of the epithelial Na⁺ channel (ENaC) in kidney tubule cells. The PI3K-dependent kinase SGK1, and a small leucine-zipper protein called GILZ1 are important mediators of aldosterone's effects, however, both also impact on a variety of other cellular processes. How specific physiologic effects are achieved has been unclear. Previous studies suggested that GILZ1 and SGK1 physically interact and functionally synergize in stimulating ENaC. SGK1 is a short-lived protein which is predominantly targeted to the endoplasmic reticulum (ER) to undergo rapid proteasome-mediated degradation via the ER-associated degradation (ERAD) system. We have found that GILZ1 redirects SGK1 away from the ER, decreasing its interaction with ER-associated ubiquitin ligases CHIP and HRD1, and selectively recruiting it to a complex that contains Raf-1 and the ubiquitin ligase Nedd4-2. These ENaC-inhibitory proteins are key targets of SGK1, and GILZ1 enhances SGK1-mediated phosphorylation and inhibition, resulting in ENaC stimulation. On the other hand, GILZ1 by reducing CHIP and HRD1-mediated ubiquitinylation decreases SGK1 degradation and prolongs its half-life. We propose that GILZ1 acts as a chaperone to alter SGK1's protein partners, resulting in enhanced SGK1 expression and increased targeting to sites of action implicated in ENaC regulation. These data provide new insight into how SGK1's manifold activities are selectively deployed through modulation of its molecular interactions and subcellular localization.

Sources of Research Support: NIH K01-DK078679 (to RS) and R01-DK56695, R01-DK85101 (to DP).

Nothing to Disclose: DP, JW, DM, I-CS, RS

OR03-1

Nocturnin Suppresses *Igf1* Expression in a Post-Transcriptional Manner Via Interaction with the 3'Untranslated Region of *Igf1* mRNA.

M Kawai¹, AM Delany², ML Adamo³ and CJ Rosen¹.

¹Maine Med Ctr Res Inst Scarborough, ME ; ²Univ of Connecticut Hlth Ctr Farmington, CT and ³The Univ of Texas Hlth Sci Ctr San Antonio, TX.

Age-related bone loss is associated with reduced insulin-like growth factor (IGF)-I and increased Pparg2 expression in bone marrow stromal cells. Previously, we showed that Pparg2 activation suppressed *Igf1* expression in a mouse stromal cell line and that women treated with rosiglitazone had reduced circulating IGF-I. However, the precise mechanism whereby Pparg2 suppresses IGF-I has remained elusive. To answer this question, we screened gene expression profiles from UAMS-33 bone marrow cells overexpressing PPAR-gamma2 and exposed to rosiglitazone. *Nocturnin* (*Noc*) was one of the top 10 genes up-regulated by PPAR-gamma2 activation. Nocturnin (*Ccrn4l*) is a circadian gene with peak expression at early night. It functions in mammalian systems by deadenylating long 3'UTRs. Based on these data, we hypothesized that *Noc* regulated *Igf1* expression by destabilizing *Igf1* mRNA through deadenylation. First, we analyzed the circadian expression profile of *Igf1* and *Noc* in whole femur. *Igf1* showed circadian rhythmicity with the nadir of expression at night when *Noc* transcripts were highest, demonstrating an anti-phase expression profile between these two genes. To test the in vivo relevance of this finding we studied *Noc*^{-/-} mice. In *Noc* deficient calvarial osteoblasts, *Igf1* expression was significantly increased, whereas *Igf1* expression was reduced in *Noc*-overexpressing MC3T3-E1 cells. Immunoprecipitation /RT-PCR analysis revealed an interaction between *Noc* protein and *Igf1* mRNA. To clarify whether the 3'UTR region of *Igf1* mRNA is recognized by *Noc*, we generated a luciferase constructs containing various lengths of the 3'UTR region in the *Igf1* message. *Noc* over-expression did not have any effect on the 170 bp short 3'UTR, which included the first poly A site. However, luciferase activity of the long form 3'UTR (6 kb) was suppressed in *Noc*-overexpressing MC3T3-E1 cells, whereas it was markedly increased in MC3T3-E1 cells expressing shRNA for *Noc*. In sum *Noc* recognizes the long form of the *Igf1* 3'UTR and apparently suppresses *Igf1* transcripts by deadenylation, possibly in collaboration with one or more RNA binding proteins. This novel post-transcriptional mechanism for regulating IGF-I may be operative during various environmental stresses including calorie restriction and post-operative catabolic conditions.

Nothing to Disclose: MK, AMD, MLA, CJR

OR03-2

Longevity and Age-Related Pathology of Mice Deficient in Pregnancy-Associated Plasma Protein-A.

Cheryl A. Conover Ph.D.¹, Laurie K. Bale¹, Jacquelyn A. Grell¹, Jessica R. Mader¹, Emily J. Mason¹, Megan A. Mason¹, Kevin P. Keenan D.V.M., Ph.D.² and Ronald J. Marler D.V.M., Ph.D.³.

¹Mayo Clin Rochester, MN ; ²Charles River Labs, Pathology Associates Frederick, MD and ³Mayo Clin Scottsdale, AZ.

Pregnancy-associated plasma protein-A (PAPP-A) is a zinc metalloproteinase that enhances insulin-like growth factor (IGF)-I bioactivity through degradation of inhibitory IGF binding proteins in the pericellular microenvironment *in vitro* and *in vivo*. Conversely, inhibition or loss of PAPP-A suppresses IGF-I receptor-mediated action without altering IGF or IGF-I receptor expression. Thus, the PAPP-A knock-out (KO) mouse is a model of reduced local IGF-I activity with normal circulating IGF-I levels and was used in this study to shed light on the role of autocrine/paracrine distinct from endocrine regulation of aging by IGF-I. PAPP-A KO mice had significantly increased mean (27%), median (27%), and maximum (35%) lifespan compared to wild-type (WT) littermates ($P < 0.0001$). End-of-life pathology was performed to assess probable cause-of-death. Incidence of hematopoietic tumors, hepatocellular carcinoma, and bronchio-alveolar carcinoma was not significantly different in the two groups of mice. However, presumably fatal neoplastic disease occurred in older aged PAPP-A KO compared to WT mice. Furthermore, PAPP-A KO mice were less likely to show degenerative changes of age, such as atrial thrombosis and nephropathy, even though the average age at death was higher. Approximately 30% of PAPP-A KO mice and 6% of WT mice died without histological evidence of lethal pathologic changes. Analyses of the pathology of PAPP-A KO and WT mice at 78, 104, and 130 weeks-of-age indicated that WT mice, in general, had more degenerative changes and tumors earlier than PAPP-A KO mice. This was particularly true for abnormalities in heart, testes, brain, kidney, spleen and thymus. In summary, the major contributors to the extended lifespan of PAPP-A KO mice are delayed occurrence of fatal neoplasias and decreased incidence of age-related degenerative changes. Thus, survival curves and pathology suggest that PAPP-A KO mice have an extended healthy lifespan.

Sources of Research Support: National Institute on Aging (R01AG028141) and The Ellison Medical Foundation.

Nothing to Disclose: CAC, LKB, JAG, JRM, EJM, MAM, KPK, RJM

OR03-3

The Aging Suppressor Klotho: A Tumor Suppressor and Modulator of the IGF-I Pathway in Human Pancreatic Cancer.

Ido Wolf M.D.^{1,2}, Lilach Abramovitz Ph.D.¹, Bella Kaufman M.D.¹ and Tamar Rubinek Ph.D.¹.

¹Sheba Med Ctr Ramat Gan, Israel and ²Tel Aviv Univ Tel Aviv, Israel.

Klotho is a transmembranal protein, composed of two subdomains, KL1 and KL2. Klotho is secreted, acts as a hormone and regulates the activity of several signaling pathways, including the insulin growth factor (IGF)-1 pathway. We have recently identified the hormone klotho as a tumor suppressor and an inhibitor of the IGF-1 in breast cancer. The IGF-I pathway plays an important role in the development of pancreatic cancer, and inhibition of the IGF-I receptor suppresses tumorigenicity and increases sensitivity of pancreatic tumors to radiation and chemotherapy. We, therefore, aimed to study the expression and activities of klotho, and its subdomain KL1, in pancreatic cancer. Immunohistochemistry revealed high klotho expression in normal exocrine pancreas (n=5), and low expression in pancreatic cancer samples (n=18; p<0.001). Klotho mRNA levels were 90-98% lower in pancreatic cancer cell lines, compared to normal pancreas. Overexpression of full length klotho, or of the KL1 domain specifically reduced colony formation and proliferation of Panc1, Colo357 and Mia-PaCa2 pancreatic cancer cell lines, but did not affect growth of the pancreatic endocrine cells Bon1 and the non-cancer cells HEK-293. Treatment with soluble human klotho (full length or KL1 domain) reduced cell viability, as measured by MTT assay, by 82% (p<0.005). Moreover, soluble klotho increased sensitivity to the cancer cells to chemotherapy. Klotho inhibited ligand-dependent activation of IGF-I and the fibroblast growth factor (FGF) pathways in the pancreatic cancer cells, while not affecting the EGF signaling pathway. For the in vivo studies, Panc1 cells were injected into flank of athymic mice and the mice were treated with daily intraperitoneal injections klotho or a control vehicle. We tested the activity of mouse klotho (10µg/kg), human klotho full length (2.5µg/kg) and KL1 protein (10µg/kg and 25µg/kg). All these forms were highly active and inhibited tumor growth. Notably, klotho administration did not affect weight or general health of the mice. Blood tests revealed no impairment of renal or hepatic functions. These data suggest klotho as a tumor suppressor and a potential novel therapy in pancreatic cancer. As klotho is an endogenous hormone, its administration to patients may be feasible and should be further explored.

Nothing to Disclose: IW, LA, BK, TR

OR03-4

Oxidation of Src by Nox4 Derived ROS Is Required for the Enhancement of IGF-I Signaling and Biological Actions in Response to Hyperglycemia in Smooth Muscle Cells (SMC).

Gang Xi PhD¹, Xinchun Shen PhD¹ and David R Clemmons MD¹.

¹Univ of North Carolina at Chapel Hill Chapel Hill, NC.

In vascular cells, high glucose induces up-regulation of NADPH oxidase (Nox) resulting in increased reactive oxygen species (ROS) production. In this study, we investigated whether the isoform Nox4 accounts for Src oxidation in SMC and if Src needs to be oxidized before it can be activated in response to IGF-I. We demonstrated that IGF-I stimulated the Src oxidation in SMC cultured in high glucose (25 mM). Inhibition of oxidation by preincubation with N-acetyl cysteine (NAC) prevented Src oxidation/activation following IGF-I treatment. Similarly, adding exogenous H₂O₂ to cells maintained in 5 mM glucose enhanced IGF-I stimulated Src autophosphorylation and this was also prevented by NAC preincubation. DPI, a Nox inhibitor, blocked the increase in ROS generation induced by high glucose and IGF-I, and it prevented IGF-I stimulated Src activation. Since Nox4 is the most abundant Nox isoform in SMC, we used shRNA to knockdown Nox4. A 75% reduction in Nox4 protein impaired high glucose and IGF-I induced ROS generation as well as Src oxidation and activation, leading to impairment of MAPK and AKT activation in response to IGF-I. Consistently, knockdown of Nox4 reduced IGF-I stimulated cell proliferation and migration in high glucose.

To understand the underlying mechanism, we examined the expression level of Nox4 and p22phox, the major subunits of Nox4 isoform oxidase. High glucose induced the expression of both in SMC. In contrast, IGF-I stimulated p22phox and Nox4 association, an event that is required for ROS generation, in high glucose. Since we have shown that IGF-I stimulated Src and Nox4 association with SHPS-1, we predicted that SHPS-1 could play a vital role in mediating Src oxidation in response to IGF-I. To support this prediction, we showed that Src oxidation/activation primarily occurred in the plasma membrane fraction following IGF-I treatment and that truncation of SHPS-1 cytoplasmic domain significantly impaired Src oxidation/activation. In addition, like Nox4, p22phox was recruited to SHPS-1 in response to IGF-I. Therefore, we postulate that high glucose induces ROS generation by increasing the Nox4 and p22phox accumulation. IGF-I stimulates an additional increase in ROS by increasing the association of Nox4 and p22phox, which occurs on the membrane localized SHPS-1 signaling complex. This localized increase in ROS leads to the enhancement of Src oxidation/activation, thereby increasing the downstream signaling and biological responses to IGF-I.

Nothing to Disclose: GX, XS, DRC

OR03-5

Dicer-Dependent microRNAs Regulate IGF-Actions in the Human Placenta.

K Forbes BSc PhD¹, JD Aplin BSc PhD¹ and M Westwood BSc PhD¹.

¹Univ of Manchester Manchester, UK.

Fetal growth restriction is associated with abnormal placental cell (cytotrophoblast) proliferation. Using an explant model of human first trimester placenta, we have demonstrated that the insulin-like growth factors (IGFs) -I and -II stimulate proliferation in cytotrophoblast and are probably essential for normal placental growth. IGF activates signalling through both Akt and ERK, so the regulation of these pathways in placenta is important for normal pregnancy outcome. The tissue contains high levels of microRNAs (miRs) some of which have been implicated in regulating IGF signalling. The production of miRs depends on processing from non-functional (pre)-miRs to mature miRs by two enzymes, Drosha and Dicer. In this study we have used Dicer siRNA to inhibit miR biogenesis in placental explants and examined the impact on IGF-stimulated proliferation in cytotrophoblast.

Dicer-specific or non-targeting siRNA (200nM) was delivered to first trimester villous tissue fragments by Nucleofector. Following transfection, tissue was maintained in culture for 72h, then treated with IGF-I or IGF-II (10nM) for a further 24h before immunohistochemical (IHC) or QPCR analysis. Following exposure to specific siRNA, Dicer protein and mRNA expression were significantly decreased. IHC analysis of cell proliferation (Ki67) revealed enhanced levels of both basal (from 17.8±2.4% to 57.8±3.2%; P<0.05, n=5) and IGF-induced proliferation (from 53.6±5.3% to 78.0±1.8% for IGF-I, and from 50.4±3.2% to 75±3.5% for IGF-II; P<0.05, n=5) following Dicer knockdown. The potential mechanism by which Dicer-dependent miRs regulate cytotrophoblast proliferation was examined by assessing the expression of molecules within the placental IGF-axis following Dicer knockdown. IHC analysis revealed no difference in IGF receptor (IGF1R) expression. However, the expression of both Akt and ERK was enhanced in the absence of Dicer. Dicer-dependent miRs regulate expression of molecules within two important pathways downstream of the IGF receptor to influence placental growth. Future investigations to define specific target genes will uncover potential therapeutic avenues for placental undergrowth which may ultimately lead to treatments for problem pregnancies.

Nothing to Disclose: KF, JDA, MW

OR03-6

Insulin-Like Growth Factor Signaling Regulates Stem/Progenitor Cells in the Epithelium of Virgin Mouse Mammary Glands.

DA Lazzarino PhD¹, L Rota MS¹, S Shushanov PhD¹, D Ludwig PhD², D LeRoith MD, PhD³ and TL Wood PhD¹.

¹New Jersey Med Sch, UMDNJ Newark, NJ ; ²Imclone Sys, a Wholly-Owned Subsidiary of Eli Lilly and Co New York, NY and ³Mt Sinai Sch of Med New York, NY.

Insulin-like growth factors (IGFs) are essential for normal pubertal epithelial growth during mammary gland development. Moreover, overexpression or constitutive activation of the IGF type 1 receptor (IGF-1R) promotes tumorigenesis in mouse models. The goal of this study was to determine if IGF signaling regulates epithelial stem and progenitor cell populations during mammary gland development. We analyzed primary mammary epithelial cells (pMECs) expressing a dominant-negative kinase-dead human IGF-1R transgene, under control of the MMTV promoter. In vivo loss of IGF signaling in the mammary epithelium resulted in a 50% reduction of cells within the CD24+CD29^{high}Sca-1⁻/lowLineage⁻ (Lin⁻), mammary stem- and committed myoepithelial-enriched pMEC fraction, as measured by flow cytometric analysis. In contrast, the CD24+CD29^{low}CD61+Lin⁻ luminal progenitor epithelial population increased 2-fold in transgenic glands. We also observed a defect in ductal branching and alveolar budding of late virgin glands, a time that correlates with multiple estrus cycles and alveolar progenitor cell expansion. Using an in vitro mammosphere culture system to enrich mammary stem/progenitor cell populations, we further demonstrated that IGF-1R signaling is essential for growth of mammospheres. Either IGF-I or IGF-II was capable of recapitulating the number and size of primary spheres obtained in standard high insulin culture media. Moreover, addition of a blocking antibody to the IGF-1R significantly reduced sphere growth. Spheres cultured under conditions of IGF-1R stimulation contained bipotential progenitors as measured by the dual expression of myoepithelial and luminal lineage markers, cytokeratin 14/cytokeratin 18, respectively. These results suggest that both mammary epithelial progenitor cell expansion and stem cell renewal are regulated by IGFs. Taken together, the data support the hypothesis that IGF signaling is required for the maintenance and renewal of stem cells in the mammary gland.

Sources of Research Support: NIH Grant DK60612 awarded to TLW.

Disclosures: DL: Employee, Lilly USA, LLC.

Nothing to Disclose: DAL, LR, SS, DL, TLW

OR04-1

Type 2 Iodothyronine Deiodinase in Mouse Skeletal Muscles.

A Marsili MD PhD¹, W Ramadan MD², MA Mulcahey BS³, SA Huang MD³, L Neves MD³, JW Harney MS¹, AM Zavacki PhD¹, JE Silva MD² and PR Larsen MD¹.

¹Brigham and Women's Hosp Boston, MA ; ²Baystate Med Ctr Springfield, MA and ³Children's Hosp Boston Boston, MA.

Skeletal muscle is a major determinant of energy expenditure. Since skeletal muscle is also a target for thyroid hormones, the role of local activation of T4 by type 2 iodothyronine-deiodinase (D2) is of interest. A recent report suggested that, unlike the effect of hypothyroidism to increase D2 in rat tissues, there was no change in human muscle D2.

The aim of this study was to evaluate factors important for D2 expression in mouse skeletal muscle. In particular we were interested in the correlation between fiber composition and D2 and whether these influence the response to hypothyroidism.

To assess fiber composition, we examined different hind limb skeletal muscles from C57/BL6 male mice (8-14 weeks of age). To induce hypothyroidism, the same mice were treated with 0.1% MMI and 1% NaClO₄ in drinking water for either 4 (short term) or 8 (long term) weeks. Untreated age and gender-matched mice were used as controls. D2 mRNA was determined by quantitative RT-PCR relative to cyclophilin. Microsomal protein from skeletal muscles was prepared by differential centrifugation; triplicate samples were incubated in an overnight D2 assay with 1 nM T4, 1 mM PTU, 25 nM T3. Products were analyzed by HPLC. Microsomes from *Dio2* null mice were used as a tissue background.

D2 activity was detectable in all muscles, and it was highest in the red, type-I, aerobic, slow twitch muscle soleus (0.5±0.07 fmol/min/mg) and lower in type II fast-twitch/anaerobic or mixed muscles (0.06±0.01 in vastus lateralis (VL); 0.08±0.01 in gastrocnemius and 0.11±0.01 in tibialis anterior (mean±SEM). Interestingly, in the largely red component of the quadriceps muscle (vastus intermedius), D2 activity was 3 fold greater (0.17±0.03) than in the white portion (VL) (p<0.01).

Four weeks of antithyroid drug treatment reduced serum T4 <0.5 µg/dl and induced a 30-40% increase (p<0.01) in D2 activity. Treatment for 8 weeks caused a 3 fold increase in D2 activity (p<0.001) in all muscles. There were no significant changes in D2 mRNA in either group.

We conclude that D2 is expressed in adult mouse skeletal muscle and is higher in type I-oxidative than in type II-glycolytic fibers. Variations in the type of muscle fibers should be kept in mind when analyzing results of muscle D2 activities. Severe hypothyroidism markedly increases D2 by a post-translational mechanism, suggesting that, at least in this species, skeletal muscle is sensitive to decrease in circulating T4.

Nothing to Disclose: AM, WR, MAM, SAH, LN, JWH, AMZ, JES, PRL

OR04-2

The Natural History of Subclinical Hypothyroidism in the Elderly: The Cardiovascular Health Study.

C M Rariy MD¹, A R Cappola MD, ScM¹, A M Arnold PhD² and L P Fried MD, MPH³.

¹Univ of Pennsylvania Sch of Med Philadelphia, PA ; ²Univ of Washington Seattle, WA and ³Columbia Univ Mailman Sch of Public Hlth New York, NY.

Background: Subclinical hypothyroidism is common in the elderly, yet the degree to which it persists over time is unknown. We examined regression, persistence, and progression of subclinical hypothyroidism over a four year period in an older population.

Methods: In 3,593 US community-dwelling men and women aged 65 and over who were enrolled in the Cardiovascular Health Study and not taking thyroid medications, serum TSH and free T4 concentrations were measured in banked specimens at 1992-1993 (Baseline) and 1996-1997 (Year 4). We identified 459 subjects with subclinical hypothyroidism (TSH=4.5-19.9 mU/L with a normal free T4 level) at baseline and analyzed their status at Year 4.

Results: Of the 459 individuals with subclinical hypothyroidism, 61% were female, 10% were non-white, and mean age was 75.6 years. 58 participants with subclinical hypothyroidism died by Year 4; their mortality did not differ from those who were euthyroid at baseline (12.6% vs. 12.9%). Among those with subclinical hypothyroidism who obtained follow-up thyroid testing or were taking thyroid medications at year 4 (n=321), 26% became euthyroid, 56% persisted, 2% progressed to overt hypothyroidism, and 16% initiated thyroid medication. In those with transient subclinical hypothyroidism, the median baseline TSH was 5.1 mU/L, and the median Year 4 TSH was 3.6 mU/L. Moreover, TSH decreased by more than 1 mU/L in >75% of those whose subclinical hypothyroidism resolved. In those with persistent subclinical hypothyroidism, the median baseline TSH was 6.1 mU/L, and the median Year 4 TSH was 6.4 mU/L. Additional data incorporating Year 2 thyroid function testing and baseline antiTPO antibodies will be presented.

Year 4 Status (n) in 459 Subclinical Hypothyroid Participants

| Baseline | Year 4 | | | | | Total |
|----------|-----------|----------|------------|------|-----------------------------|-------|
| | Euthyroid | Sub Hypo | Overt Hypo | Meds | Not on Meds, No F/U or Died | |
| TSH<10 | 81 | 164 | 4 | 37 | 128 | 414 |
| TSH≥10 | 2 | 15 | 2 | 16 | 10 | 45 |
| Total | 83 | 179 | 6 | 53 | 138 | 459 |

Conclusions: Nearly half of older individuals with endogenous subclinical hypothyroidism will not remain so four years later, due to resolution, progression, or initiation of medication. Use of a single set of thyroid function tests to define subclinical hypothyroidism may lead to significant misclassification over time and could influence the results of longitudinal studies of clinical outcomes. These data also suggest that at least one quarter of older people with subclinical hypothyroidism may not require treatment due to subsequent TSH normalization.

Sources of Research Support: R01-032317 from the NIA and contracts N01-HC-85079 through N01-HC-85086, N01-HC-35129, and N01 HC-15103 from the NHLBI.

Nothing to Disclose: CMR, ARC, AMA, LPF

OR04-3

Effects of Iatrogenic Hyperthyroidism on Body Mass in Thyroid Cancer Patients.

HN Polotsky MD¹, M Brokhin MD¹, G Omry MD¹ and RM Tuttle MD¹.

¹Memorial Sloan-Kettering Cancer Ctr New York, NY.

Background: Spontaneous hyperthyroidism is frequently associated with weight loss, whereas normalization of thyroid function in affected individuals often leads to weight gain. However, the exact role of thyroid hormones in regulation of acute and chronic weight fluctuations and maintenance is not well understood. The objective of this study was to determine the effects of iatrogenic hyperthyroidism on body mass in otherwise healthy thyroid cancer survivors.

Methods: Records of differentiated thyroid cancer patients treated at Memorial Sloan-Kettering Cancer Center were examined. Analysis was limited to patients who were treated with total thyroidectomy and radioactive iodine (RAI) remnant ablation for which complete information was available. Patients with poorly differentiated pathology, prior history of unrelated malignancy, pre-existing thyroid disease, distant metastases at presentation, external beam radiation and significant medical co-morbidities (diabetes, cystic fibrosis) were excluded. Weight and body mass index (BMI) were analyzed at baseline (immediately prior to surgery) and two post-treatment time-points (6-24 months and 3-5 years). All patients were maintained on thyrotropin suppression with the target TSH at or below the lower limit of normal (0.1-0.5mU/L). Group means were compared using Mann Whitney U test and repeated measures ANOVA as appropriate.

Results: The analytic sample included 87 thyroid cancer patients (mean age 42.4 +13.5 years, 71.2% females). Significant increases in weight, BMI and percent weight change occurred in parallel with chronological aging.

Anthropometric Characteristics of the Study Cohort before and after Therapy (Thyroidectomy with RAI Treatment)

| | Baseline | 1st follow-up 6-24 months | 2nd follow-up 3-5 years | P-value |
|------------------------|-------------|---------------------------|-------------------------|---------|
| Weight, kg | 76.3 (20.6) | 77.6 (21.7) | 80.5 (25.9) | <0.01 |
| Weight Change, % | | 1.9 (-0.8,4.4) | 3.2 (0.3,7.4) | 0.04 |
| BMI, kg/m ² | 27.0 (5.7) | 27.6 (6.2) | 28.5 (7.6) | <0.01 |
| Median TSH, mU/L | | 0.15 (0.03,0.64) | 0.10 (0.02,0.76) | |

Data are presented as mean (sd) if normally distributed or median (interquartile range) if skewed

Conclusions: In otherwise healthy differentiated thyroid cancer patients, significant weight gain occurred during the 3-5 years of follow-up despite the thyrotropin suppression. The data suggest that mild iatrogenic hyperthyroidism does not promote weight loss or prevent aging-related weight gain.

Nothing to Disclose: HNP, MB, GO, RMT

OR04-4

Pharmacogenomic Response to Thyrotropin-Releasing Hormone Stimulation in Healthy Volunteers: The Influence of a Common Type 2 Deiodinase Gene Polymorphism on Serum T3.

PW Butler MA MD¹, SM Smith BSN RN¹, JD Linderman MS BSN RN¹, RJ Brychta PhD¹, AT Alberobello PhD¹, OM Dubaz BA¹, JA Luzon BS¹, MC Skarulis MD¹, CS Cochran RN¹, RA Wesley PhD¹, F Pucino PharmD MPH^{1,2} and FS Celi MD MHSc¹.

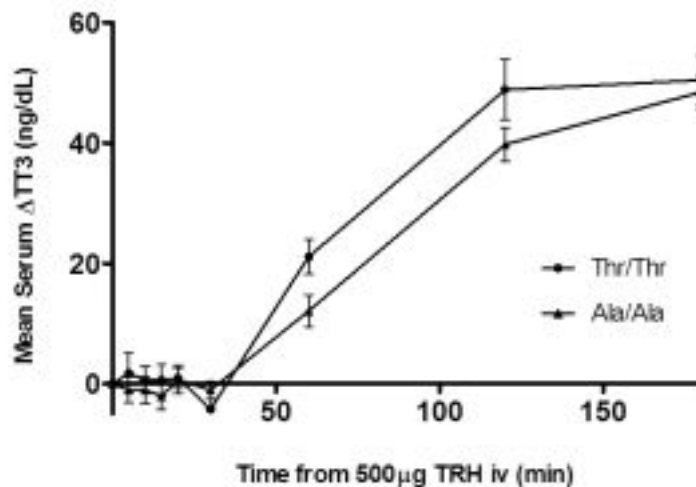
¹Natl Inst of Hlth Bethesda, MD and ²Food and Drug Administration Silver Spring, MD.

Background: Type 2 deiodinase (D2)-mediated intrathyroidal conversion plays an important role in the maintenance of circulating levels of T3 as demonstrated in states of sustained cAMP pathway activation (1). The common threonine 92 alanine (Thr92Ala) D2 polymorphism has been associated with changes in pituitary-thyroid axis homeostasis (2,3) but the results are conflicting (4). We thus sought to investigate the effects of the Thr92Ala polymorphism on thyroid hormone secretion in volunteers by using the TRH stimulation test to investigate the acute response to TSH.

Methods: 83 healthy volunteers were screened and genotyped for the Thr92Ala polymorphism by PCR/RFLP. Fifteen volunteers of each genotype (Thr/Thr, Thr/Ala, and Ala/Ala) underwent a 500 µg iv TRH test with serial measurements of serum total T3 (TT3), free T4 (fT4) and TSH over 180 minutes.

Results: Mean baseline TT3 (103 v. 105 ng/dL), fT4 (1.3 v. 1.3 ng/dL) and TSH (1.69 v. 1.24 mIU/mL) concentrations were not different between Ala/Ala and Thr/Thr groups, respectively (all p=n.s.). As shown in Figure 1, Ala/Ala subjects showed an attenuated rate of rise in serum T3 levels compared to Thr/Thr subjects (p<0.03). Stimulated delta serum TT3 levels were significantly different (p=0.03) at 60 minutes, AUC for 30-180 minutes trended in the same direction (p=0.09). Subjects attained similar maximal (180 min) TRH-stimulated TT3 levels.

Conclusion: Our results indicate that the commonly-occurring Thr92Ala D2 variant is associated with a decreased rate of acute TSH-stimulated TT3 release from the thyroid and are consistent with a diminished rate of TSH-stimulated intrathyroidal T4 to T3 conversion by D2. These data provide a proof of concept that the Thr92Ala polymorphism is associated with subtle changes in thyroid hormone homeostasis. Further studies will be necessary to investigate the effects of this polymorphism in end-organ targets of thyroid hormone action.



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Sources of Research Support: The Intramural Research Program of the NIDDK, program Z01-DK047057-02.

Nothing to Disclose: PWB, SMS, JDL, RJB, ATA, OMD, JAL, MCS, CSC, RAW, FP, FSC

OR04-5

An Undetectable Initial rhTSH-Stimulated Tg Levels 12 Months after the Initial Treatment of Thyroid Cancer Serves as a Good but Not Infallible Indicator for Cure.

J Klubo-Gwiedzinska MD, PhD¹, KD Burman MD¹, D Van Nostrand MD¹ and L Wartofsky MD¹.

¹Washington Hosp Ctr Washington, DC.

Background

Follow-up evaluation after the treatment of well-differentiated thyroid cancer (WDTC) includes cervical ultrasonography (US) and measurement of serum thyroglobulin (Tg) during TSH suppression, and after recombinant human TSH(rhTSH)-stimulation. Once rhTSH-stimulated Tg is undetectable, the timing and necessity of subsequent rhTSH testing is uncertain.

Aim

The aim of our study was to evaluate the utility of repeated rhTSH-stimulated Tg measurements in patients with WDTC who have had no evidence of disease at their initial rhTSH-stimulation test.

Material and Methods

A retrospective chart review of 278 patients with WDTC treated with total/near total thyroidectomy and radioiodine ablation was performed. Inclusion criteria: undetectable basal and initial rhTSH-stimulated Tg level, measured by immunometric assay. Exclusion criteria: detectable Tg antibodies.

Results

Pathology: 57.1% classic papillary thyroid cancers (PTC), 20.4% follicular variant of PTC, 4.2% columnar or tall cell variant of PTC, 10.4% follicular and 7.9% Hurthle cell cancer. Clinical stage at diagnosis : I in 69.6% of cases, II in 15%, III in 12.9% and IV in 2.5%.

The number of repeated rhTSH stimulation tests during the follow up period (3-11 years, mean 6.3) varied from two to seven. After having a negative initial Tg response to rhTSH, 10 patients (3.6%) had a detectable rhTSH stimulated Tg values during follow-up: 9/10 after a second rhTSH stimulation and 1/10 after the third. Of these 10, 5 had stimulated Tg between 0.5-1 ng/ml with no other evidence of disease and 5 had rhTSH-stimulated Tg level > 1 ng/ml, which was consistent with cervical or mediastinal lymph node enlargement. All patients were followed without further treatment. Disease progression was observed only in 1 patient, who had presented with stage IV thyroid cancer at initial diagnosis. Nevertheless, repeated rhTSH stimulation tests were not necessary in this case, because suppressed Tg levels rose to detectable values.

Conclusion

An undetectable rhTSH-stimulated Tg levels 12 months after the initial treatment of thyroid cancer served to indicate good prognosis for cure in 96.4% of patients. Although continued follow-up of these patients could be based only on suppressed Tg measurements, at least one additional rhTSH stimulation test would be required to detect the 3.6% of patients with residual disease.

Nothing to Disclose: JK-G, KDB, DVN, LW

OR04-6

A Study of the Rate of Thyroid Testing in Pregnancy at an Academic Boston Area Medical Center.

DL Chang MD PhD¹, AM Leung MD¹, LE Braverman MD¹ and EN Pearce MD MSc¹.

¹Boston Univ Sch of Med and Boston Med Ctr Boston, MA.

Introduction: Maternal hypothyroidism in pregnancy may lead to adverse obstetric outcomes and intellectual impairment in the offspring. However, there are conflicting views on the role of thyroid screening in pregnancy. A recent study has shown that the case-finding approach recommended in the 2007 Endocrine Society guidelines may miss up to 30% of pregnant women with overt/subclinical hypothyroidism. The present study examined the rate of thyroid testing in pregnant women at Boston Medical Center (BMC) over one year.

Design: We conducted a retrospective cohort study to examine the rate of thyroid testing in 1000 pregnant women aged 18 to 46 yr who presented to BMC's Obstetrics/Gynecology (OB/GYN) or Family Medicine (FAM) Clinics for their first prenatal visit during 2008. Demographic data (age, race, insurance), medical history (thyroid, other autoimmune disorders, obstetrics history) and thyroid function tests were assessed. Gestational age was estimated from the last menses or ultrasound dating when available.

Results: 983 patients were included in the final data analysis (17 were excluded due to improper coding). The overall median maternal age was 28 yr and median gestational age was 10.3 wk. 33 pt had prior thyroid disease, 15 pt had a history of other autoimmune diseases, and 18 pt were on thyroid medication. Thyroid function tests were carried out in 84% (826 pt) and the median gestational age at the time of testing was 9.7 wk. The majority of pt (65%, 541/826) had a serum TSH during the 1st trimester (340 pt at <9 wk, 201 pt at 9-12 wk). 918 pt (93%) were followed in the OB/GYN clinic and only 65 pt (7%) in the FAM clinic. However, the rates of thyroid testing were similar in both (84% and 86%, respectively). Of the 826 pts tested, 67 (8.1%) had a TSH > 2.5 mIU/L (63 pt, 2.5-5.5 and 4 pt, >5.5). Of these 67 pt, only 6 had a prior history of thyroid disease and 2 had type 1 DM. Based on current case-finding guidelines, 59 of the 67 pt with a TSH >2.5 might not have been tested and their overt/subclinical hypothyroidism would have gone undetected. **Conclusion:** BMC has a high rate of thyroid function testing (84%) in pregnant women. Performing targeted thyroid testing only in high risk patients would have missed 88% of pregnant women with thyroid hypofunction. Finally, our findings emphasize the value of screening for thyroid dysfunction in pregnant women since 8.1% had evidence of thyroid hypofunction.

Nothing to Disclose: DLC, AML, LEB, ENP

OR05-1

The Metabolically Healthy Obese: A Prospective Study on Risk of Development of Cardiovascular Events.

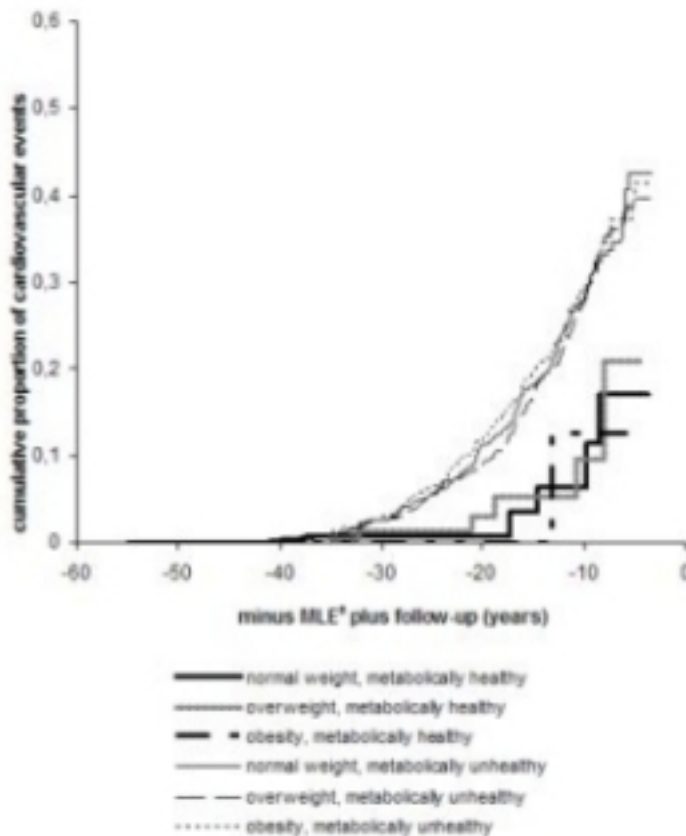
F.A.J. Verburg MD¹, A.P. van van Beek MD PhD¹, W.J. Sluiter PhD¹, S.J.L. Bakker MD PhD¹, R.T. Gansevoort MD PhD¹ and B.H.R. Wolffenbuttel MD PhD¹.

¹Univ Med Ctr Groningen, Univ of Groningen Groningen, Netherlands.

Objective. Obesity is a major health problem by its associated excess risk of cardiovascular disease. However, some obese subjects do not have concomitant impaired glucose tolerance, hypertension and dyslipidemia. There are no prospective data whether these metabolically healthy obese subjects are protected against cardiovascular disease.

Methods and Results. In the ongoing prospective Dutch PREVEND study a total of 3612 participants (43.2%) had normal weight (BMI <25 kg/m²) at baseline, 3419 (40.9%) had overweight (BMI 25-29.9 kg/m²) and 1325 (15.9%) had obesity (BMI ≥30kg/m²). In the group with normal weight 39.1% were metabolically healthy, defined as no history of cardiovascular disease, the absence of diabetes (ADA criteria) and hypertension (JNC 7 criteria) and dyslipidemia (LDL cholesterol ≥3.50 mmol/l or HDL cholesterol ≤1.03 mmol/l for men and ≤1.29 mmol/l for women or triglycerides ≥1.7 mmol/l or the use of lipid lowering drugs). In the groups with overweight and obesity 13.3% and 6.8% were metabolically healthy. During a median follow-up of 7.5 years cardiovascular events occurred in 0.6% of participants with metabolically healthy normal weight, in 1.3% of subjects with healthy overweight and in 1.1% of the healthy obese (P=NS). In metabolically unhealthy participants these percentages were 6.3%, 9.4% and 10.6% for subjects with normal weight, overweight and obesity, respectively.

The figure shows the cumulative proportion of cardiovascular events in the different groups. By projecting life-expectancy on the negative x-axis, participants with the same life-expectancy are compared in the time before (expected) death.



Cardiovascular events during follow-up in different subgroups¹

¹left censored on events vs. MLE² to eliminate bias by differences in age at baseline and differences in life expectancy because of gender and year of birth

²Median Life Expectance

In addition, Cox regression analysis revealed that Body Mass Index was not associated with an elevated cardiovascular risk (HR 1.09, p=0.473), when corrected for gender, year of birth, previous cardiovascular disease and metabolic

parameters.

Conclusions. Metabolically healthy obesity represents only a small subset of the total obese population. Metabolically healthy obese persons do not have an elevated cardiovascular risk when compared to normal weight or overweight subjects with a similar metabolic profile.

Nothing to Disclose: FAJV, APvB, WJS, SJLB, RTG, BHRW

OR05-2

AHNAK Gene Expression Is Increased in Human Obesity and Decreased after Bariatric Surgery.

I Hochberg MD/Ph.D¹, A Assalia MD², D Ben-Yosef Ph.D¹, M Armoni Ph.D¹, TP Ciaraldi Ph.D³, RR Henry MD³ and E Karnieli MD¹.

¹Rambam Hlth Care Campus and Fac of Med, Technion Haifa, Israel ; ²Rambam Hlth Care Campus Haifa, Israel and ³Veterans Affairs San Diego Healthcare Syst and Dept of Med, Univ of California San Diego, CA.

Background: AHNAK is a giant phospho-protein that is increased in adipose and muscle tissues in animal models of obesity. Previous results from our group suggest that AHNAK represses the transcription activity of GLUT4 gene leading to insulin resistance. In order to study the relevance of AHNAK to human obesity we studied the expression of AHNAK in obese patients.

Methods: We isolated adipocytes from subcutaneous (SCad) and visceral abdominal (Vad) adipose tissue biopsies obtained from 38 patients undergoing elective surgery (15/23 M/F; age 39.7±13.6 (Mean±SD); BMI 39.4±9.6 kg/m²; and HOMA 3.11±2.52). AHNAK mRNA was quantified using real-time PCR. Adipocyte diameter, as measured by microscopy, was 502±307 μ³×10⁶ for Vad and 701±423 μ³×10⁶ for SCad. AHNAK mRNA expression was also determined in visceral fat biopsies obtained from 6 patients before and after weight loss after bariatric surgery.

Results: SCad AHNAK mRNA correlated with BMI (R²=0.38; p=0.029), weight (R²=0.372; p=0.033), HOMA (R²=0.555; p=0.001), and fasting plasma glucose levels (R²=0.414; p=0.018). Further, Vad AHNAK mRNA levels positively correlated with adipocyte volume (R²= 0.493; p=0.004) and AST levels (R²=0.354; p=0.034). The correlation between AHNAK gene expression and obesity is supported by a good correlation between the extent of weight loss and the reduction of AHNAK mRNA in the 6 patients where biopsies were taken before and after weight loss (R²=0.943 ; p=0.005). Further, we found a significant correlation between AHNAK mRNA expression in Vad and SCad from the same patient (correlation coefficient 0.555; p=0.001).

AHNAK mRNA level in VAd and SCad was higher in patients with hyperlipidemia (p=0.016 and p=0.047 respectively). However, there was no correlation between AHNAK mRNA expression in adipocytes and basal insulin levels, HbA1c, or a diagnosis of diabetes.

Conclusions: The correlation between the AHNAK levels in human adipocytes, and the degree of obesity and derangement in metabolic parameters, suggests a potential role for AHNAK in the pathogenesis of insulin resistance.

Sources of Research Support: Chief Scientist Office at the Ministry of Health, Jerusalem, Israel Grant #3-00000-5563; Rambam Health Campus, Division of Medicine Fellow Grant.

Nothing to Disclose: IH, AA, DB-Y, MA, TPC, RRH, EK

OR05-3

Outcomes of Bariatric Surgery in Obese Patients Heterozygous for MC4R Mutations.

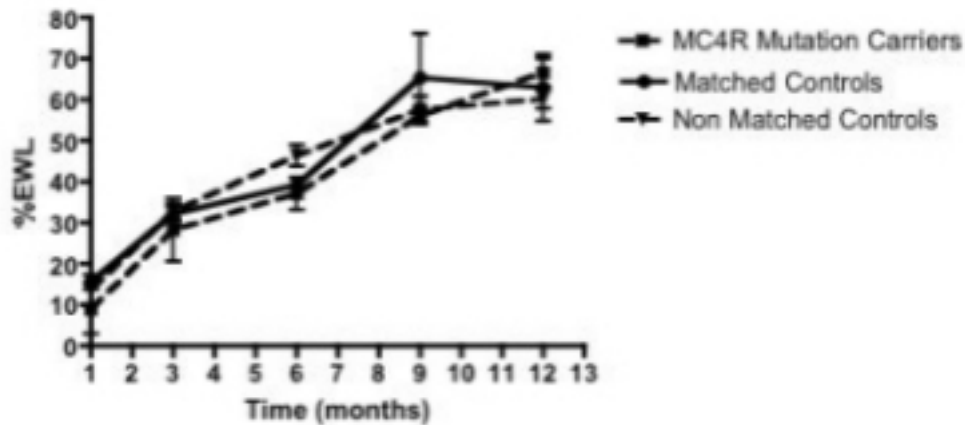
IPR Aslan MD¹, MA Calton BS¹, G Campos MD², DS Evans PhD¹ and C Vaisse MD, PhD¹.

¹Univ of California, San Francisco San Francisco, CA and ²Univ of Wisconsin Madison, WI.

The melanocortin-4 receptor (MC4R) is essential for central regulation of energy homeostasis and food intake. Heterozygous mutations are the most frequent genetic cause of obesity, with a global prevalence of approximately 2.5% in severely obese individuals (1). It was suggested that patients with MC4R mutations may have poorer outcomes after bariatric surgery (2); however, the large majority of patients in that report carried MC4R variants that were not associated with obesity and had no demonstrated functional effect on the protein.

Objective: To describe clinical outcomes and weight loss after Roux-en-Y Gastric bypass (RYGB) in severely obese patients (Body Mass Index, BMI > 35) with heterozygous functionally relevant melanocortin-4 receptor (MC4R) gene mutations.

Patients and Methods: Ninety-two severely obese patients who had undergone bariatric surgery were screened for MC4R mutations. Of these patients, 4 were heterozygous for functionally relevant MC4R mutations. We compared percent excess weight loss (%EWL) in the 4 MC4R mutation carriers with that of two control groups; Matched controls: 8 patients matched for BMI, gender, age and presence of diabetes mellitus; and non matched controls: the remaining 80 patients who underwent RYGB and for whom follow-up data were available.



Results: Four different heterozygous MC4R gene mutations were found: Cys271Phe, Pro299Ser, Arg236Cys (two patients had this mutation). In these patients with MC4R mutations, %EWL after bariatric surgery (66% EWL) was not significantly different compared to matched controls (70% EWL) and BMI controls (60% EWL) after one year.

Conclusion: This is the first study to demonstrate weight loss outcomes after Roux-en-Y Gastric bypass in patients with functionally relevant MC4R mutations. This study suggests that patients with heterozygous MC4R mutations may also benefit from bariatric surgery and that weight loss may be independent of the presence of heterozygous MC4R mutations.

(1) Farooqi IS et al., N Engl J Med 2003; 348: 1085-95.

(2) Potoczna N et al., J Gastrointest Surg 2004; 8:971-981.

Sources of Research Support: Genentech Endocrinology Fellowship Grant ;AHA Postdoctoral Fellowship Grant; National Institutes of Health R01 DK 60540 Awards; ADA mentor-based Postdoctoral Fellowship Award.

Nothing to Disclose: IPRA, MAC, GC, DSE, CV

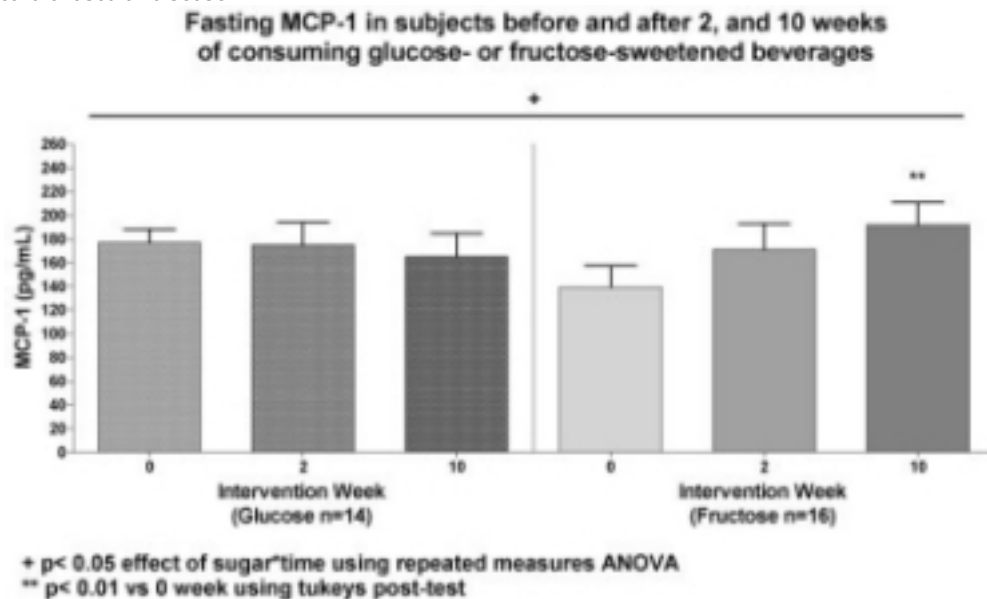
OR05-4

Consuming Fructose-, but Not Glucose-, Sweetened Beverages Increases Fasting Concentrations of MCP-1 in Older, Overweight/Obese Men and Women.

CL Cox¹, KL Stanhope¹, AA Bremer¹, NL Keim² and PJ Havel¹.

¹Univ of California, Davis Davis, CA and ²United States Dept of Agriculture, Western Human Nutrition Res Ctr Davis, CA.

We have recently reported that consuming fructose for 10 weeks, at 25% of energy requirements, leads to increased intra-abdominal fat deposition and a more atherogenic lipid profile in overweight (BMI 25-35; age 43-70) adults compared with isocaloric consumption of glucose (1). To determine if fructose consumption also increases MCP-1, a marker of inflammation within adipose tissue associated with visceral fat accumulation and atherosclerosis, we measured fasting plasma MCP-1 concentrations before and after 2 and 10 weeks of consumption of fructose- or glucose-sweetened beverages in these same subjects. During the 2-week baseline phase of the study subjects resided at the Clinical Research Center (CCRC) and consumed an energy-balanced diet (55% complex carbohydrate, 30% fat, 15% protein). Subjects were then discharged from the CCRC and consumed their usual diets *ad libitum*, along with either glucose- or fructose-sweetened beverages (25% of energy requirements) for 8 weeks. Subjects returned to the CCRC for inpatient procedures following 2 weeks of intervention and for the final 2 weeks of the study during which they consumed the beverages as part of an energy balanced diet (25% sweetened beverage, 30% complex carbohydrate, 30% fat, 15% protein). Fasting concentrations of MCP-1 increased from 144 to 178 pg/mL after 2 weeks (+28 ± 10%) and increased further to 199 pg/mL after 10 weeks (+84 ± 33%; P = 0.03 sugar*time) of intervention in subjects consuming fructose, but were not increased in subjects consuming glucose (-2 ± 9% at 2 weeks; -8 ± 11% at 10 weeks). There was a significant influence of the number of metabolic syndrome risk factors (MSRF) (0, 1, 2, or 3) on the change of MCP-1 (MSRF*time P = 0.037) and a significant 3-way interaction between sugar, number of MSRFs, and time (sugar*MSRF*time P = 0.041). These results indicate that, within the fructose group, subjects having fewer MSRFs exhibited larger increases of MCP-1 over time. These findings thus suggest that sustained fructose consumption promotes inflammation within adipose tissue, which has been linked to insulin resistance and increased risk of cardiovascular disease.



1) Stanhope KL et al., J Clin Invest 2009;119:1322

Sources of Research Support: National Institutes of Health grants HL-075675 and UL1 RR024146.

Nothing to Disclose: CLC, KLS, AAB, NLK, PJH

OR05-5

Effects of Macronutrient Manipulation on Short-Term Metabolic Response to Overfeeding.

MS Thearle MD¹, N Pannaciuoli MD, PhD², S Bonfiglio PA¹ and J Krakoff MD¹.

¹NIDDK/NIH Phoenix, AZ and ²Bristol Myers Squibb Princeton, NJ.

Background: The thermic effect of food (TEF) consists of both obligatory and facultative components. Studies have indicated that diets with unbalanced macronutrient content, especially low protein diets, lead to a higher energy cost of weight gain. To explore the role macronutrients play in stimulating a facultative response during short term overfeeding in humans, we investigated the energy expenditure (EE) response to 24 hours of overfeeding with diets of varying macronutrient content.

Methods: Subjects with normal glucose regulation were overfed twice energy balance requirements or fasted for 24 hours while in a respiratory chamber (n=14: 71% male; age 35±9 y; BMI 28.3±5 kg/m²; %body fat 33.3±11.2%; mean±SD). Diets were given in random order, with an intervening 3 day washout period, and with the following variations in macronutrient content: 50% carbohydrates (C), 30% fat (F), 20% protein (P) (BOF); 51% C, 46% F, 3% P (LPF); 75% C, 5% F, 20% P (CNP); 75% C, 22% F, 3% P (CLP); 20% C, 60% F, 20% P (FNP). 24h EE, sleeping EE and TEF (defined as the diet related EE as percent of daily caloric intake) were compared to baseline measurements during eucaloric feeding using one way ANOVA.

Results: The greatest increases in 24h EE occurred during diets with higher protein content (%difference over baseline: BOF: 7.3% (95% CI 4.2, 10.3%), p<0.01; CNP 12.4% (9.4, 15.5%), p<0.0001; FNP 7.1% (4.1, 10.1%), p<0.01). There was no difference between EE during low protein diets (3%P) and eucaloric feeding. Sleeping EE also increased after the CNP and FNP diets (%difference over baseline: CNP 11.1% (6.6, 15.5%), p=0.004; FNP 12.5% (8.0, 16.9%), p=0.0009). The average TEF of the overfeeding diets did not differ from the TEF during energy balance (9.1±7.1%). TEF was reproducible with an intraclass correlation coefficient between the 3 normal protein chambers of 0.7 (p<0.0001). TEF during overfeeding was negatively associated with %body fat even after adjustment for sex, age, race and diet such that a 1% increase in %body fat was associated with a 0.45±0.17% decrease in TEF (p=0.02).

Conclusions: Although 24h EE increased with overfeeding on the higher protein diets, the percent of caloric intake used for processing nutrients did not change. However, sleeping EE increased during the CNP and FNP diets indicating recruitment of a thermogenic process. Further, TEF with overfeeding was negatively associated with adiposity measures indicating a possible role in weight regulation.

Sources of Research Support: Intramural Research Program of the NIH/NIDDK.

Nothing to Disclose: MST, NP, SB, JK

OR05-6

Effect of Diet Composition on Weight Loss in Insulin Resistant People.

RA Plodkowski MD^{1,2}, ST St Jeor PhD¹, QT Nguyen DO¹, GCJ Fernandez PhD^{1,3} and VB Dahir PhD^{1,3}.

¹Univ of Nevada Sch of Med Reno, NV ; ²VA Sierra Nevada Hlth Care Syst Reno, NV and ³Univ of Nevada Reno, NV.

Background: There has been controversy regarding the best macronutrient composition for calorie restricted diets for weight loss. Many professionals and health advocacy groups recommend a low fat diet but low carbohydrate diets have also been popular. However, one diet may not give optimal weight loss for all individuals. This study was conducted to determine if diet composition would impact the amount of weight loss in insulin resistant (IR) participants.

Methods: Females (n=45) between 18 and 65 years of age and a BMI 30-40 kg/m² were recruited from the general population. After informed consent was performed, fasting insulin levels were used to identify IR subjects (>15 uIU/mL). The IR subjects were randomly assigned to either a low fat diet (LF: 60% carbohydrate, 20% fat and 20% protein) or a low carbohydrate diet (LC: 45% carbohydrate, 35% fat and 20% protein) administered utilizing calorie controlled foods. The primary outcome, change in body weight (kg), was recorded for each subject at 4, 8 and 12 weeks. A repeated measures mixed model ANOVA was used to test the mean differences in the change in body weight from baseline between the LF and LC treatment groups at 4, 8 and 12 weeks. Based on the minimum information criterion, the first-order autoregressive (AR(1)) covariance structure gave the best fit and was used to model the correlation between the three repeated measurements.

Results: No statistically significant baseline differences were observed between the LF and LC groups for mean age (51.1 versus 49.4 years, p=0.58) or baseline body weight (95.2 kg versus 101.5 kg, p=0.0883), respectively. As a group, all IR subjects (LF and LC) significantly lost weight at 4, 8 and 12 weeks (p <0.0001). When the two different groups were compared, the differences between the LF and LC group in weight loss (kg) became significant at 12 weeks: 3.23 versus 4.81 kg at 4 weeks (p=0.10); 5.64 versus 7.14 kg at 8 weeks (p=0.12); and 7.34 versus 9.33 kg at 12 weeks (p=0.04), respectively.

Conclusion: This study showed that for people with insulin resistance (>15 uIU/mL) a low carbohydrate diet yielded significantly more weight loss compared with a low fat diet at 12 weeks. These data have potential widespread applications for clinical practice when counseling people with insulin resistance regarding lowering carbohydrate intakes to help improve weight loss as part of a calorie restricted diet. (ClinicalTrials.gov Identifier: NCT01034046)

Nothing to Disclose: RAP, STSJ, QTN, GCJF, VBD

OR06-1

Potent, Orally Available Inhibitors of Glucosylceramide Synthase Improve Glucose Tolerance.

S Richards Ph.D.¹, A Hanel Ph.D.¹, E Koltun Ph.D.¹, K Ogilvie Ph.D.¹, KA Won Ph.D.¹, L Mattheakis Ph.D.¹, P Finn Ph.D.¹, P Kearney Ph.D.¹ and CJ Larson Ph.D.¹.

¹Exelixis South San Francisco, CA.

Recent publications suggest insulin resistance in tissue may be in part due to the presence of elevated levels of a particular group of lipids, called glycosphingolipids. Glucosylceramide synthase (GCS) is a key enzyme in the glycosphingolipid biosynthetic pathway. Medicinal chemistry optimization of high throughput screening hits has resulted in potent, selective, orally available GCS inhibitors that reduce levels of the ganglio-series of glycosphingolipids in liver, fat, and muscle cells, and normalize metabolic parameters in vivo. Data from an example lead are presented. EXEL-0346 inhibits GCS enzymatic activity in biochemical and cellular assay formats (IC₅₀'s of 2.2 and 14.9 nM, respectively), while showing no activity against a panel of over 40 other pharmacological targets, including glycosidases, known off-target liabilities of other inhibitors of this enzyme. EXEL-0346 reduces formation of glucosylceramides and downstream metabolites in liver, muscle, and fat cells, and in vivo in both lean and DIO mice after a single dose. EXEL-0346 normalizes numerous metabolic parameters in multiple rodent studies. In DIO mice, as well as lowering glucosylceramide levels in target tissues, chronic oral dosing with EXEL-0346 improves oral glucose tolerance and fasting glucose and insulin after 14 days. In addition, EXEL-0346 treatment reverses the fatty liver phenotype induced by high fat diet, including relief of centrilobular microvacuolar fat as measured by oil Red O staining, reduced hepatic triglyceride and cholesterol measures, and normalization of ALT and AST levels. Reductions in circulating triglycerides are also observed. In parallel to these changes, improved insulin signaling in liver, fat, and muscle can be measured. EXEL-0346 chronic oral dosing also lowered glucosylceramide levels while improving various metabolic measures in ZDF rats, including oral glucose tolerance, fasting glucose, fasting insulin, and HbA1c levels. EXEL-0346 likewise improves hepatic hypertriglyceridemia. When compared to lean normal rats, insulin resistant ZDF rats exhibit a greater number of islets, greater islet size, more beta cells per islet and larger beta cells in the pancreas; EXEL-0346 reduces these parameters towards those observed in normal rats, lean rats. Thus inhibition of GCS, as exemplified by the described lead EXEL-0346, represents a potentially novel therapeutic strategy for the treatment of diabetes and other pathologies of metabolic syndrome.

Nothing to Disclose: SR, AH, EK, KO, KAW, LM, PF, PK, CJL

OR06-2

Postprandial ApoB48 Levels Determined Using ELISA in Diabetic and Normal Subjects.

A Leonard BSc¹, T Kyaw Tun MB BCh¹, P Gaffney PhD¹, J Sharma MB BS¹, J Gibney MB BCh, MD¹ and G Boran MB BCh, MD¹.

¹Adelaide & Meath Hosp, Incorporating the NCH Tallaght, Ireland.

Background

The contribution of chylomicrons to postprandial hypertriglyceridaemia has not been fully elucidated. This is partly due to lack of availability of a standardised method for quantification of apolipoprotein (apo) B48, which is specific for intestinally-derived lipoproteins. In this study we used a novel ELISA method for apo B48 quantification in fasting and postprandial samples. The impact of type 2 diabetes, improved glycaemic control, statin medication and gender on intestinally derived lipoproteins was assessed.

Research Design and Methods

Blood samples were drawn before and following a physiological meal. Apo B48 and triglyceride concentrations were assessed at each time point. Forty nine patients with type 2 diabetes and sixty control patients were enrolled in the study. Forty one diabetic patients were studied following intensification of diabetes control through lifestyle measures and oral antihyperglycaemic drugs.

Results

Compared to normal subjects, apo B48 levels at the 6-hour time-point were greater in the diabetic patients and did not differ between those treated and not treated with statins. Female controls had a significantly lower apo B48 area under the curve compared to male counterparts following adjustment for HOMA-IR. Compared to normal subjects ($13.04 \pm 7.67 \mu\text{g/ml}$) apo B48 concentrations at the 6-hour time-point was significantly higher in patients with diabetes ($17.73 \pm 13.46 \mu\text{g/ml}$) ($p=0.037$). This did not differ between those treated and not treated with statins. Apo B48 levels did not change following intensification of glycaemic control. At completion of study protocol with improved glycaemic control twenty four patients were taking statin medication. These patients had a significantly improved apo B48 at 6-hour time-point ($p=0.008$) compared to patients not taking statin medication.

Conclusion

The human apo B-48 ELISA kit allows specific determination of intestinally-derived apolipoprotein B in plasma. This method will enable more detailed analysis of postprandial dyslipidaemia in normal and diabetic subjects.

Nothing to Disclose: AL, TKT, PG, JS, JG, GB

OR06-3

Consuming Fructose-, or HFCS-, but Not Glucose-Sweetened Beverages for 2 Weeks Increases Nighttime Triglyceride and Fasting LDL and Apolipoprotein-B (apoB) Concentrations in Young Men and Women.

KL Stanhope Ph.D, R.D.¹, AA Bremer M.D., Ph.D.¹, NL Keim Ph.D.², GX Chen B.S.¹, T Fong B.S.¹, VI Lee B.S.¹, RI Menorca B.S.¹, K Nakajima Ph.D.³ and PJ Havel Ph.D, D.V.M.¹.

¹Univ of California Davis Davis, CA ; ²United States Dept of Agriculture, Western Human Nutrition Res Ctr Davis, CA and ³ Tufts Univ Sch of Med Boston, MA.

We have reported that 10 weeks of fructose consumption at 25% of energy requirements increases plasma concentrations of postprandial TG, and fasting apoB and LDL in older, overweight/obese adults, while glucose consumption does not (1). To determine the relative effects of glucose and fructose consumption in younger, healthier adults, as well as the effects of high fructose corn syrup (HFCS) consumption (55% fructose, 45% glucose), a commonly used dietary sweetener, we measured lipid parameters in young (27 ± 7 years) adults before and after consumption of glucose-, fructose- or HFCS-sweetened beverages at 25% of energy requirements for 2 weeks ($n = 14/\text{group}$). Baseline procedures were conducted while subjects resided at the Clinical Research Center (CCRC) and consumed an energy-balanced diet (55% complex carbohydrate, 30% fat, 15% protein). Subsequently, they consumed their usual diets *ad libitum* along with the assigned sweetened beverage for 2 weeks. Subjects returned to the CCRC and intervention procedures were conducted while they consumed the sweetened beverages as part of an energy-balanced diet (25% sugar-sweetened beverage, 30% complex carbohydrate, 30% fat, 15% protein). Similar to what we reported for older, overweight/obese adults (1), consumption of fructose increased the 24-h TG AUC ($\Delta = +531 \pm 154 \text{ mg/dl} \times 24\text{h}$, $P < 0.001$) compared with consumption of complex carbohydrate at baseline, and compared with consumption of glucose ($-216 \pm 109 \text{ mg/dl} \times 24\text{h}$) in younger, leaner ($\text{BMI} = 25.5 \pm 3.9 \text{ kg/m}^2$) subjects. Consumption of HFCS-sweetened beverages also tended to increase 24-h TG AUC ($+200 \pm 164 \text{ mg/dl} \times 24\text{h}$). Consumption of both fructose ($+50 \pm 11 \text{ mg/dl}$, $P < 0.0001$) and HFCS ($+41 \pm 8 \text{ mg/dl}$, $P < 0.001$) resulted in the formation of an additional peak of plasma TG concentrations at 22:00-24:00h that was not apparent during baseline and that was significantly higher than in subjects consuming glucose ($+18 \pm 10 \text{ mg/dl}$). Fasting LDL concentrations were also significantly increased in subjects consuming fructose ($+11.6 \pm 3.5 \text{ mg/dl}$, $P < 0.01$) and HFCS ($+22.7 \pm 7.0 \text{ mg/dl}$, $P < 0.001$), but were unchanged in subjects consuming glucose ($-0.7 \pm 3.0 \text{ mg/dl}$), as were fasting apoB concentrations (fructose: $+13.8 \pm 4.0 \text{ mg/dl}$, $P < 0.05$; HFCS: $+18.6 \pm 5.6 \text{ mg/dl}$, $P < 0.0001$; glucose: $+1.9 \pm 2.5 \text{ mg/dl}$). These results indicate that consuming beverages containing HFCS (a mixture of glucose and fructose) increases LDL and apoB equivalently to those containing 100% fructose in young adults.

(1) Stanhope KL et al., JCI 2009; 119:1322-1334.

Sources of Research Support: National Institutes of Health grants 5R01HL091333 and UL1RR024146.

Nothing to Disclose: KLS, AAB, NLK, GXC, TF, VIL, RIM, KN, PJH

OR06-4

Fructose Inhibits Hepatic Triglyceride Hydrolysis.

Haibo Liu M.D.¹, Danshan Huang Ph.D.¹, Leah Phan B.S.¹, Vamsi Chavakula M.D.² and Anthony Heaney Ph.D., M.D.¹.

¹David Geffen Sch of Med at UCLA Los Angeles, CA and ²Harvard Med Sch Boston, MA.

Obesity, diabetes and hyperlipidemia are coexisting conditions frequently associated with nonalcoholic fatty liver disease. Increased dietary refined carbohydrate, particularly fructose positively correlates with higher risk of metabolic syndrome in humans. Increased hepatic flux of fructose leads to hepatic triglyceride accumulation but the molecular mechanisms are not fully understood. Mammalian cell triglyceride lipolysis depends on levels of the enzyme adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). To investigate the mechanism of fructose induced hepatic triglyceridemia, twelve Nu/Nu mice were fed either a high-fructose (60%, HF) or high-glucose (60%, HG) or standard chow diet for 12 weeks. Intraperitoneal glucose challenge was performed at baseline and at monthly intervals, and plasma insulin and hepatic triglyceride content and ATGL expression were measured. We previously reported that these HF and HG fed mice exhibited impaired glucose-stimulated insulin secretion and glucose disposal after an intraperitoneal glucose challenge. Histological examination of the livers demonstrated severe hepatic steatosis in fructose-fed mice. Additionally, hepatic triglyceride content was higher and ATGL expression was significantly lower in fructose-fed mice compared to glucose- or standard diet-fed mice $p < 0.02$. *In vitro*, in both human hepatoblastoma HepG2 and primary cultures of murine hepatocytes, ATGL expression and insulin-mediated phosphorylation of HSL decreased following fructose-treatment alone or when low concentration (550 μ M) fructose was added to cells treated with diabetic range high glucose (11.1 μ M) concentrations.

These results provide important insight into the mechanisms of fructose-induced hepatic hypertriglyceridemia and indicate that fructose suppresses hepatic ATGL expression and HSL activity resulting in reduced hepatic triglyceride hydrolysis and hepatic triglyceridemia. They have particular relevance to diabetic patients as our data demonstrates that low concentrations of fructose interferes with hepatic triglyceride handling in the setting of hyperglycemia.

Nothing to Disclose: HL, DH, LP, VC, AH

OR06-5

Induction of Apolipoprotein A-I Gene Expression by Glucagon-Like Peptide-1 and Exendin-4.

JOE M Chehade MD¹, Rosalyn Alcade MD¹, Emad Naem MD¹, Senan Sultan MD¹, Abdul-Razzak Alamir MD¹, Michael J. Haas PhD¹, Norman C. Wong MD² and Arshag D. Mooradian MD².

¹Univ of Florida – Jacksonville Coll of Med Jacksonville, FL and ²Univ of Calgary Calgary, Canada.

Diabetic dyslipidemia is an important risk factor for the development of macrovascular complications. Recent clinical trials suggest that diabetics treated with glucagon-like peptide-1 (GLP-1) have improved plasma lipid profile, including an increase in plasma high-density lipoprotein cholesterol (HDLc) levels. To determine if GLP-1 (7-36 amide) and the GLP-1-like insulinotropic peptide exendin-4 regulate expression of apolipoprotein A-I (apo A-I), the primary anti-atherogenic component of HDLc, HepG2 hepatocytes and Caco-2 intestinal cells, representative of tissues that express the majority of plasma HDLc, were treated with increasing amounts of each peptide and apo A-I protein secretion was measured in the conditioned medium. Apo A-I secretion increased from 595 ± 93 (mean \pm SD) arbitrary units (A.U.) to 707 ± 13 , 756 ± 52 , 939 ± 79 , and 1425 ± 43 A.U. in cells treated with 0, 0.1, 1.0, 10, 100 and 1000 nM GLP-1, respectively, and from 673 ± 43 A.U. to 674 ± 57 , 953 ± 52 , 950 ± 157 , 1171 ± 23 , and 1183 ± 107 A.U. in cells treated with 0, 0.1, 1.0, 10, 100 and 1000 nM exendin-4, respectively. These increases in protein accumulation were accompanied by similar increases in apo A-I mRNA levels and apo A-I promoter activity. In contrast, treatment of Caco-2 cells with either GLP-1 or exendin-4 had no effect on apo A-I secretion. It is concluded that GLP-1- and exendin-4-mediated increase in HDLc is at least partly due to changes in hepatic expression of apo A-I.

Sources of Research Support: Amylin Pharmaceuticals Inc.

Nothing to Disclose: JMC, RA, EN, SS, A-RA, MJH, NCW, ADM

OR06-6 Treatment with Omega-3 Fatty Acids but Not Exendin-4 Improves Hepatic Steatosis.

D G Bulchandani MD¹, J S Nachnani MD¹, B Herndon PhD¹, A Molteni MD, PhD¹ and L M Alba MD¹.

¹Univ of Kansas-Sch of Med Kansas City, KS.

Purpose / Background

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease, affecting both children and adults in United States. There is no proven effective therapy for fatty liver. The aim of this study was to evaluate the biochemical and histological effects of omega-3 fatty acids and exendin-4 on the liver in an animal model of nonalcoholic fatty liver disease.

Methods

Rodents fed a methionine and choline deficient (MCD) diet have been extensively studied as a model of steatosis and steatohepatitis. Sixty three ~8-week-old outbred Sprague-Dawley male rats were used for this study. Of the 63 rats, ten animal served as MCD baseline, ten were treated with exendin-4 and ten were fed omega-3 fatty acids (10% fish oil as the fat source of the MCD diet); each group had a paired group receiving control diet. Three animals were used as procedure control. At 75 day necropsy, tissues and serum were harvested and livers were formalin fixed for histology.

Results

The diet was exceptionally efficient at producing fatty livers in the MCD controls, which had a liver steatosis score of 38 ± 6.7 (of 50 possible). Treatment with exendin-4 showed no improvement, with a steatosis score of 44 ± 5.16 , $p=0.07$ not significant between groups. The animals receiving MCD with 10% fish oil (high omega 3 fatty acids) substituted for the 10% corn oil in the standard MCD diet had a liver steatosis score of 15.6 ± 13.46 , $p<0.001$ when compared to either controls or exendin-4 groups. Adiponectin levels were decreased significantly by exendin-4 but not fish oil. Fish oil significantly increased serum AST, and exendin-4 had no effect. Neither changed serum insulin, leptin, ALT, or GLP-1. Conclusion: In a model of steatohepatitis induced by absence of methionine and choline, a significant improvement in liver pathology with increased AST was produced by substituting oil rich in omega 3 fatty acids for corn oil in the diet. Exendin-4, the GLP-1 agonist, produced a significant drop in adiponectin but no pathology improvement. These data raise the possibility that omega 3 fatty acids may have the potential to be a treatment option in patients with fatty liver disease and steatohepatitis.

Sources of Research Support: Saint Lukes Foundation Grant; awarded to DB,JN,LA.

Nothing to Disclose: DGB, JSN, BH, AM, LMA

OR07-1

Early Menopause Is Associated with Cardiovascular Disease Events: The Multi-Ethnic Study of Atherosclerosis (MESA).

MF Wellons MD¹, D Vaidya MD², D Herrington MD³, PJ Schreiner MD⁴ and P Ouyang MD².

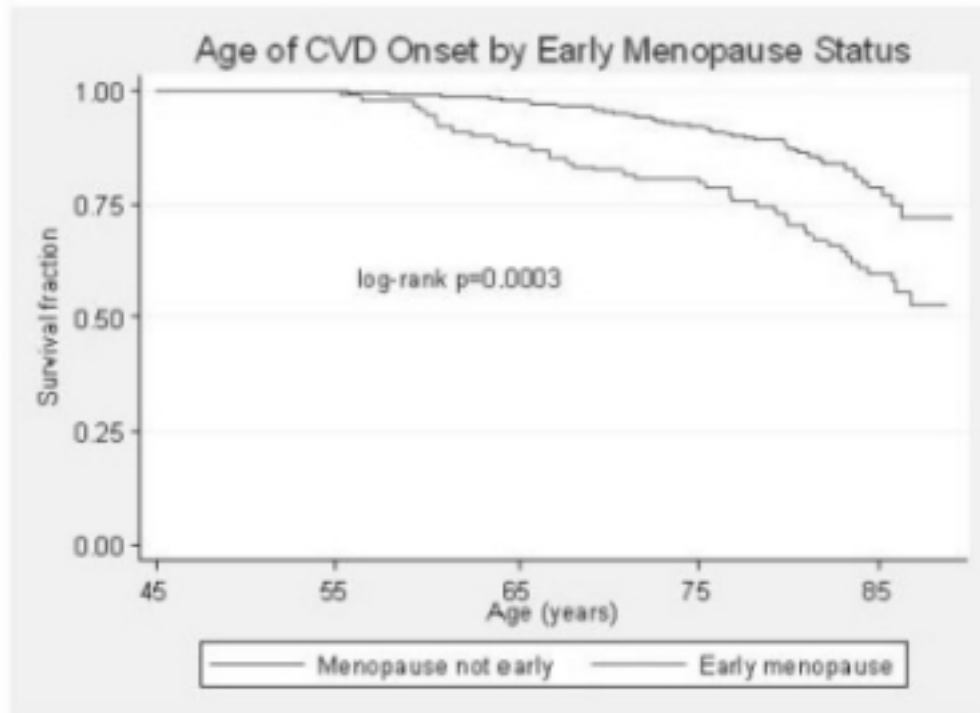
¹Univ of Alabama Birmingham, AL ; ²Johns Hopkins Med Inst Baltimore, MD ; ³Wake Forest Univ Winston-Salem, NC and ⁴Univ of Minnesota Minneapolis, MN.

Early menopause is associated with increased cardiovascular disease (CVD) mortality in some white populations, but not consistently. We hypothesized that within a large multi-ethnic population of women, early menopause (<age 46) would be associated with CVD events.

Methods: We included 2509 women (ages 45-84 at baseline, 987 White, 331 Chinese, 641 Black, 550 Hispanic) from MESA. We excluded women reporting a hysterectomy without oophorectomy prior to menopause, as pre vs. postmenopausal status could not be determined. Age at menopause was defined by self-reported age at menopause ("natural menopause") or age at bilateral oophorectomy ("surgical menopause"). The primary exposure was "early menopause" (natural or surgical menopause <age 46). Women not reporting menopause at baseline were considered unexposed to early menopause. CVD events included MI, resuscitated cardiac arrest, definite angina, probable angina (if followed by revascularization), stroke, stroke death, coronary heart disease death, or other atherosclerotic/CVD death. We used Kaplan-Meier statistics to estimate the proportion of women experiencing CVD events with age as the underlying timescale, allowing for delayed entry at enrollment (curve plotted from 55 years, below which there are no events). Adjusted survival age analyses included race/ethnicity, education, smoking (current, past, never), hypertension, total cholesterol, HDL-C, diabetes, and whether the menopause was natural or surgical. Additional analyses included ever-HRT use and BMI.

Results: 693/2509 (28%) women reported early menopause: early surgical menopause 247/2509 (10%) and early natural menopause 446/2509 (18%). Kaplan-Meier curves show that women with early menopause had an increased risk for CVD events (logrank p=0.0003). After adjustment for risk factors using Cox models, this association remains statistically significant (HR 2.11, 95% CI 1.33, 3.33). Further adjustment for HRT and BMI produce virtually identical results (2.12, 95% CI 1.35, 3.33).

Conclusion: Early age at menopause (natural or surgical) is positively associated with CVD events, independent of cardiovascular risk factors and HRT use.



Nothing to Disclose: MFW, DV, DH, PJS, PO

OR07-2

Tetrahydrobiopterin Decreases Large Artery Stiffening in Estrogen-Deficient Postmenopausal Women.

Lela S Mansoori M.D.¹, Amie L Meditz M.D.¹, Chelsea M Bergman B.S.¹, Wendy M Kohrt Ph.D¹ and Kerrie L Moreau Ph.D¹.

¹Univ of Colorado Denver Hlth Scis Ctr Aurora, CO.

Background: Large artery stiffness increases with advancing age and/or menopause and is associated with increased risk of cardiovascular disease (CVD). Oxidative stress contributes to increased arterial stiffness in estrogen-deficient postmenopausal women. The mechanism by which oxidative stress modulates arterial stiffness is unknown but may be related to the suppression of tetrahydrobiopterin (BH4), an essential co-factor for nitric oxide synthase (eNOS) to produce nitric oxide, resulting in an “uncoupling” of eNOS and impaired endothelial vasodilatory function. We hypothesized that BH4 administration will decrease large artery stiffness in estrogen-deficient postmenopausal women, but will have no effect in premenopausal women. **Methods:** Carotid artery compliance (ultrasound) was measured in eumenorrheic premenopausal (n=8; 34±6 y; mean±SD) and postmenopausal (n=20; 57±4) women, before and 3 hours after the oral administration of BH4 (10 mg/kg body weight). **Results:** Baseline arterial compliance was 54% lower in postmenopausal than in premenopausal women (0.62±0.20 vs. 1.36±0.39 mm²/mm Hg×10⁻¹, p<0.0001). BH4 administration increased carotid artery compliance in postmenopausal women by 15% (0.71±0.17 mm²/mm Hg×10⁻¹; P<0.001), but had no effect in premenopausal women (1.30±0.27 mm²/mm Hg×10⁻¹; P=0.62), possibly because of adequate BH4 bioavailability. **Conclusion:** These results suggest that reduced BH4 bioavailability contributes to the increased arterial stiffness in estrogen-deficient postmenopausal women and may be one of the mechanisms mediating the increase in large artery stiffness with oxidative stress.

Sources of Research Support: NIH Grant RO1 AG027678; Center for Women's Health Research University of Colorado Denver Junior Faculty Research Development Award; Women's Health Research University of Colorado Denver Pilot Project Grant.

Nothing to Disclose: LSM, ALM, CMB, WMK, KLM

OR07-3

Endometrial Safety and Clinical Pharmacokinetics of an Ultra-Low Dose Estradiol Vaginal Tablet for Treatment of Menopausal Women with Vaginal Atrophy.

James Simon MD¹, Robert Gut MD, PhD², Jeffrey Goldstein DO², John Germak MD² and Lila Nachtigall MD³.

¹George Washington Univ Sch of Med Washington, DC ; ²Novo Nordisk, Inc Princeton, NJ and ³New York Univ Sch of Med New York City, NY.

Objective: To evaluate the endometrial safety and pharmacokinetic (PK) profile of a 10 mcg estradiol (E2) vaginal tablet for the treatment of menopausal women with vaginal atrophy. The results from 3 clinical trials (2 safety, 1 PK) were analyzed.

Methods: Results from subjects in an open-label trial (n=336) were pooled with those from the treatment arm of a double-blinded, placebo controlled trial (n=205). All subjects received 10 mcg E2 vaginal tablets, daily for 2 weeks followed by twice weekly for a total of 12 months. Endometrial biopsies (EBx) were performed at baseline and end-of-trial (EOT) from which an endometrial hyperplasia rate was determined and compared to the reported background (untreated) incidence rate of 0-1%. In a separate 12-week PK study, 29 women (age 60-70) with vaginal atrophy were treated with 10 mcg E2 vaginal tablets (daily for first 2 weeks followed by twice weekly for 10 weeks). Blood samples were obtained at 12 time points over 24 hours at baseline (Day-1) and Days 1, 14, 82 and 83. Plasma E2 concentrations were measured by gas chromatography mass spectrometry. E2 levels were assessed as mean area under the curve (AUC₍₀₋₂₄₎) and mean plasma concentrations (C_{ave}=AUC₍₀₋₂₄₎/24) for each 24 hour period.

Results: 469 subjects had EOT EBx with the following results: 86% atrophic or inactive, 13% non-evaluable, 1% polyps and 0.2% were "weakly proliferative". One case each of complex hyperplasia without atypia (subject exposed to trial drug for only 9 days) and endometrial adenocarcinoma, Grade 2 Stage 1B (subject without an evaluable baseline Bx) were reported. In total, 2 events of hyperplasia/carcinoma were reported in 386 evaluable biopsies (incidence rate 0.52%).

Summary of Serum Estradiol Levels

| Day | AUC ₍₀₋₂₄₎ (pg.h/mL) | C _{ave} (pg/mL) | | Median |
|-----|---------------------------------|--------------------------|---------|--------|
| | Mean | Geom Mean | Minimum | |
| -1 | 75.6 | 3.15 | 0 | 3.71 |
| 1 | 225 | 9.39 | 1.86 | 10.28 |
| 14 | 157 | 6.56 | 1.36 | 7.30 |
| 82 | 44.9 | 1.87 | 0 | 2.48 |
| 83 | 111 | 4.64 | 0 | 4.31 |

Conclusion: The endometrial hyperplasia/carcinoma incidence rate of 0.52%, compared to reported background (untreated) incidence rate of 0-1%, showed no increased risk in postmenopausal women undergoing treatment with 10mcg E2 vaginal tablets for 12 months. Although E2 levels increased following administration of vaginal tablets in the first two weeks of treatment, average plasma concentrations always remained within the normal postmenopausal range.

Disclosures: JS: Speaker, Novo Nordisk. RG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. LN: Speaker, Novo Nordisk.

OR07-4

Heritability of Circulating Testosterone and SHBG in Adult Women from the Framingham Heart Study.

AD Coviello MD¹, WV Zhuang PhD², KL Lunetta PhD², J Ulloor PhD¹, A Zhang PhD¹, D Karasik PhD³, DP Kiel MD, MPH³, S Bhasin MD¹, RS Vasan MD^{1,4} and JM Murabito MD^{1,4}.

¹Boston Univ Sch of Med Boston, MA ; ²Boston Univ Sch of Public Hlth Boston, MA ; ³Inst of Aging Res, Hebrew SeniorLife, Harvard Med Sch Boston, MA and ⁴Framingham Heart Study Framingham, MA.

Background: Circulating testosterone and sex-hormone binding globulin (SHBG) levels are influenced by many factors. However, little is known about the genetic contribution to their concentrations in women: the heritability of circulating testosterone and SHBG has not been established in women.

Objective: To estimate the heritability of circulating total and free testosterone and SHBG in women in families of the Framingham Heart Study (FHS).

Methods: Women in the FHS Offspring and Generation 3 not using exogenous hormones including estrogens, progestins, and androgens were eligible for this investigation. Women who were pregnant or who had undergone bilateral oophorectomy were excluded. Testosterone levels were measured using liquid chromatography tandem mass spectrometry (LC-MSMS), a state of the art method for measuring total testosterone in the low circulating levels occurring in women. SHBG was measured by an immunofluorometric assay (Delphia-Wallac, Inc.). Free testosterone was calculated by the laws of mass action equations. Serum hormones were transformed by rank-normalization to minimize skewness. Covariates considered in the analysis included age, age², BMI, BMI², diabetes, smoking, and menopausal status. Heritability estimates (SOLAR software) were adjusted for significant covariates.

Characteristics of Women from the Offspring and Generation 3 Cohorts in the Framingham Heart Study

| | OVERALL | OFFSPRING GENERATION 2 | GENERATION 3 |
|---------------------------|-------------|------------------------|--------------|
| Sample, n | 2677 | 1071 | 1614 |
| Age, years | 49 ± 14 | 62 ± 10 | 41 ± 8 |
| BMI, kg/m ² | 27 ± 6 | 28 ± 6 | 26 ± 6 |
| Post-menopause, % | 38% | 80% | 10% |
| Smoker, % | 14% | 12% | 16% |
| Diabetes, % | 5% | 10% | 2% |
| Total Testosterone, ng/dl | 29.6 ± 18.3 | 32.1 ± 21.8 | 27.9 ± 15.3 |
| Free Testosterone, pg/ml | 3.2 ± 2.2 | 3.6 ± 2.4 | 2.9 ± 1.9 |
| SHBG, nmol/L | 82.0 ± 44.1 | 74.2 ± 38.8 | 87.2 ± 46.6 |

Results: 2677 women were included representing 868 sister pairs and 688 mother-daughter pairs (Table 1). Heritability estimates for free testosterone were 0.258 (SD 0.052), total testosterone 0.261 (SD 0.053), and SHBG 0.560 (SD 0.051) (all $p < 1.0 \times 10^{-7}$).

Conclusion: Circulating testosterone and SHBG levels show strong heritability patterns after adjusting for age, BMI, diabetes, smoking, and menopausal status. These significant heritability estimates support a strong genetic component to circulating sex steroid levels and underscore the importance of further work to determine the specific genes that contribute to variation in sex steroid profiles in women.

Nothing to Disclose: ADC, WVZ, KLL, JU, AZ, DK, DPK, SB, RSV, JMM

OR07-5

Aromatase Inhibition Causes Increased Amplitude but Not Frequency of Hypothalamic-Pituitary Output in Normal Women.

A Kucherov BA¹, AJ Polotsky MD, MS¹, M Menke MD¹, B Isaac RN¹, B McAvey MD¹, E Buyuk MD¹, A Bradford PhD², C Hickmon BS¹, B Babbs BS² and N Santoro MD².

¹Albert Einstein Coll of Med Bronx, NY and ²Univ of Colorado, Denver Sch of Med Aurora, CO.

Although aromatase inhibitors are increasingly being used for ovulation induction, their impact upon the hypothalamic-pituitary axis and luteinizing hormone (LH) pulsatility is not described. In order to better understand this relationship, we administered 2.5mg letrozole daily for 7 days to 4 eumenorrheic (non-PCOS), early follicular phase women of normal BMI (mean = 20.47 ± 0.68 kg/m²). The women were sampled with daily, first morning voided urine collections, thrice weekly blood sampling, and an 8-hour, q10 minute blood sampling session, with a bolus of GnRH given at 4 hours. The first 4-hours of the q10 minute blood sampling sessions were compared to the patterns previously observed in 12 normal weight, mid-reproductive aged, early follicular phase, normal BMI (mean = 20.8 ± 1.7 kg/m²) controls¹ using the same assay system. LH was measured using a well-characterized immunofluorometric assay (LH Spec; DELFIA; Pharmacia; Gaithersburg, MD). Parameters of LH pulsatility were determined using two objective methods.^{2,3} Group means were compared using t tests.

Results: Mean LH and LH pulse amplitude more than doubled in women who had taken letrozole compared to controls, but the LH pulse frequency did not differ between women taking letrozole and controls.

Table 1

| | Letrozole | SD | Control | SD | P Value |
|----------------------------|------------|-----|---------|-----|-----------------|
| | N = 4 | | N = 12 | | |
| Age, years | 32.3 | 5.7 | 24.9 | 4.8 | 0.07 |
| BMI, kg/m ² | 20.6 | 0.6 | 20.8 | 1.7 | 0.82 |
| LH Pulse Frequency/4 hours | 1.5 | 1.0 | 2.4 | 1.7 | 0.32 |
| LH Pulse Amplitude | 4.5 | 2.7 | 1.6 | 0.7 | <0.01 |
| LH, Mean IU/Liter | 8.7 | 1.4 | 3.4 | 0.7 | <0.01 |

Conclusions: Our results indicate that the release of negative feedback inhibition of estradiol on the hypothalamic-pituitary axis in normal women by aromatase inhibitors creates an amplitude-related increase in endogenous hypothalamic-pituitary drive. The finding that mean LH and LH pulse amplitude, but not frequency, increased after letrozole suggests a possible pituitary site of action.

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(2) Wilshire GB, Loughlin JS, Brown JR, Adel TE, Santoro N. Diminished function of the somatotrophic axis in older reproductive-aged women. *J Clin Endocrinol Metab.* 1995;80:608-13.

(3) Kushler RH, Brown MB. A model for the identification of hormone pulses. *Stat Med.* 1991;10:329-40.

Sources of Research Support: NIH U54 HD058155 Center for the Study of Reproductive Biology; K24 HD041978 to NS.

Nothing to Disclose: AK, AJP, MM, BI, BM, EB, AB, CH, BB, NS

OR07-6

Variability of the Reproductive Phenotype in Women with Congenital GnRH Deficiency.

ND Shaw MD^{1,2}, CK Welt MD¹, SB Seminara MD¹, KA Martin MD¹, MG Au MBE, MS¹, LC Plummer BS¹, VA Hughes¹, N Pitteloud MD¹, WF Crowley, Jr MD¹ and JE Hall MD¹.

¹Massachusetts Gen Hosp Boston, MA and ²Children's Hosp Boston Boston, MA.

Context: Traditional teaching holds that one should suspect the diagnosis of GnRH deficiency or isolated hypogonadotropic hypogonadism (IHH), in an adolescent girl with absent thelarche, primary amenorrhea, and low gonadotropins. Recent studies, however, point to a broader phenotypic spectrum in female IHH with some women demonstrating incomplete puberty. We sought to determine whether there was a relationship between the reproductive phenotype and activity of the GnRH pulse generator in a large cohort of women with IHH.

Methods: 71 women seen at Massachusetts General Hospital for presumed GnRH deficiency were studied. Women were >17 years old with absent or incomplete puberty, no other pituitary hormone deficiencies, and no history of excessive exercise or disordered eating. Patients underwent brain imaging to exclude hypothalamic or pituitary lesions, formal smell testing (n=48), genetic testing (n=62), and a 12-hour frequent sampling study, measuring serum LH as a marker of spontaneous GnRH secretion.

Results: At the time of evaluation, women were 28 ± 9 (mean \pm SD) years old with a BMI of 24.3 ± 0.8 kg/m² and were predominantly Caucasian. 46% were anosmic or hyposmic and 32% harbored a mutation in one of the genes known to be associated with IHH. 60% had a history of spontaneous thelarche, but only 8% had one or more menses (range 1-6). On frequent sampling, 72% had no LH pulses, 17% had low amplitude and/or low frequency pulses, and 11% had normal pulsatility. Of the 8 patients with normal pulsatility, 3 were anosmic, 1 had a cleft lip/palate, and 2 had mutations in genes known to be associated with IHH (*TACR3* and *PROKR2*). The presence of pulsatile GnRH secretion was associated with menarche ($p < 0.05$), but not with thelarche or olfactory phenotype.

Conclusion: GnRH deficient women present with a range of clinical phenotypes prior to treatment. Of note, over half of the patients had evidence of breast development at the time of diagnosis and some had a history of vaginal bleeding, findings that were independent of olfactory status. Menarche, even when limited to one period, was associated with evidence of GnRH activity, while thelarche was not. These results caution physicians against dismissing the diagnosis of GnRH deficiency in a woman with early breast development or menarche.

Sources of Research Support: U54HD028138, R01HD42708, M01RR1066 and T32HD007396.

Nothing to Disclose: NDS, CKW, SBS, KAM, MGA, LCP, VAH, NP, WFC, JEH

OR08-1

Changing Trends in Osteoporosis-Associated Mortality in the United States: 1990 to 2006.

A Melamed¹, F Sorvillo², JS LoPresti¹, D Foote¹ and N Jacob¹.

¹Keck Sch of Med of the Univ of Southern California Los Angeles, CA and ²Sch of Public Hlth, UCLA Los Angeles, CA.

Introduction: Osteoporotic fracture is associated with an increased risk of death (1). In the United States, incidence of hip fracture among those 65 years and older has declined since 1995 (2). To evaluate whether declining fracture incidence has led to a reduction in mortality, we utilized the National Center for Health Statistics' large, publicly available, and population-based multiple-cause-of-death (MCO) dataset to analyze secular trends in osteoporosis-associated mortality from 1990 to 2006.

Methods: Osteoporosis-associated deaths occurring in the United States from 1990 through 2006 were identified from MCO data using ICD-9 (733.0) and ICD-10 (M80.-M82.) codes. Population-based mortality rates were calculated using bridged-race populations estimates. Mortality rates were standardized to the US census 2000 population. Secular trends were assessed with Poisson regression.

Results: Between 1990 and 2006 there were 39,755,734 deaths in the United States, of which 184,562 were osteoporosis-associated (0.5% of all deaths). Those whose death certificates listed osteoporosis were predominately female (88.5%) and white (95.2%), and the median age of death was 86 years. Annual age-adjusted osteoporosis-associated mortality rates (with 95% confidence intervals) for this period are shown in the figure below. Incidence of osteoporosis-related deaths rose by 6.2 % (95% CI 6.0 - 6.4) per year from 1990 to 2000 (p for trend < 0.001); remained essentially flat between 2000 and 2002 (p for trend = 0.11); and declined by 4.2% (95% CI 3.7 - 4.7) annually from 2002 to 2006 (p for trend < 0.001). The time trends evident in the overall US population persisted in both men and women.

Conclusions: After many years of apparent growth, the rate of osteoporosis-associated mortality in the US leveled off in 2000 and began to decline in 2002. Since case fatality for fractures did not decline during this period (2), it is likely that the downward trend in osteoporosis-associated mortality is related to declining fracture incidence. These data suggest that continued screening and treatment of osteoporosis may lead to further reductions in preventable mortality.



(1) Johnell O et al., Osteoporos Int 2004; 15:38-42

(2) Brauer CA et al., JAMA 2009; 302:1573-1579

Nothing to Disclose: AM, FS, JSL, DF, NJ

OR08-2

Application of a Fracture Risk Tool Using Data from a Large Integrated Healthcare Delivery System.

JC Lo MD¹, AR Pressman PhD¹, M Chandra MS¹ and B Ettinger MD¹.

¹Kaiser Permanente Northern California Oakland, CA.

BACKGROUND: Fracture risk assessment can be useful in identifying high risk subgroups who may benefit from aggressive osteoporosis management. We evaluated the potential utility of the Fracture Risk Calculator (FRC) for predicting 10-year hip fracture risk within a "real world" clinical population.

METHODS: Clinical data were extracted from Kaiser Permanente Northern California electronic databases for women aged 50-85 years whose hip BMD was measured during 1997-2003. Variables included hip BMD z score, race/ethnicity, age, BMI, smoking, prior fracture, rheumatoid arthritis, glucocorticoid use, secondary osteoporosis factors. Missing BMI was set to 25 kg/m² and other missing data were set to null. We excluded women who used 3 or more bisphosphonate prescriptions during follow-up (22%). Predicted 10-year hip fracture risks were calculated using the web-based FRC in automated batch mode (Foundation for Osteoporosis Research and Education). Observed hip fracture probabilities (product-limit survival estimate) were ascertained up to 10 years after the BMD test date, and these data were compared with those predicted by FRC.

RESULTS: Among 76,188 women with hip BMD (mean age 62.3 ± 8.6 yr), mean total hip BMD z-score was +0.4. The proportion of women with FRC-predicted 10-year hip fracture risk ≥ 3% increased markedly with age: 2.4% (age 50-59 yr) to 96.4% (age 80-85 yr). During mean follow-up of 7 years, 1205 women suffered a hip fracture. In all six risk categories (see Table) the FRC tool performed well with some underestimation in the higher risk groups. Ratios of observed to expected probabilities ranged from 1.1- 1.7. Missing data, a frequent problem when using administrative data sources, could contribute to an underestimation of fracture risk.

CONCLUSION: A relatively simple tool can be applied to estimate fracture risk burden within large aggregate populations and may prove useful in identifying high risk subgroups for further evaluation. The discriminant power of this tool in individual cases remains to be examined.

| FRC 10yr Hip Fx Probability (%) | Number of women (N) | Observed Hip FX (N) | Mean FRC 10y Probability (mean%) | Observed 10y Hip Fx Probability (%) |
|---------------------------------|---------------------|---------------------|----------------------------------|-------------------------------------|
| < 1 | 45,884 | 110 | 0.4 | 0.4 |
| 1 - 2.9 | 17,767 | 236 | 1.7 | 2.2 |
| 3- 4.9 | 5,106 | 164 | 3.8 | 5.2 |
| 5 - 6.9 | 2,503 | 129 | 5.9 | 8.1 |
| 7 - 9.9 | 1,956 | 170 | 8.3 | 14.2 |
| 10 + | 2,972 | 396 | 17.8 | 22.3 |

Disclosures: BE: Advisory Group Member, Lilly USA, LLC, Teva.

Nothing to Disclose: JCL, ARP, MC

OR08-3

Use of SSRIs May Impact Bone Density in Adolescents and Young Women with Anorexia Nervosa.

M. Misra MD, MPH^{1,2}, M. Le Clair¹, N. Mendes BA¹, K.K. Miller MD¹, E.A. Lawson MD¹, E. Meenaghan NP³, T. Weigel MD³, S. Ebrahimi PhD⁴, D. Herzog MD¹ and A. Klibanski MA¹.

¹Massachusetts Gen Hosp and Harvard Med Sch Boston, MA ; ²MassGen Hosp for Children and Harvard Med Sch Boston, MA ; ³McLean Hosp Belmont, MA and ⁴Cambridge Eating Disorders Ctr Cambridge, MA.

Objectives: Alterations in serotonin levels impact bone metabolism in animal models, however, the impact of pharmacological manipulation of serotonin through use of selective serotonin reuptake inhibitors (SSRIs) has not been examined in adolescents. SSRIs are commonly used in adolescents and young women with anorexia nervosa (AN), a condition that predisposes to low bone mineral density (BMD). Our objective was to determine whether SSRI use is associated with low BMD in AN.

Methods: We examined Z-scores for spine, hip, femoral neck and whole body (WB) BMD, spine bone mineral apparent density and whole body BMC/height (Ht) in adolescents and young women with AN 12-21 years old who had never been on SSRIs (SSRI=0), on SSRIs for <6 months (SSRI<6M), and on SSRIs for >6 months (SSRI>6M).

Results: Subjects who had been on an SSRI>6M had lower spine, femoral neck and WB BMD Z-scores than subjects on an SSRI<6M. Hip BMD and WB BMC/Ht Z- scores were lower in subjects on an SSRI>6M compared with the other two groups (Table). Duration of SSRI use, duration since AN diagnosis and duration of amenorrhea were negative predictors of BMD Z-scores, whereas BMI was a positive predictor. In a regression model including these covariates, duration of SSRI use remained an independent and negative predictor of BMD Z-scores.

Bone density Z-scores in adolescents and young women with anorexia nervosa who had never used SSRIs, used SSRIs for less than 6 months or for greater than 6 months

| Z-scores | Never on SSRIs (n=86) | SSRI use < 6 M (n=37) | SSRI use > 6 M (n=20) | p |
|-------------------|-----------------------|-----------------------|-----------------------|--------|
| Spine BMD | -1.16 ± 1.05 | -0.83 ± 0.82* | -1.70 ± 0.77 | 0.006 |
| Spine BMAD | -1.38 ± 0.89 | -1.19 ± 0.85 | -1.62 ± 0.67 | Ns |
| Hip BMD | -0.67 ± 0.94* | -0.45 ± 0.73* | -1.31 ± 0.91 | 0.003 |
| Femoral neck BMD | -0.81 ± 1.03 | -0.59 ± 0.79* | -1.35 ± 0.83 | 0.02 |
| Whole body BMD | -0.49 ± 1.03 | -0.17 ± 0.90* | -0.85 ± 1.07 | 0.05 |
| Whole body BMC/Ht | -0.84 ± 0.72* | -0.59 ± 0.60* | -1.41 ± 0.75 | 0.0002 |

*p <0.05 when compared with SSRI use > 6 M after controlling for multiple comparisons

Conclusion: Duration of SSRI use>6 months is associated with low BMD Z-scores in adolescents and young adults with AN independent of BMI and amenorrhea.

Sources of Research Support: In part by NIH grants 1 UL1 RR025758-01, DK 062249 and K23 RR018851.

Disclosures: AK: Principal Investigator, Pfizer, Inc.

Nothing to Disclose: MM, MLC, NM, KKM, EAL, EM, TW, SE, DH

OR08-4

Early Growth Hormone Introduction Is Associated with Higher Cortical Bone Mineral Density in Turner Syndrome.

Z Sumnik¹, O Soucek¹, M Snajderova¹, S Kolouskova¹, Z Hlavka² and J Lebl¹.

¹Charles Univ, 2nd Fac of Med Prague, Czech Republic and ²Charles Univ, Fac of Mathematics and Physics Prague, Czech Republic.

INTRODUCTION: Increased rate of fractures has been reported in patients with Turner syndrome (TS). Low bone mineral density is considered to be among potential causes of increased bone fragility in these patients. **OBJECTIVE:** The objectives of our study were to describe the bone mineral density (BMD) and bone geometry at the radius in girls with TS and to analyze the impact of growth hormone (GH) therapy and estrogen replacement on their bone. **DESIGN:** Sixty seven girls with TS aged 6-18 years treated currently or in the past with GH and/or estrogens were examined using peripheral quantitative computerized tomography. Results were compared to reference data. **RESULTS:** Height-adjusted cortical BMD was decreased in young girls with TS but then normalized during growth (mean Z-scores -1.23, -0.59 and 0.16 for prepubertal, pubertal and postpubertal group, respectively, $p < 0.01$). Height-, age- and cortical thickness-adjusted cortical BMD was negatively correlated to the age when GH therapy was initiated ($p < 0.02$), and positively correlated to the estrogen administration ($p < 0.01$). Trabecular BMD was normal prior to estrogen substitution, but decreased thereafter (mean Z-scores were -0.16, -0.65 and -1.35, $p < 0.01$). Bone cross-sectional area at the diaphysis was enlarged in all age groups. **CONCLUSIONS:** Height-adjusted cortical BMD is low before puberty in TS, but it normalizes during growth. An early GH therapy start, as well as estrogen replacement, is associated with higher cortical BMD. Further studies should investigate potential causality of this relation.

Sources of Research Support: Ministry of Health of Czech Republic, Grant No. 64203.

Nothing to Disclose: ZS, OS, MS, SK, ZH, JL

OR08-5

Biomarkers of Bone Metabolism and Serum Free Estradiol (E2) Levels in Medically Castrated Older Men Treated with MK-0773 (MK), Testosterone (T), or Placebo (PBO) for 12 Weeks.

SA Stoch MD¹, EJ Friedman MS¹, Y Zhou PhD¹, H Zhu PhD¹, P Larson MS¹, B Binkowitz PhD¹, J Chodakewitz MD¹ and JA Wagner MD, PhD¹.

¹Merck & C, Inc Whitehouse Station, NJ.

BACKGROUND/AIMS: GnRH agonist (GnRHa) therapy causes a hypogonadal phenotype associated with a decrease in bone mineral density (BMD). Since T is commonly contraindicated in males receiving GnRHa, alternatives to address the hypogonadal phenotype represent a therapeutic opportunity. Selective Androgen Receptor Modulators (SARMs) target the androgen receptor to elicit desired effects (increase lean body mass) without the mechanism-based liabilities (e.g. prostate proliferation). This study was designed to qualify the SARM profile of MK in 68 healthy older men given Lupron by evaluating lumbar spine BMD and urinary N-telopeptide/creatinine (NTx/Cr). Given the role of E2 in bone metabolism, serum E2 levels were also measured.

METHODS: The study design was previously reported (1). Briefly, subjects received monthly Lupron injections prior to and after randomization to treatment; 75 mg MK twice daily, 200 mg T every 14 days, or PBO for 12 weeks. BMD was assessed on Days -28, 1, and 85. Samples to assess serum E2 and urine NTx/Cr were collected throughout the study.

RESULTS: Sixty-two subjects (60 ± 6 yrs) completed the study. Four month mean change from baseline (Day -28) results are presented in the table below. MK and PBO subjects experienced a decrease in BMD and an increase in bone resorption (NTx/Cr). BMD was restored to baseline while NTx/Cr was suppressed in T subjects. In all treatment groups, serum E2 levels dropped to the castrate range following the first injection of Lupron and remained at nadir throughout the study for the MK and PBO groups, while returning to baseline within 8 weeks in subjects who started T treatment.

Conclusion: This study demonstrates that the non-aromatizable SARM, MK, did not reverse the bone phenotype induced by Lupron and further underscores the pivotal role of E2 in maintaining normal bone physiology in men. Our data suggest that non-aromatizable SARMs may not be an optimal approach to reverse hypogonadal-induced bone loss.

| | Treatment | N | Mean Change ^a (SD) |
|---------------------------------------|-----------|----|-------------------------------|
| Lumbar Spine BMD (g/cm ²) | MK | 20 | -0.0056 (0.0383) |
| | T | 22 | 0.0111 (0.0340) |
| | PBO | 20 | -0.0079 (0.0262) |
| Urine NTx/Cr (nmol(BC)/mmol(Cr)) | MK | 20 | 4.24 (10.6376) |
| | T | 22 | -14.2227 (8.5273) |
| | PBO | 20 | 5.765 (13.6966) |
| % Free E2 | MK | 17 | -0.7465 (0.3240) |
| | T | 20 | 0.4990 (0.6970) |
| | PBO | 18 | -0.71494 (0.3149) |

^a Four month mean change (Day -28 to Day 85).

(1) Stoch et al., Endocrine Society Meeting, June 11, 2009, S21-4.

Disclosures: EJF: Employee, Merck & Co. YZ: Employee, Merck & Co. HZ: Employee, Merck & Co. PL: Employee, Merck & Co. BB: Employee, Merck & Co.

Nothing to Disclose: SAS, JC, JAW

OR08-6 Multimodality Diagnosis and Treatment of Tumor-Induced Osteomalacia.

P Andreopoulou MD¹, CM Millo MD², JC Reynolds MD², MH Kelly RN, MS¹, BA Brillante RN, MBA¹, FM Wodajo MD³, R Chang MD², CC Chen MD² and MT Collins MD¹.

¹NIDCR, NIH Bethesda, MD ; ²NIH Bethesda, MD and ³Inova Fairfax Hosp, VCU Sch of Med Falls Church, VA.

Tumor induced osteomalacia (TIO) is an acquired hypophosphatemic disorder caused by FGF23-secreting tumors. We present data from a single center study of 23 subjects with presumed TIO referred to the NIH after previous evaluation at other institutions.

Laboratory data were as follows: phosphorus 1.2-2.3 mg/dl (mean 1.8, nl 2.5-4.8), FGF23 68-1605 pg/ml or RU/ml (nl 10-50 pg/ml or 19-108 RU/ml). Genetic testing was performed when indicated. Diagnostic imaging included FDG-PET/CT, whole body SPECT- Octreotide, 99Tc Sestamibi (Tc-MIBI) Scintigraphy, MRI, and when indicated, selective venous sampling for FGF23. All subjects had an octreotide scan. 21/23 subjects had FDG-PET and 16/23 had Tc-MIBI. FDG-PET was the most sensitive method of tumor localization. It was true positive in 10/21 and false positive in 2/21 subjects. Octreotide scan was positive in 10/23 subjects. However, it was true positive only in subjects with positive FDG-PET. Octreotide scan was positive and FDG-PET negative in a subject with an intracranial tumor. One subject with a positive octreotide scan did not undergo an FDG-PET scan. 7/21 patients had uptake in both octreotide and FDG-PET. One subject's octreotide scan took place after resection of the culprit tumor and prior to histological diagnosis. Octreotide scan was negative in 2 cases of falsely positive FDG-PET scans. Tc-MIBI was positive (5/16) but only in subjects with uptake on both the octreotide and FDG-PET scans. 14/23 subjects underwent selective venous sampling for diagnostic determination of an FGF23 gradient. In 10/23 tumor was identified and resected for cure (1/23 is awaiting surgery), in 3/23 tumor was found but subjects have either recurrent metastases (2/23), a partial resection still requiring treatment (1/23). 1/23 is post radiation of an intracranial lesion. In 8/23 no tumor was found despite multiple rounds of imaging and venous catheterization. Even with wide resections, tumor margin was positive in 5/13 surgical cases. 2 subjects presented late following the initial surgery (>10 and 5 yrs post-op) with pulmonary metastases. The diagnosis of TIO often requires a multimodality approach. FDG-PET/CT seems to be the best single imaging modality. Correlation with the CT findings available on the FDG-PET/CT and the Octreotide increases specificity. Aggressive wide resection is recommended for the initial operation. There may be cases of non-tumoral "TIO" due to other sources of FGF23.

Nothing to Disclose: PA, CMM, JCR, MHK, BAB, FMW, RC, CCC, MTC

S19-1

The Effects of Short- and Long-Term Denosumab Treatment on CTX Profile: Results from 6 Years of Continuous Denosumab Administration.

PD Miller MD¹, EM Lewiecki MD², MA Bolognese MD³, R Weinstein MD⁴, B Ding PhD⁵, RB Wagman MD^{6,7}, J San Martin MD⁵ and M McClung MD⁸.

¹Colorado Ctr for Bone Res Lakewood, CO ; ²New Mexico Clin Res & Osteoporosis Ctr Albuquerque, NM ; ³Bethesda Hlth Res Ctr Bethesda, MD ; ⁴Diablo Clin Res, Inc Walnut Creek, CA ; ⁵Amgen Inc Thousand Oaks, CA ; ⁶Amgen Inc San Francisco, CA ; ⁷Stanford Univ Sch of Med Stanford, CA and ⁸Oregon Osteoporosis Ctr Portland, OR.

Denosumab (DMAb) is an investigational, fully human monoclonal antibody that inhibits RANKL, an essential mediator of osteoclast formation, function, and survival. DMAb has been shown to reduce the risk of vertebral, hip, and nonvertebral fractures in the 3-year FREEDOM trial (1). In a phase 2 study, DMAb increased bone mineral density (BMD) and reduced bone turnover markers over 4 years of treatment (2). At the end of each DMAb dosing interval of 6 months, attenuation of reduction in CTX had been observed (2). This 4-year phase 2 study has been extended for 4 additional years, and 6 years of data are now available to further characterize changes in CTX over time. In the parent study, postmenopausal women with a DXA T-score between -1.8 and -4.0 (lumbar spine) or -1.8 and -3.5 (total hip or femoral neck) were randomized to receive placebo, alendronate (ALN), or 1 of 7 different doses of DMAb. After 2 years on study, subjects were reallocated to maintain, discontinue, or discontinue and reinitiate DMAb; discontinue ALN; or maintain placebo for 2 more years (2). In the study extension, all subjects received open-label DMAb 60 mg subcutaneously every 6 months. Here we compare the degree of reduction in CTX within the dosing interval in the parent and extension studies in years 1 and 5, time points when 1- and 6-month post-dose CTX levels were obtained, and report BMD changes through 6 years of treatment.

For the 124 subjects who received continuous DMAb treatment, median reductions in CTX levels were 89% and 91% at 1-month post-dose in years 1 and 5, respectively. Median reductions in CTX 6 months post-dose were 72% in year 1 and 48% in year 5. During year 6, CTX levels 6-months post-dose showed a similar degree of reduction: 43% and 55% at months 6 and 12, respectively. Continuous treatment with DMAb for 6 years was associated with mean BMD increases from parent study baseline of 13.3%, 6.1%, and 1.9% for the lumbar spine, total hip, and 1/3 radius, respectively. We conclude that short- and long-term DMAb administration resulted in consistently robust post-dose CTX reduction with quantitative CTX increases at the end of the dosing interval. This dynamic profile in serum CTX was associated with continued BMD gains through 6 years of DMAb treatment.

1. Cummings SR et al. N Engl J Med 2009;361:756-765
2. Miller PD et al. Bone 2008;43:222-229

Sources of Research Support: Amgen Inc.

Disclosures: PDM: Scientific Board Member, Amgen, Procter & Gamble, Roche, Lilly, Merck, Novartis, Sanofi-Aventis; Principal Investigator, Amgen, Procter & Gamble, Roche, Lilly, Merck, Novartis, Sanofi-Aventis, Takeda; Advisory Group Member, Lilly, Merck, Procter & Gamble, Sanofi-Aventis, Roche, Amgen, Novartis, GlaxoSmithKline; Consultant, Amgen, Procter & Gamble, Roche, Lilly, Merck, Novartis, Sanofi-Aventis, GlaxoSmithKline; Speaker, Amgen, Procter & Gamble, Roche, Lilly, Merck, Novartis, GlaxoSmithKline, Sanofi-Aventis. EML: Principal Investigator, Amgen, Lilly, GlaxoSmithKline, Novartis, Pfizer, Procter & Gamble, Roche, Sanofi-Aventis, Wyeth; Scientific Board Member, Amgen, Lilly, Novartis, Roche, GlaxoSmithKline, Wyeth; Speaker Bureau Member, Lilly, Novartis, Roche, Genzyme; Owner, Procter & Gamble, Teva. MAB: Speaker, Amgen, AstraZeneca, Novartis Pharmaceuticals, Lilly USA, LLC, GlaxoSmithKline; Investigator, Amgen, Lilly USA, LLC, Merck & Co., Roche Pharmaceuticals, Procter & Gamble; Investigator, Takeda. BD: Employee, Amgen; Owner, Amgen. RBW: Employee, Amgen; Owner, Amgen. JSM: Employee, Amgen; Owner, Amgen. MM: Investigator, Amgen; Lilly USA, LLC, Merck & Co., Novartis Pharmaceuticals, Procter & Gamble; Consultant, Amgen; Lilly USA, LLC, Merck & Co., Novartis Pharmaceuticals, Procter & Gamble; Speaker, Amgen; Lilly USA, LLC, Novartis Pharmaceuticals, Sanofi-Aventis.

Nothing to Disclose: RW

S19-2

Progesterone for Vasomotor Symptoms: A 12-Week Randomized, Masked Placebo-Controlled Trial in Healthy, Normal-Weight Women 1-10 Years Since Final Menstrual Flow.

JC Prior MD^{1,2} and CL Hitchcock PhD^{1,2}.

¹Univ of British Columbia Vancouver, Canada and ²Vancouver Coastal Hlth Res Inst Vancouver, Canada.

Vasomotor symptoms (VMS, hot flushes/night sweats) are the leading reason midlife women seek therapy. Oral medroxyprogesterone is effective for VMS in multiple controlled trials and equivalent to estrogen, the gold standard, in a 1-y parallel, masked trial. We hypothesize that oral micronized progesterone (OMP) effectively treats VMS.

Trial Design: Four weeks baseline, then 12 weeks blinded therapy.

Methods: Randomized, masked, placebo-controlled trial of OMP conducted at an academic center between June 2003 and Oct 2009. Healthy, non-obese, non-smoking community women with moderate to severe VMS were eligible if no ovarian hormone therapy for 6 mo. Women completed questionnaires, physical measurements and fasting blood tests. Throughout the study, women recorded VMS intensity (0-4) and number in a daily diary; VMSScore is the sum across day and night of intensity-times-number.

Interventions: After baseline, eligible women received randomized, blinded therapy to be taken as 3x100 mg OMP (Prometrium®) or identical placebo immediately before sleep each evening. Therapy was recorded on the diary.

Outcome: The planned primary outcome was average daily VMSScore during the last 28 days of therapy, with VMS number also assessed.

Randomization and masking: Randomization was computer-generated, administered and maintained by an independent pharmacist until data were cleaned prior to analysis. Participants and all study team members were masked.

Results: 133 women were randomly allocated to receive OMP (n=75) or placebo (n=58). The intent-to-treat analysis included all 114 women (OMP n=68, placebo n=46) with at least 7 days diary data during both baseline and therapy. Women were aged 55.0±4.4 (mean±SD), 4.3±2.6 y from last flow, BMI 24.7±2.8, waist circ. 78.4±6.7 cm. Most (91%) identified as white. Baseline VMSScore was 17.0±1.0 and number was 6.8±0.3 per day.

OMP was more effective than placebo in treating VMS (95% CI of VMSScore diff: 1.8-6.7, p=0.001; ANCOVA with baseline VMSScore as covariate), with a 56% decrease from baseline on OMP and 28% on placebo. Similarly, VMS number was significantly lower on OMP than on placebo (95% of diff: 0.8-2.5), with a 48% decrease on OMP and 22% decrease on placebo. No serious adverse events occurred.

Conclusion: Oral micronized progesterone is effective therapy for hot flushes and night sweats in healthy women 1-10 years since final menstrual flow.

Trial Registry: www.clinicaltrials.gov NCT00152438.

Sources of Research Support: Private individual donations to the Centre for Menstrual Cycle and Ovulation Research (CeMCOR). Active drug and placebo provided by Schering (Canada) and by Besins Healthcare.

Nothing to Disclose: JCP, CLH

S19-3

Metformin during Pregnancy in Polycystic Ovary Syndrome: Results of a RCT.

E Vanky MD PhD^{1,2}, S Stridsklev MD^{1,2}, R Heimstad MD PhD^{1,2}, PR Romundstad PhD² and SM Carlsen MD PhD^{1,2}.

¹St Olavs Hosp Trondheim, Norway ; ²Norwegian Univ of Sci and Technology Trondheim, Norway ; ³Norwegian Univ of Sci and Technology Trondheim, Norway ; ⁴Norwegian Univ of Sci and Technology Trondheim, Norway and ⁵St Olavs Hosp Trondheim, Norway.

Background: Women with polycystic ovary syndrome (PCOS) have an increased incidence of pregnancy complications. Retrospective and non-randomized studies report beneficial effects of metformin on pregnancy complications. The present study was designed to test the hypothesis that metformin use in pregnancy reduces preeclampsia, preterm delivery and/or gestational diabetes mellitus (GDM) in PCOS.

Methods: The Metformin treatment in pregnant PCOS women (PregMet) study is a prospective, randomized, double-blind, multicentre trial comparing metformin 2000mg daily with placebo.

Inclusion criteria: PCOS diagnosed according to The Rotterdam Consensus criteria, age 18 - 45 years, gestational week 5 - 12 at inclusion and singleton viable fetus.

Exclusion criteria: ALAT > 90 IU/L, creatinine > 130 µmol/L, alcohol abuse, previously diagnosed diabetes mellitus or fasting s-glucose > 7.0 mmol/L at inclusion and use of glucocorticoids or drugs known to interfere with metformin.

Three-hundred-forty-seven PCOS women, with altogether 363 pregnancies were informed about the study; 32 did not meet inclusion criteria, 58 declined and 16 women participated twice. Two-hundred-seventy-three pregnancies were randomly assigned to metformin or placebo treatment.

Primary outcomes: incidence of preeclampsia, preterm delivery and GDM or the composite of these three disorders.

Secondary outcomes: weight, heart rate and blood pressure development in pregnancy, and the mode and length of delivery.

Results: Results: There were no differences in the prevalence of preeclampsia, preterm delivery and GDM or a composite of these three disorders between the metformin and placebo groups. Women in the metformin group gained less weight during pregnancy compared to those in the placebo group. No difference was found in other secondary outcomes, such as heart rate and blood pressure development in pregnancy, and length and mode of delivery.

Primary end points according to treatment group

| | Metformin n (%) | Placebo n (%) | Risk diff % | 95% CI | p-value |
|------------------|-----------------|---------------|-------------|--------------|---------|
| Preeclampsia | 10/135 (7.4) | 5/135 (3.7) | 3.7% | -1.7 to 9.2 | 0.18 |
| Preterm delivery | 5/135 (3.7) | 11/135 (8.2) | -4.4% | -10.1 to 1.2 | 0.12 |
| GDM# | 22/125 (17.6) | 21/124 (16.9) | 0.8% | -8.6 to 10.2 | 0.87 |
| Composite | 35/135 (25.9) | 33/135 (24.4) | 1.5% | -8.9 to 11.3 | 0.78 |

GDM diagnosed after inclusion

Conclusion: Metformin medication in polycystic ovary syndrome, from first trimester to delivery, does not reduce pregnancy complications.

Nothing to Disclose: EV, SS, RH, PRR, SMC

S19-4

Blood Pressure and Cardiovascular Disease Risk in the Veterans Affairs Diabetes Trial (VADT).

RJ Anderson MD¹, G Bahn MS², D Kaufman MS², TE Moritz MS², C Abaira MD³ and WC Duckworth MD⁴

¹VA Med Ctr Omaha, NE ; ²Hines VA Hosp Hines, IL ; ³VA Med Ctr Miami, FL and ⁴VA Med Ctr Phoenix, AZ.

The Veterans Affairs Diabetes Trial (VADT) is a prospective, randomized study of 1791 veterans with T2DM. The primary goal was to determine whether intensive glucose control prevented major cardiovascular disease (CVD) events. Blood pressure (BP) control was an essential component for reduction of CVD risk.

Objective: To determine whether baseline and follow-up (On-Study) systolic blood pressure (SBP), diastolic blood pressure (DBP), and SBP combined with DBP predict CVD events.

Design: BP and other CVD risk factors were controlled uniformly in both the intensive and standard glucose control groups. All who entered the trial with new or treated hypertension received stepped treatment to maintain BP below 130/80 mmHg. The primary outcome was the time from randomization to the first occurrence of myocardial infarction, stroke, congestive heart failure, surgery for vascular disease, inoperable coronary disease, amputation for ischemic gangrene or CV death.

Results: Baseline and On-Study BP significantly predicted CVD events whether analyzed as separated SBP and DBP, or as combined SBP and DBP. For patients with separated SBP ≥ 140 mmHg, the risks were significant at baseline (HR=1.508 P=0.0004) and On-Study (HR=1.469, P=0.0016). Separated DBP < 70 mmHg increased CVD events at baseline (HR=1.482, P=0.0007) and On-Study (HR=1.491, P=0.0002). Analysis of combined blood pressure categories indicated high risk for CVD events for SBP ≥ 140 and DBP < 70 mmHg at baseline (HR=1.785, P=0.029) and On-Study (HR=2.042, P=0.0029) and DBP < 70 mmHg with all other levels relative to the target BP (105-129/70-79 mmHg). Prehypertensive SBP (120-139 mmHg) with DBP ≥ 70 mmHg was not associated with increased risk in these patients at baseline and On-Study. Sensitivity analyses indicated further significant risk of primary outcomes with DBP < 60 mmHg combined with SBP < 105 mmHg (HR=2.825, P=0.0001).

Conclusions: The increased risk of CVD events with SBP ≥ 140 mmHg supports the urgency for treatment of Stage 1 systolic hypertension in these patients with T2DM. However, prehypertensive SBP with a DBP ≥ 70 mmHg was not associated with increased CVD risk. Increased risk with DBP < 70 mmHg, even when combined with SBP in target ranges, is a new finding for T2DM patients. Additional analyses indicate that DBP < 60 mmHg is a critical DBP below which risk is further increased. The findings emphasize that lowering DBP to < 70 mmHg in these patients elevated CVD risk and should be avoided.

Disclosures: CA: Speaker, Novo Nordisk.

Nothing to Disclose: RJA, GB, DK, TEM, WCD

S19-5

Once-Daily, Low-Dose, Controlled-Release Phentermine/Topiramate Demonstrates Significant Improvement in Weight, Related Risk in Overweight/Obese Patients with Comorbidities.

WT Garvey MD¹, CH Bowden MD² and WW Day PhD².

¹Univ of Alabama at Birmingham Birmingham, AL and ²Vivus, Inc Mountain View, CA.

A Phase 3, double-blind, randomized controlled study (CONQUER) was conducted to confirm the efficacy and safety of a low-dose, controlled-release formulation of phentermine/topiramate (PHEN/TPM) for weight loss in overweight/obese adults, and to determine related risk reduction. A total of 2487 subjects with BMI 27-45 kg/m² and ≥2 comorbidities (eg, glucose intolerance, hypertension, dyslipidemia) were randomly assigned in a 2:1:2 ratio to receive placebo (n=994), PHEN 7.5 mg/TPM 46 mg (7.5/46 [n=498]), or PHEN 15 mg/TPM 92 mg (15/92 [n=995]). Least squares (LS) mean percent weight loss at Week 56 was significantly greater in both PHEN/TPM groups vs placebo in the ITT-LOCF population (N=2448): 1.2%, 7.8%, and 9.8% for placebo, 7.5/46, and 15/92, respectively (*P*<0.0001 vs placebo). Secondary analyses for cardiometabolic risk factors demonstrated significant, dose-related improvements from baseline in lipids, glycemic parameters, and insulin sensitivity in PHEN/TPM subjects; these improvements were significantly greater than with placebo, with the exception of LDL change in the 7.5/46 group (Table).

LS Mean Changes From Baseline in Cardiometabolic Parameters at Week 56 (ITT with LOCF)

| Parameter | Placebo | 7.5/46 | 15/92 |
|--------------------------------------|---------|--------|--------|
| LDL % change | -4.1 | -3.7 | -6.9* |
| HDL % change | 1.2 | 5.2* | 6.8* |
| Total cholesterol (TC) % change | -3.3 | -4.9* | -6.3* |
| TC/HDL ratio change | -0.19 | -0.43* | -0.52* |
| Triglycerides % change | 4.7 | -8.6* | -10.6* |
| HbA1c % change | 0.1 | -0.0* | -0.1* |
| Fasting glucose (mg/dL) | 2.3 | -0.1* | -1.3* |
| Fasting insulin (μU/mL) | 0.7 | -3.5* | -4.0* |
| HOMA-IR | 0.46 | -0.93* | -1.07* |
| Whole-body insulin sensitivity index | 0.50 | 1.71* | 1.98* |

**P*<0.05 vs placebo

The PHEN/TPM groups also had greater LS mean improvements from baseline in inflammatory markers C-reactive protein, adiponectin, and fibrinogen (*P*<0.05 vs placebo).

In addition to achievement of significant weight loss from baseline, treatment with PHEN/TPM improved cardiometabolic parameters that increase the risk of type 2 diabetes and cardiovascular disease. The greater weight loss seen with 15/92 was associated with greater improvements in markers of risk. Treatment was well-tolerated; adverse events were generally mild. PHEN/TPM appears to be effective in the treatment of obesity and improvement in cardiometabolic disease risk.

Sources of Research Support: Vivus, Inc.

Disclosures: WTG: Researcher, Merck & Co., Abbott Laboratories, Genzyme Corporation; Owner, Merck & Co., Abbott Laboratories, Genzyme Corporation, Pfizer, Inc., Lilly USA, LLC, Schering Plough, Novartis Pharmaceuticals, Vivus USA; Consultant, Vivus USA; Speaker Bureau Member, Merck & Co. CHB: Employee, Vivus USA. WWD: Employee, Vivus USA; Owner, Vivus USA.

S19-6

5- α Reduction of Testosterone to Dihydrotestosterone Is Not Required in Mediating Its Effects on Muscle and Sexual Function.

KM Lakshman MD MPH¹, S Basaria MD¹, P Knapp¹, TG Travison PhD¹, NA Mazer MD¹, S Hanka¹, N Mohammed¹, P Dao¹, J Ullloor¹, J Krasnoff PhD¹, R Miciek¹, A Elmi¹, N LeBrasseur PhD¹, T Storer¹ and S Bhasin MD¹.

¹Boston Univ Boston, MA.

Background: Testosterone (T), the main circulating androgen in men, acts directly in many target tissues. However its effects on certain tissues require 5- α reduction to dihydrotestosterone (DHT). We do not know whether 5- α reduction to DHT is essential for mediating T effects on skeletal muscle and sexual function. Therefore, we determined the effects of graded doses of T on lean body mass (LBM), fat mass (FM), muscle strength and sexual function in the absence and presence of a potent 5- α -reductase inhibitor, dutasteride.

Methods: 102 healthy eugonadal men (age 18-50 yrs) were given monthly Lupron injections to suppress endogenous T and randomized to weekly injections of 50, 125, 300 or 600 mg T enanthate for 20 weeks. Subjects within each group were further randomized to receive either dutasteride (2.5 mg/d) or placebo. Outcomes were measured at baseline and week 20.

Results: The dutasteride and placebo groups did not differ significantly in any baseline characteristics. Serum T levels increased dose-dependently in both dutasteride and placebo groups with no significant differences between the groups (Dose effect, $P < 0.0001$; Dutasteride effect, $P = 0.4$). LBM increased dose-dependently ($P < 0.0001$) in the dutasteride (+ 0.6, 3.7, 5.8, 7.0 kg) and placebo (+ 0.8, 3.4, 5.7, 8.1 kg) group with no significant differences between the two groups ($P = 0.56$). FM decreased in a dose-dependent manner in the dutasteride (+ 0.99, -1.4, -2.2, -2.07 kg) and placebo group (-1.0, -0.3, -4.7, -3.1 kg; Dose effect, $P < 0.0001$; Dutasteride effect $P = 0.19$). Both groups showed significant increase in muscle strength measured as 1RM strength in leg and chest press in the 125, 300 and 600 mg dose groups (Dose effect, $P < 0.0001$) with no significant difference between the groups ($P = NS$). Sexual function, measured as change in the International Index of Erectile Function score, worsened in the 50 and 125 mg groups, but showed improvement in the 300 and 600 mg groups (T dose effect, $P = 0.19$) without any differences between the dutasteride and placebo groups ($P = 0.83$). Overall, there was no significant change in the PSA levels in either group (Dose effect, $P = 0.10$; Dutasteride, $P = 0.15$).

Conclusion: In healthy men, 5- α reduction to DHT is not essential for mediating T effects on muscle mass and strength, and sexual function. Combined administration of T plus a 5-alpha reductase inhibitor should be explored as a potential function promoting anabolic therapy that might spare the prostate.

Sources of Research Support: Glaxo SmithKline for supplying Dutasteride for this study.

Nothing to Disclose: KML, SB, PK, TGT, NAM, SH, NM, PD, JU, JK, RM, AE, NL, TS, SB

P1-1

Novel Synthetic Agonists Exchange for Phospholipids Bound to the Ligand Binding Pockets of SF-1 or LRH-1, and Selectively Modulate Receptors Activity.

F Cai MD, MS¹, RD Blind PhD¹, J Stec PhD², DD Belsham PhD⁴, TM Willson PhD³, RJ Richard PhD² and HA Ingraham PhD¹.

¹Univ of California, San Francisco San Francisco, CA ; ²Univ of Southampton Southampton, UK ; ³GlaxoSmithKline Research Triangle Park, NC and ⁴Univ of Toronto Toronto, Canada.

The nuclear receptors SF-1 (NR5a1) and LRH-1 (NR5a2) are important regulatory factors in steroid and bile acid homeostasis, respectively, and both receptors play important roles in embryogenesis and organogenesis. Although, phospholipids occupy and exchange into the ligand binding pocket of SF-1, it remains unclear whether phospholipids function as bona fide regulatory ligands. A series of synthetic small molecules were found to bind either the SF-1 and/or LRH-1 ligand binding domains (LBDs) using a fluorescence resonance energy transfer (FRET)-based assay for co-activator peptide binding. These new compounds were then tested for their ability to 1) displace bound phosphatidyl glycerol (PG) from purified LBD protein and 2) regulate known SF-1 and LRH-1 targets using tetracycline-inducible cell lines. Several compounds were able to specifically displace PG from purified SF-1 LBD. In HEK293 cells, known NR5a target genes (StAR and SHP) were activated in a dose dependent manner by several compounds suggesting that they function as NR5a agonists. Using another embryonic hypothalamic cell line, we have identified several new SF-1 targets that are either markedly activated or repressed by these new compounds; these targets include several developmental genes and those involved in phosphatidyl inositol metabolism. Based on our collective biochemical and cellular data we suggest that these new selective NR5a agonists function by stabilizing NR5a receptors. Thus, these new compounds increase the effective dosage of NR5a receptors and as such could be of therapeutic or experimental value.

Nothing to Disclose: FC, RDB, JS, DDB, TMW, RJR, HAI

P1-2

PKR and the RISC Proteins PACT, TRBP, and Dicer Are SRA-Binding Nuclear Receptor Coregulators.

A Redfern¹, N Ikeda¹, S Colley¹, X Li¹, D Beveridge¹, M Epis¹, C Foulds², L Stuart¹, V Russell¹, K Ramsay¹, BW O'Malley² and Pj Leedman^{1,3}.

¹Western Australian Inst for Med Res Perth, Australia ; ²Baylor Coll of Med Houston, TX and ³Univ of Western Australia Perth, Australia.

Transcriptional signaling is tightly controlled by nuclear receptor (NR) coregulators. There are emerging links between the NR pathway and members of the RNA-Induced Silencing Complex (RISC). In the cytoplasm, the RISC contains double-stranded RNA-binding proteins, including PACT (protein kinase RNA [PKR] activator), TRBP (TAR RNA-binding protein), and Dicer that process pre-microRNAs (pre-miRNAs) into microRNAs (miRNAs) which in turn target specific mRNA species for regulation. Recently, the first step in miRNA maturation was shown to be hormonally suppressed by activated ER α through its association with the nuclear Drosha processing complex (1). Here we show that PACT, TRBP and Dicer together with PKR, are SRA (steroid receptor RNA activator)(2)-binding NR coregulators that target steroid-responsive promoters and regulate NR signaling. SRA facilitates recruitment of these proteins to target promoters and also associates with them in the cytoplasm. Argonaute 2 (Ago2), another member of cytoplasmic RISC, also associates with SRA and TRBP in the nucleus. Interestingly, specific pre-miRNAs were found to associate with members of the RISC in the nucleus. These data demonstrate a new functional nuclear repertoire for PKR, PACT, TRBP and Dicer as coregulators of SRA-mediated NR signaling. Furthermore, the association of members of RISC with pre-miRNAs in the nucleus and SRA in the cytoplasm provides evidence for new linkages between NR-mediated transcription and the factors involved in miRNA processing.

(1) Yamagata, et al., Mol Cell 2009; 36:340

(2) Lanz et al., Cell 1999; 97:17.

Sources of Research Support: National Health and Medical Research Council, Australia.

Nothing to Disclose: AR, NI, SC, XL, DB, ME, CF, LS, VR, KR, BWO, PjL

P1-3

AM251 Induces Expression of EGF Receptor and Its Ligands through Inhibition of Estrogen Receptor-Related Receptor α .

Jennifer L Fiori Ph.D.¹, Mitesh Sanghvi Ph.D.¹, Sutapa Kole B.S.¹, Ruin Moaddel Ph.D.¹ and Michel Bernier Ph.D.¹.

¹Natl Inst of Hlth (NIH)/Natl Inst on Aging (NIA) Baltimore, MD.

Changes in specific cannabinoid receptor (CB1R and CB2R) signaling have been shown to occur during carcinogenesis and dysregulation of the epidermal growth factor receptor (EGFR) has been associated with many aspects of the carcinogenic process such as growth, migration, and angiogenesis in various tumor types. This study examined potential crosstalk of CB1R with EGFR leading to oncogenic signaling. Human pancreatic carcinoma cells (PANC-1) were incubated with the specific CB1R inhibitor, AM251, to monitor its effect on the expression of relevant genes. A time (6-24 h)-, and dose (0-5 μ M)-dependent increase in EGFR and HB-EGF mRNA levels was observed in PANC-1 cells following treatment with AM251, but not the CB2R inhibitor AM630. Incubation with the CB1R agonist Win55,212-2 (5 μ M) had only a modest inhibitory effect, consistent with the fact that PANC-1 cells expressed very low levels of CB1R. This responsiveness to AM251 was associated with elevated steady-state EGFR protein levels and significant EGF-induced activation of cell surface EGFRs and intracellular signaling pathways. AM251 altered cell morphology and had enhancing effects on the responses of EGF toward proliferation, migration, and colony formation in soft agar. To identify potential non-CB1R-mediated mechanisms responsible for AM251 action, we examined several signaling pathways and established that estrogen receptor-related receptor (ERR) α , whose expression correlates to a poor prognosis in various tumors, transduced the AM251 signal. The synthetic ERR α inverse agonist, XCT790, mimicked AM251's ability to induce EGFR and HB-EGF expression, and cell pretreatment with biochanin A (a selective ERR α agonist) blocked AM251 actions. AM251 was found to displace diethylstilbestrol from the ERR α ligand binding domain immobilized on a chromatographic support. In summary, these data suggest that AM251 upregulates EGFR expression and signaling via a novel non CB1R-mediated pathway involving inhibition of ERR α .

Sources of Research Support: Intramural Research Program of the National Institutes of Health (NIH) / National Institute on Aging (NIA).

Nothing to Disclose: JLF, MS, SK, RM, MB

P1-4

SHP Activity Is Positively Regulated by PRMT5 through Arginine Methylation at a Naturally-Occurring Mutation Site.

D Kanamaluru¹, Z Xiao PhD², T D Veenstra PhD² and J K Kemper PhD³.

¹Univ of Illinois at Urbana-Champaign Urbana, IL ; ²Natl Cancer Inst-Frederick Frederick, MD and ³Univ of Illinois at Urbana-Champaign Urbana, IL.

An orphan nuclear receptor and transcriptional corepressor, Small Heterodimer Partner (SHP), plays an important role in maintaining metabolic homeostasis by repressing the activity of many nuclear receptors. Recently, our group reported that SHP inhibits the transcription of CYP7A1, a key gene in hepatic bile acid biosynthesis, by coordinately recruiting chromatin modifying cofactors including mSin3A/histone deacetylase1 (HDAC1), G9a and Swi/Snf-Brm to the CYP7A1 promoter. In line with its important role as a metabolic regulator, naturally-occurring mutations in the SHP gene have been reported in human subjects that are associated with mild obesity and diabetes. However the molecular basis underlying the mechanism by which the mutations lead to metabolic disorder is unknown. Since several Arginine mutations, including R57W, were identified as naturally occurring mutants, we examined the importance of Arginines in SHP function. R57W mutant showed reduced interaction with mSin3a, HDAC1 and Brm, but not with G9a, and impaired repressive activity. Tandem mass spectrometric analysis revealed that Arg 57 in SHP is indeed methylated by PRMT5. SHP interaction with PRMT5 was increased after bile acid treatment in HepG2 cells and mouse liver. Overexpression of wild type PRMT5 increased SHP-mediated repression, whereas a dominant negative PRMT5 mutant or downregulation of PRMT5 using siRNA reversed it. Overexpression of R57W mutant in mouse liver through adenoviral delivery method resulted in increased expression of metabolic genes involved in bile acid and fatty acid synthesis in a gene-selective manner, which may be due to differential recruitment of corepressors by SHP in a promoter-specific manner. We are studying the effects of R57W on metabolic outcomes by measuring bile acid levels and serum and liver triglyceride levels. We are also studying in-vivo effects of downregulation of PRMT5 on SHP function using adenoviral-mediated overexpression of siPRMT5.

Nothing to Disclose: DK, ZX, TDV, JKK

P1-6

Acid Ceramidase (ASAH1) Mediated Repression of Steroidogenic Factor 1 (SF-1) Regulates Steroidogenic Gene Transcription in H295R Adrenocortical Cells.

NC Lucki¹ and MB Sewer PhD².

¹Georgia Inst of Technology Atlanta, GA and ²Univ of California, San Diego La Jolla, CA.

Adrenocortical steroidogenesis is regulated by adrenocorticotropin (ACTH) primarily through a cAMP/protein kinase A (PKA)-dependent pathway. ACTH signaling leads to cholesterol import and transport as well as the transcriptional activation of genes required for cortisol biosynthesis. ACTH signaling also stimulates the activity and regulates the transcription of several enzymes involved in sphingolipid (SL) biosynthesis. These enzymes act as effectors during ACTH/cAMP signaling to regulate the cellular concentrations of bioactive SLs, including sphingosine (SPH) and sphingosine-1-phosphate (S1P), both of which modulate steroidogenesis. Acid ceramidase (encoded by ASAH1) is one such enzyme that hydrolyzes ceramide (cer) into SPH and a fatty acid. We have previously identified an important link between ACTH/cAMP signaling and the SL metabolic pathway by demonstrating that ACTH rapidly promotes SL metabolism, including cer turnover and S1P secretion. In addition, silencing ASAH1 expression induces the transcription of CYP17, a gene involved in cortisol production. We also have identified SPH as an antagonist ligand for steroidogenic factor 1 (SF-1), a nuclear receptor that regulates the transcription of most steroidogenic genes, including CYP17. Given that SF-1 is predominantly nuclear, we hypothesized that SPH production and ligand binding occur in the nucleus. In this study, we show that ASAH1 and its coactivator protein saposin D are localized in the nuclei of H295R adrenocortical cells. In addition, ACTH/cAMP signaling acutely induces nuclear ceramidase activity, which is concomitant with transient decreases in nuclear ceramide concentrations as quantified by liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Further, ASAH1 represses cAMP- and SF-1-dependent CYP17 reporter gene activity. Significantly, we demonstrate that ASAH1 binds directly to SF-1 via an LxxLL motif that lies between amino acids 17-30 on the α subunit of the enzyme and that this motif is required for repression of SF-1. These findings suggest that SPH binding to SF-1 is facilitated by direct protein-protein interactions between the receptor and the cer metabolizing enzyme and identify ASAH1 as a novel class of coregulatory proteins.

Nothing to Disclose: NCL, MBS

P1-7

The Effect of Thyroid Hormone on the FXR/SHP-Dependent Regulatory Mechanism of CYP7A1 Gene.

HY Ahn MD¹, EK Lee MD¹, SH Kim¹, DJ Park MD, PhD¹, SY Kim MD, PhD¹, BY Cho MD, PhD¹, DD Moore MD, PhD² and YJ Park MD, PhD¹.

¹Seoul Natl Univ Coll of Med Seoul, Republic of Korea and ²Bayer Coll of Med Houston, TX.

Hepatic cholesterol 7 α hydroxylase (CYP7A1), the rate-limiting enzyme for cholesterol catabolism and bile acid synthesis, plays an important role in the removal of excess cholesterol. It is well-known that the farnesoid X receptor (FXR)/small heterodimer partner (SHP)/ liver receptor homolog (LRH)-1 -dependent mechanism is the most important for the transcriptional regulation of CYP7A1 gene. Bile acid activation of FXR has been shown to repress the expression of CYP7A1 via increasing the expression of SHP. The increased abundance of SHP causes it to associate with LRH-1, an obligate factor required for transcription of CYP7A1, thus inhibits the transcriptional activity of LRH-1. Recently, two TR(thyroid hormone receptor)-binding sites were identified in the far upstream 5' flank region. There is an inverse relationship between thyroid hormone and serum cholesterol, and the murine CYP7A1 gene is highly inducible by thyroid hormone in vivo, thus the presence of thyroid hormone response element (TRE) suggest direct thyroid hormone regulation to the murine CYP7A1 gene. However, the regulatory mechanism is still unclear.

In this study, we demonstrated that the treatment of thyroid hormone (T3) in C57BL/6 mouse repressed the expression SHP and increased the CYP7A1. Both in mouse primary hepatocytes and human hepatoma HepG2 cells, the same results were observed after T3 treatment. The overexpression of LRH-1, which is also an important transcriptional regulator of SHP, increased the promoter activity of SHP in HepG2 cells, and the activity was more increased with a co-treatment of TR β and retinoid X receptor (RXR), which disappeared after T3 treatment. These findings suggest that there exists an indirect mechanism in the regulation of thyroid hormone of the CYP7A1 gene. However, when we analyzed the expression of CYP7A1 gene using SHP-null mouse, the increments of CYP7A1 gene by T3 were still observed, suggesting the other regulatory mechanism other than the T3-SHP-dependent mechanism also exists. In conclusion, thyroid hormone can regulate the expression of CYP7A1 gene, as well as directly, through the SHP-dependent mechanisms. The regulation of SHP by T3 seems to be resulted from interaction between T3/TR β and LRH-1.

Nothing to Disclose: HYA, EKL, SHK, DJP, SYK, BYC, DDM, YJP

P1-8

Interaction of Nuclear Receptor Coactivator 4 with Tubulin and Localization to the Microtubule Network and Mitotic Spindle.

A Kollara PhD^{1,2} and TJ Brown PhD^{1,2}.

¹Samuel Lunenfeld Res Inst Toronto, Canada and ²Univ of Toronto Toronto, Canada.

Several nuclear receptor coregulatory proteins have been shown to localize both in the cell cytoplasm and nucleus. The retention of these coregulatory proteins in the cytoplasm is suggestive of functions in addition to their activity in the cell nucleus to regulate receptor transcriptional activity. For example, the nuclear Steroid Receptor Coactivator 3 (SRC3) exhibits histone acetyltransferase (HAT) activity, whereas cytoplasmic SRC3, which associates with microtubules, is devoid of HAT activity. Nuclear Receptor Coactivator 4 (NcoA4) is a ubiquitously expressed coactivator that interacts with and enhances the transcriptional activity of several nuclear receptors including the androgen, estrogen, progesterone, vitamin D, peroxisome proliferator-activated isoforms α and γ and aryl hydrocarbon receptor. Unlike the p160 coactivators, NcoA4 lacks a recognized nuclear localization signal and immunohistochemical studies indicate cytoplasmic localization of NcoA4 in the presence or absence of ligand-activated receptor, suggesting additional actions of NcoA4 in the cell cytoplasm. In the present study, we tested whether NcoA4 interacts with and co-localizes to the microtubule filaments, particularly the mitotic spindle. Co-immunoprecipitation studies with T47D human breast cancer and COS African green monkey kidney cells demonstrated interaction of NcoA4 with α -tubulin. This interaction was observed both with endogenously expressed NcoA4 and cyan fluorescent-tagged NcoA4 fusion protein. Deconvolution fluorescence microscopy of T47D and COS cells immunostained for NcoA4 and α -tubulin revealed punctuate NcoA4 staining associated with the microtubule network in cells at prophase. In dividing cells, intense NcoA4 staining was associated with the mitotic spindle whereas at telophase, intense NcoA4 staining was noted at the midbodies extending beyond the spindle staining for tubulin. In addition, co-localization of NcoA4 with γ -tubulin in the centrosomes of dividing cells was also detected. These findings indicate that NcoA4 is a novel microtubule-associating protein and furthermore a novel centrosomal protein. Thus, in addition to its role as a nuclear receptor coactivator, NcoA4 may play a role in microtubule dynamics associated with mitotic spindle assembly and function. Further studies to determine the functional role of the NcoA4- α -tubulin interaction are in progress.

Sources of Research Support: National Sciences and Engineering Research Council of Canada.

Nothing to Disclose: AK, TJB

P1-9

Role of Rb Family in Vitamin D Receptor-Mediated Anti-Tumor Effects in Ovarian Cancer Cells.

J Tang Ph.D.¹, P Li Ph.D.¹, AKW Tse Ph.D.¹, SV Nicosia M.D.^{1,2}, X Zhang Ph.D.^{1,2} and W Bai Ph.D.^{1,2}.

¹USF Coll of Med Tampa, FL and ²H Lee Moffitt Cancer Ctr Tampa, FL.

Epithelial ovarian cancers (OCa) remain a highly lethal malignancy worldwide. Our published studies have shown that active vitamin D (VD) compounds suppress the growth of multiple OCa cells and that the vitamin D receptor (VDR) mediating the anti-tumor effects represents a novel molecular target for the development of new drugs for OCa. However, about half of human OCa is found to be resistant to VD-induced growth suppression. Understanding the underlying mechanisms is essential to develop strategies to overcome the resistance. Here, we present our findings that support an important role for members of the Rb family in VD action, suggesting that the functional loss of Rb proteins may contribute to the resistance. The VDR transcriptional activity and levels of expression are lower in VD-resistant OCa cells as comparison to sensitive ones. Interestingly, the Rb is hyperphosphorylated in VD-resistant cells. Transfection of Rb into OCa cells increases the VDR transcriptional activity, which is suppressed by T antigen. Consistently, knockdown of the Rb family with shRNA in VD-sensitive OCa cells reduces the levels of the VDR expression and the cell's growth response to VD suppression. Similar data were obtained in Rb/p107/p130 triple knockout mouse embryonic fibroblasts. Co-immunoprecipitation analyses reveal a complex formation between the VDR and the three members of the Rb family, which is decreased by Rb phosphorylations and enhanced by VD treatment. We thus conclude that members of Rb family act redundantly to regulate VD sensitivity in OCa cells. Our studies suggest that compounds that restore the functional status of Rb proteins can be used in combination with active VD compounds for OCa prevention/treatment.

Sources of Research Support: R01 grant from NCI (CA111334) and a team science project grant from the Florida Department of Health (09KT-03).

Nothing to Disclose: JT, PL, AKWT, SVN, XZ, WB

P1-10

Identify Natural and Synthetic Ligands/Activators for Testicular Nuclear Receptor 4.

LY Fang MS¹, SJ Lin MS¹, S Liu PhD¹, YF Lee PhD¹ and C Chang PhD¹.

¹Univ of Rochester Med Ctr Rochester, NY.

Testicular Nuclear Receptor 4 (TR4) was classified as an orphan nuclear receptor which contains many physiological functions in fertility, neuron development, metabolism, bone development, and skeleton muscle. Recently we identified TR4's ligands/activators in controlling lipid uptake in macrophages. We found PUFAs, PUFA metabolites, and Thiazolidinedione (TZD) can function as TR4 ligands/activators in regulating TR4 transactivation activities. In peptide mapping assays, PUFA metabolites 15HETE and 13 HODE, but not TZD, show direct binding with TR4 that changes TR4 conformation. TZD-rosiglitazone is an anti-diabetic drug and is the ligand of Peroxisome proliferator-activated receptor gamma (PPAR γ). Although TZD can not change TR4 conformation, we show our discovery that TR4 mRNA and protein levels are increased upon TZD treatment. We further studied *in vivo* CD36 DNA pull down assay showing that TZD can increase TR4 CD36 promoter binding ability. Moreover, we found TR4 can interact with PPAR γ upon TZD treatment based on mammalian two-hybrid experiments which suggest TZD effect might go through TR4 and PPAR γ interaction. Taken together, we found PUFA metabolites can bind and change TR4 conformation to promote its activity while TZD can stimulate TR4 function by increasing TR4 mRNA and protein levels and also by increasing TR4 DNA binding ability in PPAR γ dependent manner. These results suggesting TR4 can be regulated in PPAR γ independent or dependent pathways depending on its upstream signals and provide a therapeutic approach to target TR4 or PPAR γ differentially in metabolic syndrome.

Sources of Research Support: This work was supported by National Institutes of Health Grants DK073414 and a George Whipple Professorship endowment.

Nothing to Disclose: LYF, SJL, SL, YFL, CC

P1-11

Estrogen Receptor Agonist Effects of ICI 182,780 in Rat Serotonergic Neurons.

N Hengen MD, PhD¹.

¹Shenandoah Univ Bernard J Dunn Sch of Pharmacy Winchester, VA.

Estrogens, including the most active endogenous estrogen 17 β -estradiol (E₂), have been shown to play an important role in numerous brain functions, including the function of the serotonergic system. Serotonergic neurons, particularly the serotonin transporter (SERT) and serotonin autoreceptors (5-HT_{1A} and 5-HT_{1B}), play a role in the pathophysiology of depression, which is notably more prevalent in females than in males. We used the RN46A cell line, derived from embryonic rat serotonergic raphe cells, to determine the effects of E₂ on SERT and 5-HT_{1B} gene expression. Cells were grown in DMEM/F12 medium with 10% FBS for 24 hrs, followed by serum-free conditions (B27 supplement in DMEM/F12) for the next 24 hrs, after which they were treated with E₂ in the concentration range from 10⁻¹¹ to 10⁻⁹M for 24 hrs. Quantitative real-time PCR with 18S RNA as a normalizer gene was used to compare SERT and 5-HT_{1B} gene expression between treatment groups. E₂ dose-dependently decreased both SERT and 5-HT_{1B} gene expression, with a maximum effect at 10⁻⁹M (~62% control for SERT, ~58% for 5-HT_{1B}). When the cells were treated with E₂ in the presence of "pure" estrogen receptor (ER) antagonist ICI 182,780 (10⁻⁷M) or with ICI 182,780 alone, ICI 182,780 not only failed to block the observed effects of E₂ on SERT and 5-HT_{1B} gene expression, but instead caused significant E₂-like effects on its own. This effect was confirmed when RN46A cells were treated with ICI 182,780 for 24 hrs at a dose range from 10⁻⁹ to 10⁻⁷M, and ICI 182,780 dose-dependently decreased both SERT and 5-HT_{1B} gene expression, with a maximum effect at 10⁻⁷M (~30% control for SERT, ~57% for 5-HT_{1B}). The observed ER agonist effects of ICI 182,780 are most likely mediated through ER β , because RN46A cells, like rat serotonergic neurons *in vivo*, express ER β , but neither ER α nor the progesterone receptor. Interestingly, ICI 182,780 in the same dose range used previously, showed no effect on ER β gene expression (~91% control with 10⁻⁷M treatment for 24 hrs), compared with dose-dependent down-regulation of ER β gene expression caused by E₂ (~53% control with 10⁻⁹M treatment for 24 hrs). ER agonist effects of ICI 182,780 shown both here and in some other neuronal systems, suggest tissue-specific mixed ER agonist/antagonist activity of ICI 182,780. In addition, because RN46A cells express only ER β , but not ER α , they provide a unique cell model system for examining the neuron-specific ER β -mediated effects of ICI 182,780.

Sources of Research Support: West Virginia IDeA Network of Biomedical Research Excellence (WV-INBRE) 2008-2009 Pilot Grant (P2ORR016477), awarded by the National Center for Research Resources.

Nothing to Disclose: NH

P1-12

Improved Nuclear Receptor Binding Assays for HTS by Conversion from FP to TR-FRET Readout.

KW Vogel PhD¹, BD Marks BS¹, KR Kupcho BS¹, KL Vedvik BS¹ and TM Hallis PhD¹.

¹Invitrogen Madison, WI.

Nuclear receptors (NRs) are ligand-dependent transcription factors that are important targets for drug discovery. Out of the top 200 best selling drugs in 2006, those targeting nuclear receptors had sales of over 23.5 billion dollars. Disease areas targeted by these drugs include metabolic disorders such as diabetes and high cholesterol, inflammation disorders such as asthma and allergies, and other endocrine diseases relating to women and men's health. Historically, NR binding assays for high-throughput screening (HTS) have been limited to radioligand binding assays or more recently to fluorescence polarization (FP) assays. However, time-resolved fluorescence resonance energy transfer (TR-FRET) has gained the interest of drug researchers as applied to assays for HTS. This is because TR-FRET has the advantage of resistance to interference from autofluorescent compounds or scattered light from precipitates that can cause false hits. It is also known to provide assays with increased sensitivity. Since an FP assay's ability to resolve potent binders is limited by the affinity of the receptor for the tracer, it is desirable to have tracers with low dissociation constants. This is not an issue for TR-FRET assays, where the sensitivity is more closely tied to the receptor concentration used in the assay and the readout allows for very low receptor concentrations (see Table below). Here, we will show data and discuss the differences in sensitivity for the conversion of currently available FP binding assays for AR, GR, ER alpha and beta, PR, and VDR to the improved TR-FRET format using similar core assay reagents.

Comparison of Optimized FP and TR-FRET Assay Conditions

| Nuclear Receptor | [Receptor] (nM) | | [Tracer] (nM) | | [Antibody] (nM) |
|------------------|-----------------|------------------|---------------|-----|-----------------|
| | TR-FRET | FP | TR-FRET | FP | TR-FRET |
| ER alpha | 0.5 | 15 ^a | 3 | 4.5 | 2 |
| ER beta | 0.3 | 10 ^a | 3 | 4.5 | 2 |
| PR | 0.5 | 40 | 10 | 2 | 5 |
| GR | 0.4 | 4 ^a | 2 | 1 | 2 |
| AR | 1 | 10 | 10 | 2 | 5 |
| VDR | 0.05 | 0.7 ^a | 0.2 | 1 | 5 |

^a Indicates full length receptor used in the FP assay and LBD used in the TR-FRET assay. Others used the LBD for both FP and TR-FRET assays.

Disclosures: KWV: Employee, Life Technologies Corp. BDM: Employee, Life Technologies Corp. KRK: Employee, Life Technologies Corp. KLV: Employee, Life Technologies Corp. TMH: Employee, Life Technologies Corp.

P1-13

Estrogen-Dependent ER-alpha Activation Is Required for Normal Branching Morphogenesis of the Mouse Prostate Gland.

EL Calderon MS¹, KW Sinkevicius PhD², M Laine², GL Greene PhD² and GS Prins PhD¹.

¹Univ of Illinois at Chicago Chicago, IL and ²Univ of Chicago Chicago, IL.

Increasing evidence indicates that estrogens acting through estrogen receptors (ER) play an important role during prostate gland development and abnormal growth associated with prostatic diseases including BPH and cancer. Studies with ER knock-out mice have shown that developmental effects of estrogens on the prostate gland are mediated through ER α and not through ER β (Prins et al, Can Res 2001). Recent studies using ACTB-Cre/ER α -/- mice demonstrated reduced branching morphogenesis of the prostate glands suggesting a critical role for ER α in this process (Chen et al, Endocrinology 2009). ER α actions can be mediated through both estrogen-dependent and estrogen-independent activation, the later effects being driven through growth factor induced phosphorylation of ER α at serine 118. Since multiple growth factor pathways are involved in regulating prostate morphogenesis and differentiation, the present study sought to determine whether estrogen-dependent and/or ligand-independent actions of ER α are involved in prostate gland development. To do so, we examined the prostate glands of male estrogen nonresponsive ER α knock-in (ENERKI) mice. These animals have a point mutation (G525L) in the ligand-binding domain of ER α that interrupts interaction with endogenous estrogens but does not affect growth factor activation of ligand-independent ER α pathways (Sinkevicius, Endocrinology 2008). Prostate lobes from peri-pubertal (day 30) male ENERKI and wt littermates mice were analyzed morphologically and histologically for branching and differentiation endpoints. Branch tip number in microdissected ventral prostates was significantly reduced from 74 ± 4 in wt mice to 58 ± 3 in ENERKI males ($p < 0.05$). While histologic inspection revealed normal epithelial cell differentiation, the lumens were markedly enlarged in ENERKI prostates as compared to wt males. Together these findings suggest that ER α activated by endogenous estrogens is involved in branching morphogenesis and planar cell division controlling circumferential growth of the prostatic ducts.

(1) Prins GS, et al, Cancer Research 2001; 61:6089

(2) Chen M, et al, Endocrinology 2009; 150:251

(3) Sinkevicius KW, et al, Endocrinology 2008; 149:2970

Sources of Research Support: DK40890 and CA89089.

Nothing to Disclose: ELC, KWS, ML, GLG, GSP

P1-14

The Role of WBP1 in Steroid Hormone Receptor Regulation.

AM Saeed BS¹ and Z Nawaz PhD¹.

¹Univ of Miami Miller Sch of Med Miami, FL.

Steroid hormone receptors (SHRs) bind steroid hormones in various target tissues and act as ligand-activated transcription factors, initiating gene transcription in a highly regulated manner. Proper SHR-mediated transcription requires a host of regulatory factors. Among these factors is an ever growing group of proteins known as coactivators. Coactivators enhance transcription by localizing to the SHR-target promoter regions where they aid in mediating chromatin modifications or act as scaffolding proteins to recruit the basal transcriptional machinery. At promoter regions, coactivators assemble into large multi-protein complexes, which contain SHRs and other proteins. Critical to the localization of coactivators into these multi-protein complexes are specific protein-protein interactions. This project investigates a particular protein-protein interaction motif (the PY-motif) and its role in SHR-signaling. The PY-motif binds proteins that contain a specific domain known as the WW-domain. The objective of this study is to identify and characterize a particular PY-motif containing protein called WBP1 (WW-Domain Binding Protein 1) as a novel coactivator of SHRs. Data presented here demonstrates that overexpression of WBP1 enhances SHR-mediated gene transcription for both estrogen and androgen receptors. In contrast, RNAi-mediated knockdown of WBP1 dampens SHR-signaling. Collectively, these data implicate WBP1 as a modulator of SHRs and WBP1 is likely functioning as a coactivator. We also examined whether WBP1 is required for downstream functional consequences of SHR-signaling. Our data indicates that WBP1 is required for both estrogen-dependent cell cycle progression and cell proliferation. Future work will be carried out to determine the precise molecular mechanism by which WBP1 modulates SHR-signaling and cell cycle progression. The results presented here and the future work will bring to light a novel mechanism of SHR-regulation.

Nothing to Disclose: AMS, ZN

P1-15

Phosphorylation of Human Estrogen Receptor-beta at Serine 105 Inhibits Cell Migration and Invasion in Prostate and Breast Cancers.

Hung-ming Lam PhD¹, CV Suresh Babu PhD¹, Yong Yuan PhD¹, Ying-wai Lam PhD¹, Jiang Wang PhD¹, Shuk-mei Ho PhD¹ and Yuet-kin Leung PhD¹.

¹Univ of Cincinnati Cincinnati, OH.

Estrogen receptor-beta (ER-beta) is involved in the progression of prostate and breast cancer. Ligand- or growth factor-mediated phosphorylation triggered ER-beta activation. However, up until now, no direct phosphorylation sites have been identified and validated in the human ER-beta. This study aimed to identify estrogen-regulated phosphorylation sites in the full-length human ER-beta and explore the biological consequences of such phosphorylation in prostate and breast cancer cells.

Three distinct serine phosphorylation sites in the AF-1 of the human ER-beta (S75, S87 and S105) were identified as targets of ERK 1/2- and p38-phosphorylation by mass spectrometry. Phosphorylation at S75 and S105 resulted in augmentation of ER-beta transactivation activity induced by estrogen. Immunohistochemical analyses using S105 phospho-specific antibody revealed that S105-phosphorylated ER-beta was readily detected in both prostate and breast cancer specimens. Endogenous ER-beta phosphorylation at S105 was demonstrated in PC-3 and MDA-MB-231 cells by activation of ERK1/2 and p38 pathways. However, 17beta-estradiol (E2) induced ERK1/2 phosphorylation and subsequent ER-beta phosphorylation at S105 in MDA-MB-231 but not PC-3 cells. In concordance, the phospho-mimetic mutant (S105E) only augmented E2-induced ERE-transactivation in MDA-MB-231 but not in PC-3 cells. Finally, when S105E mutant was overexpressed in PC-3 and MDA-MB-231 cells, cellular migration and invasion were inhibited but cell growth and cell cycle progression remained unchanged.

This study was the first to identify estrogen-regulated phosphorylation sites in the human ER-beta and establish that they are direct targets of ERK1/2 or p38. It also demonstrated a role for such phosphorylation in the inhibition of cancer cell migration and invasion.

Sources of Research Support: ES006096, ES015584, CA15776, CA112532.

Nothing to Disclose: H-ML, CVSB, YY, Y-WL, JW, S-MH, Y-KL

P1-16

Eph-Ephrin Signaling during Folliculogenesis and Its Potential Dysregulation in Estrogen Receptor β -Null Mice.

AV Buensuceso¹ and BJ Deroo PhD^{1,2}.

¹The Univ of Western Ontario London, Canada and ²Lawson Hlth Res Inst London, Canada.

The formation of a preovulatory follicle requires follicle-stimulating hormone (FSH) and intra-ovarian estrogen, which synergize to promote granulosa cell (GC) differentiation. Estrogen receptor β (ER β) is a nuclear transcription factor essential for estrogen signaling in GCs, and a lack of ER β results in incomplete FSH-mediated GC differentiation. Ovulation in ER β -null female mice is impaired, resulting in fewer and smaller litters than wildtype females (1). Previous microarray studies from our laboratory indicated that several genes are regulated by FSH in an ER β -dependent manner in GCs. One of these genes, *Efna5*, encodes a membrane-associated protein (ephrinA5) that interacts with several members of the largest-known family of receptor tyrosine kinases, the Eph receptors. Eph receptors and ephrins have been implicated in numerous physiological processes in both developing and adult tissues, and are overexpressed in some cancers (2). However, little is known about the involvement of the Eph-ephrin system in folliculogenesis and the regulation of this system by ER β . Using qPCR and immunoblot analysis, we observed an increase in *Efna5* expression in GCs isolated from FSH-treated wildtype mice, but not in GCs from FSH-treated ER β -null mice. In addition to *Efna5*, FSH increased mRNA levels of *Epha5*, which encodes an Eph receptor with a high affinity for ephrinA5. Interestingly, FSH induced similar ER β -dependent increases in the expression of three genes that encode Eph receptors with high affinities for ephrinA5, suggesting that FSH coordinately increases compatible ephrin ligand-receptor levels in GCs during the formation of a preovulatory follicle. Similar patterns of FSH-mediated Eph receptor expression were observed in primary GCs treated with FSH in culture. In addition, FSH-mediated increases in the expression of *Efna5* and *Epha5* were detected in the GFSHR-17 rat granulosa cell line. EphrinA5 was localized specifically to cumulus cells by immunofluorescence, while ephrinB1 was detected in both granulosa and thecal cells. These findings suggest the involvement of ephrin signaling in folliculogenesis in mice, and the dysregulation of this signaling system in GCs of ER β -null mice. Consequently, disruption of ephrin signaling downstream of estrogen action may play a role in human infertility conditions.

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(2)Pasquale EB, *Cell* 2008; 133(1):38-52

Nothing to Disclose: AVB, BJD

P1-17

Post-Transcriptional Regulation of Estrogen Receptor Beta Isoforms in Prostate Cancer.

YK Leung PhD¹, MT Lee MPhil¹, J Wang MD PhD¹ and SM Ho PhD¹.

¹Univ of Cincinnati Cincinnati, OH.

Estrogen receptor beta (ER β) isoforms are distinct from ER β 1 in terms of expression level and their potential functions in prostate cancer (PCa). The mechanism of how these isoforms are differentially regulated remains largely unknown. Recent knowledge on ER β promoters, promoter 0N and 0K, may not provide enough information to explain the differences as epigenetic changes on promoter 0N do not seem to silence all ER β isoforms expression and promoter 0K appears to play minimum regulatory role in several in vitro models. In this study, we began with investigating how ER β isoforms are regulated at the transcriptional level. We first analyzed the 5' untranslated regions (UTRs) of all ER β transcripts in order to map all possible promoters for ER β . 5' UTRs derived from promoters 0N and several exon 0K-derived transcripts with differences in size were identified. Such variation in length was due to random insertion of 9 different exon 0Xs between exons 0K and 1. Sequence analyses revealed that multiple translation regulatory elements including upstream open reading frames (uORFs) and internal ribosome entry sites (IRESs) exist on those extended 5'UTRs. To determine the role of these elements on ER β gene expression, we performed site-directed mutagenesis to investigate their functions on luciferase-based reporter assays. Our results showed that the presence of uORFs or IRESs on 5'UTR significantly inhibits or promotes, respectively reporter expression via alteration of protein translation efficiency. In other words, besides regulated by traditional transcriptional events, the expression of ER β and its isoforms are further post-transcriptionally controlled by the existence of exon 0Xs. We then applied nested PCRs to determine the promoter usage of each ER β isoform. Unexpectedly, all ER β isoforms, including ER β 1, were found to be transcribed from the promoter 0K. In order to gain insight onto the clinical relevance of this study, we analyzed the expression of exon 0K in 14 matched pairs of benign and cancer tissues. Not every matched pair shows utilization of exon 0K but it was shown to be preferentially utilized in some cancers when compared to adjacent benign tissues. These findings were consistent with our in situ hybridization study that exon 0K was also detected in both cancer foci as well as in some adjacent normal glands. Our data suggest that ER β and its isoforms are heavily dysregulated in PCa at multiple levels.

Sources of Research Support: Research supported in part by ES006096, ES015584, CA015776, and CA112532.

Nothing to Disclose: YKL, MTL, JW, SMH

P1-18

Selective Activation of Estrogen Receptor β Target Genes by 3,3'-Diindolylmethane.

OI Vivar PhD^{1,2}, EF Saunier PhD², DC Leitman PhD^{1,2}, GL Firestone PhD¹ and LF Bjeldanes PhD¹.

¹UC Berkeley Berkeley, CA and ²UCSF San Francisco, CA.

3,3'-Diindolylmethane (DIM) is a natural compound found in cruciferous vegetables that has anti-proliferative and estrogenic activity. However, it is not clear if the estrogenic effects are mediated through ER α , ER β or both ER subtypes. We investigated whether the estrogenic activities of DIM are ER β -selective. DIM stimulated ER β but not ER α activation of an estrogen response element upstream of the luciferase reporter gene. DIM also selectively activated multiple endogenous genes through ER β . DIM did not bind to ER β , indicating that it activates genes by a ligand-independent mechanism. DIM causes ER β to bind regulatory elements and recruit the SRC-2 coactivator, which leads to the activation of ER target genes. Silencing of SRC-2 inhibited the activation of ER target genes, demonstrating that SRC-2 is required for transcriptional activation by DIM. Our results demonstrate that DIM is new class of ER β -selective compounds, because it does not bind to ER β , but instead it selectively recruits ER β and coactivators to target genes, representing a novel mechanism for achieving receptor selectivity.

Nothing to Disclose: OIV, EFS, DCL, GLF, LFB

P1-19

Repression of Estrogen Receptor β Function by Putative Tumor Suppressor DBC1.

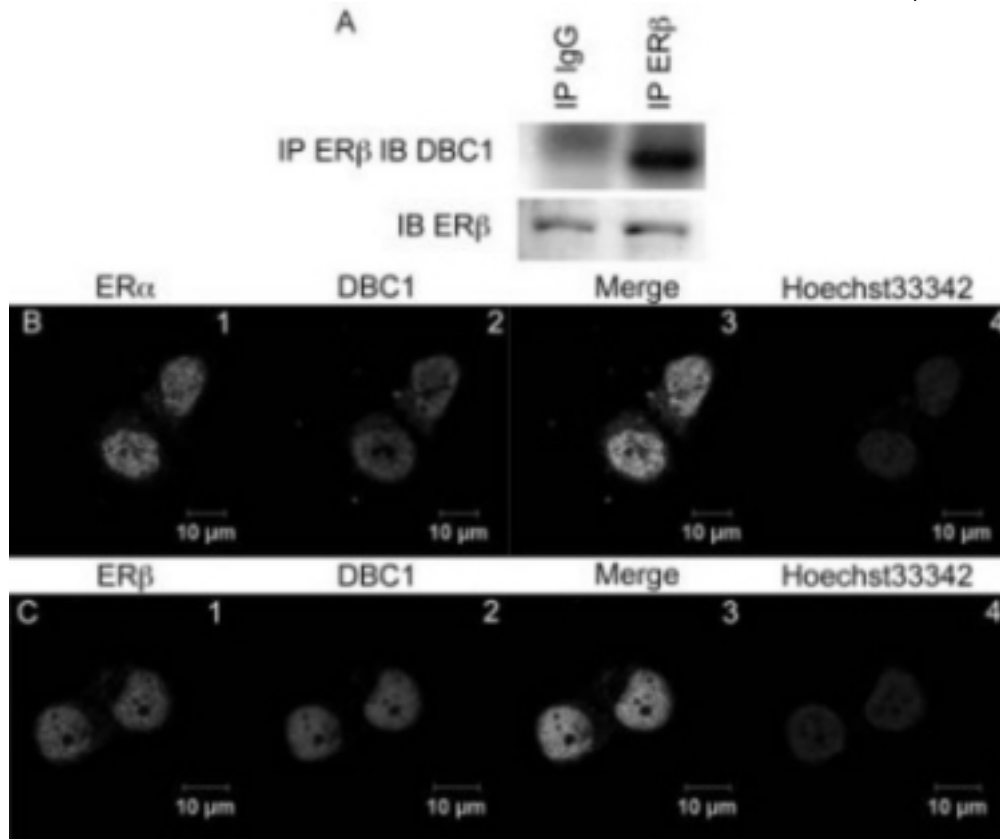
Osamu Hiraike M. D., Ph. D.¹, Satoshi Koyama M. D.¹, Haruko Hiraike M. D.¹, Shunsuke Nakagawa M. D., Ph. D.¹, Yuichiro Miyamoto M. D.¹, Michihiro Tanikawa M. D.¹, Kenbun Sone M. D.¹, Shigeaki Kato Ph. D.², Tetsu Yano M. D., Ph. D.¹ and Yuji Taketani M. D., Ph. D.¹.

¹The Univ of Tokyo Tokyo, Japan and ²Inst of Molecular and Cellular Bioscis, The Univ of Tokyo Tokyo, Japan.

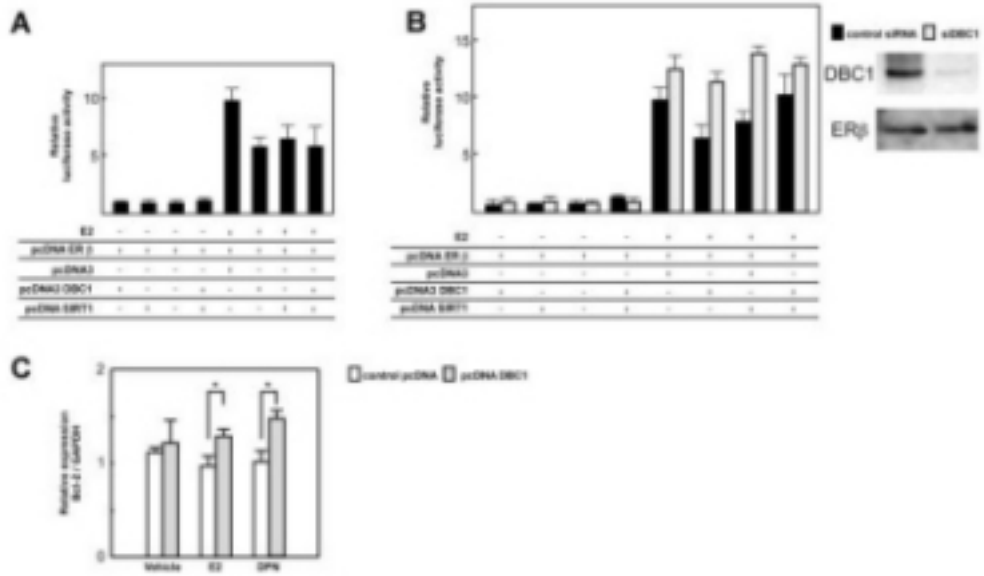
It has been well established that estrogen is involved in the pathophysiology of breast cancer. Estrogen receptor (ER) α appears to promote the proliferation of cancer tissues, while ER β can protect against the mitogenic effect of estrogen in breast tissue. The expression status of ER α and ER β may greatly influence on the development, treatment, and prognosis of breast cancer.

Previous studies have indicated that the deleted in breast cancer 1 (DBC1/KIAA1967) gene product has roles in regulating functions of nuclear receptors. The gene encoding DBC1 is a candidate for tumor suppressor identified by genetic search for breast cancer. Caspase-dependent processing of DBC1 promotes apoptosis, and depletion of the endogenous DBC1 negatively regulates p53-dependent apoptosis through its specific inhibition of SIRT1. In addition, DBC1 modulates ER α expression and promotes breast cancer cell survival by binding to ER α .

Here we report an ER β -specific repressive function of DBC1. Immunoprecipitation and immunofluorescence studies show that ER β and DBC1 interact in a ligand-independent manner similar to ER α . In vitro pull down assays revealed a direct interaction between DBC1 amino-terminus and activation function-1/2 domain of ER β .



Although DBC1 shows no influence on the ligand-dependent transcriptional activation function of ER α , the expression of DBC1 negatively regulates the ligand-dependent transcriptional activation function of ER β in vivo, and RNA interference-mediated depletion of DBC1 stimulates the transactivation function of ER β . These results implicate the principal role of DBC1 in regulating ER β -dependent gene expressions.



Nothing to Disclose: OH, SK, HH, SN, YM, MT, KS, SK, TY, YT

P1-20

Small Molecule Inhibitors of Progesterone Receptor Action in Breast Cancer Cells.

IO Aninye M.S.¹, KC Berg¹, MT Cherian M.S.¹, AR Mollo¹, AL Etheridge¹, PJ Hergenrother Ph.D.¹, EM Wilson Ph.D.², SK Nordeen Ph.D.³ and DJ Shapiro Ph.D.¹.

¹Univ of Illinois at Urbana-Champaign Urbana, IL ; ²Univ of North Carolina Sch of Med Chapel Hill, NC and ³Univ of Colorado Denver Aurora, CO.

The progesterone receptor (PR) acts in part through the binding of liganded PR to DNA and regulation of the expression of progesterone-responsive genes. PR plays vital roles in a diverse set of biological processes, from homeostasis to reproduction. Much has been learned about PR action using progestins and anti-progestins that bind in the receptor's ligand-binding pocket. To identify new probes for PR action that act outside of the ligand-binding pocket, we used high-throughput screening. Analysis of structure-activity relationships for inhibition of PR and estrogen receptor (ER) identified a structural motif that results in preferential inhibition of PR compared to ER. We identified three small molecules that retained their ability to inhibit PR across a range of progesterone concentrations, suggesting that they act outside of the ligand-binding pocket. These small molecules preferentially inhibit PR-mediated induction of the mouse mammary tumor virus (MMTV)-luciferase reporter and endogenous alkaline phosphatase activity in T47D human breast cancer cells and exhibit a much lower ability to inhibit ER, glucocorticoid receptor (GR) and androgen receptor (AR). In vitro, these compounds do not inhibit binding of PR to progesterone response element DNA. This novel class of inhibitors provides a promising start for further optimization and therapeutic development and will be useful in dissecting mechanisms of PR action.

Sources of Research Support: Ruth L. Kirschstein NRSA NIDDK Fellowship awarded to IOA.

Nothing to Disclose: IOA, KCB, MTC, ARM, ALE, PJH, EMW, SKN, DJS

P1-21

Progesterone Receptor Interaction with Signaling Molecules outside the Nucleus Detected in Live Cells by a Bimolecular Fluorescence Complementation (BiFC) Assay.

V Boonyaratanakornkit Ph.D.¹, J Thirugnanasampanthan B.S.¹, Z Songyang Ph.D.¹ and DP Edwards Ph.D.¹.

¹Baylor Coll of Med Houston, TX.

A subpopulation of steroid hormone receptors resides outside the nucleus and is capable of interacting with signaling complexes. These interactions mediate rapid steroid activation of signaling cascades independent of nuclear transcription and play roles in mediating the proliferative and cell survival effects of steroids. Despite several advances in this field, studies are lacking to demonstrate direct interaction of steroid receptors with signaling molecules and to identify subcellular interaction sites in live cells. We have applied a BiFC assay to address these questions for progesterone receptor (PR). The BiFC assay is based on the principle that two non-fluorescence fragments of a fluorescent protein form a stable fluorescent complex when brought together by two interacting proteins fused to the fragments. Thus, BiFC is amenable to detection of transient protein-protein interactions. The N- or C-terminal fragment (YN or YC) of YFP was fused to the N- or C-terminus of PR-B respectively (nYN-PRB or cYC-PR-B). Since c-Src is a well-studied steroid receptor interacting signaling molecule, it was tagged at the C-terminus with YC (cYC-Src). PR and c-Src fusion constructs were transfected into the ER/PR null MCF-7 cells (MCF7C4-12) or 293T cells, and YFP signals were detected by flow cytometry and fluorescence microscopy. No YFP signal was detected in cells expressing either nYN-PRB or cYC-Src alone, whereas cells co-expressing nYN-PRB and cYC-Src conferred a robust fluorescence signal that was minimally affected by progesterone (PG). YFP signals were localized in punctate perinuclear areas of the cytoplasm and at the plasma membrane with no detectable signal in the nucleus. No YFP signal was detected in cells expressing nYN-PR-B containing point mutations in the polyproline motif that mediates binding of PR to c-Src SH3 domain. As further evidence for specificity of the BiFC assay, cells co-expressing YN or YC fused to PR-B, gave an exclusively PG-dependent nuclear YFP signal consistent with homodimerization of PR, a known PG-dependent nuclear event. BiFC screening of a small library of SH3 domain containing genes identified 7 other proteins that interact with PR outside the nucleus including Yes-1, Hck, Fyn, PI-3 kinase-p85 α , Grb2, Crk and Vav1. Interestingly, the localization of YFP signals were distinct for some of these proteins, suggesting that PR is capable of interacting with signaling complexes located at different subcellular sites.

Sources of Research Support: American Cancer Society RSG-0607401-TBE awarded to VB; DOD DAMD W81XWH-08-1-0226 awarded to JT; NIH DK04930 awarded to DPE.

Nothing to Disclose: VB, JT, ZS, DPE

P1-22

Antiprogestin RU486 Stabilizes Serine-294 Phosphorylated Progesterone Receptor.

J Ali-Khan DVM, M.Sc¹, L Amazit PhD¹, A Guiochon-Mantel MD, PhD¹, M Lombes MD, PhD¹ and H Loosfelt Pharm D, PhD¹.

¹INSERM U693 Le Kremlin Bicetre, France.

The human progesterone receptor (PR), a ligand-inducible transcription factor, exists as two isoforms PRA and PRB. Transcriptional regulation of PR target genes by agonist ligands is coupled to PR down-regulation via proteasome pathway. Both of these phenomena are functionally linked to phosphorylation of PR on serine 294 by agonist ligands. Paradoxically, PR stabilization through inhibition of proteasome dramatically decreases PR activity. Antiprogestins act essentially by modifying PR interactions with coactivators/corepressors, however, their impact on PR stability/turnover is not clear. We investigated the effects of antiprogestin RU486 (mifepristone) in this particular context, using uterine and breast cellular models expressing either PRA or PRB. Phosphorylation on serine 294, transcriptional activation and subsequent degradation of both isoforms were observed after agonist R5020 treatment. However, RU486 also induced rapid (15 min) but surprisingly, stable (up to 24h) phosphorylation of PR on serine 294 independently of ERK1/2 MAPK activities. In contrast to R5020, RU486 stabilized PRA and PRB expression through ERK1/2 MAPK dependent mechanism as RU486 induced PRB degradation in the presence of specific MEK1/2 inhibitor, U0126. Transient transfections of ubiquitin expression plasmid accelerated the degradation of PRB in the absence or presence of R5020. However, under similar conditions, PRB levels did not decrease in RU486 treated cells suggesting that PR-RU486 complex might be insensitive to ubiquitinylation and degradation. In sum, both PR agonist R5020 and antagonist RU486 induce serine 294 PR phosphorylation through a MAPK-independent pathway. An additional phosphorylation step dependent of MAPK activity impedes degradation of phosphorylated-Ser294 PR-RU486 complex. This unknown phosphorylation event possibly results in the inhibition of PR interaction with ubiquitin ligase factors required to direct PR towards degradation. Our results reveal that RU486 acts through a major mechanism consisting in the repression of PR degradation that might impair the cycling and renewal of PR-coregulators complex on target gene promoter.

Sources of Research Support: Inserm, University Paris-Sud, Doctoral Scholarship from Higher Education Commission, Pakistan (to JAK) and the Association pour la Recherche contre le Cancer (ARC).

Nothing to Disclose: JA-K, LA, AG-M, ML, HL

P1-23

Progestins Induce Breast Cancer Cell Proliferation through the AP-1 Transcription Factor.

MC Diaz Flaque Msc¹, W Beguelin Msc¹, CJ Proietti PhD¹, MA Rivas Msc¹, M Tkach Msc¹, EH Charreau PhD¹, R Schillaci PhD¹ and PV Elizalde PhD¹.

¹Inst de Biol y Med Experimental Buenos Aires, Argentina.

Breast cancer is a major worldwide health problem that affects a significant number of women. Accumulating evidence has shown the involvement of the progesterone receptor (PR) in breast cancer development. In its classical mechanism of action, PR acts as a ligand-induced nuclear transcription factor. In addition to its direct transcriptional effects, PR activates signal transduction pathways in breast cancer cells through a rapid or nongenomic mechanism, which results in cell proliferation. Interestingly, progestins induce the expression of key regulators of cell cycle progression which do not contain a classical progesterone response element (PRE) in their promoters, such as cyclin D1. We and others have also shown that progestins are able to induce cyclin D1 expression in breast cancer cells. Our present findings demonstrate that the synthetic progestin medroxyprogesterone acetate (MPA) is able to induce an increase in the levels of c-jun and c-fos phosphorylation and AP-1 transcriptional activation. The transcription factor AP-1 consists of two family members Fos and Jun. AP-1 binds to specific DNA sequences called TPA response elements (TRE) in their regulatory regions. Our chromatin immunoprecipitation (ChIP) assays demonstrated that progestin stimulates c-jun, c-fos, and PR recruitment to the TRE site of the cyclin D1 promoter. The simultaneous binding of the three proteins to the cyclin D1 promoter upon progestin stimulation was shown by using sequential ChIP assays. These data identify, for the first time, the interaction between AP-1 and PR regulating cyclin D1 transcription by tethering to DNA-bound at TRE site. Furthermore, we found that the inhibition of c-fos and c-jun activation by the use of dominant negative forms of these proteins (A-Fos and TAM-67 respectively) completely blocked progestin-induced cyclin D1 expression and *in vitro* breast cancer cell growth. Finally, we addressed the effect of targeting AP-1 in *in vivo* MPA-dependent growth of C4HD progestin-dependent murine mammary tumor. Transfection of C4HD cells with TAM-67 or A-Fos DN expression vectors significantly inhibited these cells' ability to form tumors in syngeneic mice when compared to empty vector-transfected C4HD cells. This study enhances our current understanding of how progestins induce AP-1 factors function and PR association in breast cancer cells and provide a solid rationale for study selective AP-1 inhibitors for the treatment and prevention of breast cancer.

Nothing to Disclose: MCDF, WB, CJP, MAR, MT, EHC, RS, PVE

P1-24

A Novel Point Mutation in the Helix 10 of the Human Glucocorticoid Receptor Causes Generalized Glucocorticoid Resistance by Disrupting the Structure of the Ligand-Binding Domain.

N Nader PhD¹, BE Bachrach MD², DE Hurt PhD³, S Gajula MD², A Pittman MD², R Lescher MD² and T Kino MD, PhD¹.

¹Eunice Kennedy Shriver Natl Inst of Child Hlth and Human Development Bethesda, MD ; ²Univ of Missouri Children's Hosp Columbia, MO and ³Natl Inst of Allergy and Infectious Diseases Bethesda, MD.

Generalized glucocorticoid resistance syndrome is a rare, familial or sporadic condition characterized by partial insensitivity to glucocorticoids, caused by mutations in the glucocorticoid receptor (GR) gene. Most of the reported cases are adults, demonstrating symptoms associated with mineralocorticoid and/or adrenal androgen excess caused by compensatively increased secretion of the adrenocorticotrophic hormone. We identified a new 2-year old female case of generalized glucocorticoid resistance syndrome. The patient (TJ) presented with a generalized seizure associated with hypoglycemia and hypokalemia. She also had hypertension, premature pubarche and accelerated bone age, while dexamethasone effectively suppressed these clinical manifestations. The patient's GR gene had a heterozygotic mutation (G → A) at nucleotide position 2141 (exon 8), which resulted in substitution of arginine (R) by glutamine (Q) at amino acid position 714 in the ligand-binding domain (LBD) of the GR α . Molecular analysis revealed that the mutant receptor (GR α R714Q) had significantly impaired transactivation activity shifting the dexamethasone titration curve rightward, and demonstrated dominant negative activity to the wild type GR α -induced transactivation of a glucocorticoid-responsive promoter. GR α R714Q showed a 2-fold reduction in affinity to dexamethasone, and had attenuated transactivation activity of the activation function (AF)-2 and reduced binding to a p160 nuclear receptor coactivator. Computer-based structural analysis revealed that replacement of arginine by glutamine at position 714 broke its salt bridge with glutamic acid (E) at position 662, while the negative charge of this glutamic acid was instead balanced by the formation of a stable new salt bridge with R704 of helix 9. This change induced a 3.0 Å displacement of R704 in the mutant receptor and released helix 10 from its former salt bridge constraint, which lead to further conformational changes in the ligand-binding pocket and in the AF-2 transactivation surface, consequently decreasing binding affinity of the mutant receptor to ligand and to the LXXLL coactivator motif. Taken together, dexamethasone treatment was effective to control the premature pubarche, hypoglycemia, hypertension and hypokalemia in this child case, wherein arginine 714 played a key role in the proper formation of the ligand-binding pocket and the AF-2 surface of the GR α LBD.

Nothing to Disclose: NN, BEB, DEH, SG, AP, RL, TK

P1-25

Glucocorticoid Ultradian Rhythmicity Directs Cyclical Gene Pulsing of the Hippocampal CLOCK Gene Period 1.

BL Conway-Campbell¹, RA Sarabdjitsingh², MA McKenna¹, JR Pooley¹, JA Douthwaite¹, YM Kershaw¹, OC Meijer², ER deKloet² and SL Lightman¹.

¹Univ of Bristol Bristol, UK and ²Leiden Univ Med Ctr Leiden, Netherlands.

In vivo glucocorticoid (GC) secretion follows a distinctive ultradian pattern, the functional significance of which is presently unknown (1). GCs act on target cells via intracellular glucocorticoid receptors (GRs) which bind to DNA at specific sequences termed glucocorticoid response elements (GREs) to modulate transcription (2). Notably, GR is widely expressed in brain, including hippocampus where GCs are thought to regulate memory and learning processes (3). Previously we have shown, in cell lines and in rat liver, that Period 1 gene transcription precisely tracks the individual GC pulses (4). Now we interrogate whether the same phenomenon occurs in brain, in particular the hippocampus. We hypothesize that GC pulses will regulate transcriptional pulsing of Period 1 in hippocampal neurons, thus maintaining a rapidly responsive system able to react to both the onset and the decline of the circadian active phase.

We report that corticosterone pulses drive transient hippocampal GR nuclear localization, detected with confocal microscopy. We show concomitant GR activation and DNA binding, determined by synthetic GRE oligonucleotide binding and verified for the Period 1 promoter region by chromatin immunoprecipitation assays. Each pulse induces "bursts" of transcription of the Period 1 gene, measured by hnRNA qPCR. The net effect of pulsatile GC exposure on accumulation of the mature *Per1* transcript was also assessed, revealing a plateau of *Per1* mRNA levels throughout the timecourse of pulsatile exposure. After the last pulse was applied, the timecourse was continued for 2 hours to simulate the approach of the animal's daily rest phase. Here, the mRNA levels dropped rapidly to baseline, indicating a relatively short half-life for hippocampal *Per1*.

The ultradian pulse timing appears to work perfectly for the optimal steady-state expression of *Per1* throughout the active phase, diminishing rapidly at the inactive phase. Any perturbation in the frequency of the corticosterone pulses, described in chronic inflammatory disease (5) and major recurrent depression (6, 7); or change in the duration of GR activation during synthetic GC treatment (4) would potentially have dramatic effects of the levels of *Per1* (8) in the hippocampus, and may therefore exert subsequent effects on memory and learning related brain function.

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Sources of Research Support: Wellcome Trust Programme Grant (074112/Z/04/Z) to S.L. The qPCR was performed on an Applied Biosystems 7500 System funded by a Wellcome Trust Equipment Grant (075548/Z/04/Z).

Nothing to Disclose: BLC-C, RAS, MAM, JRP, JAD, YMK, OCM, ERd, SLL

P1-26

The Human Glucocorticoid Receptor β Single Nucleotide Polymorphism GR9 β A3669G Alters Gene Expression.

CM Jewell¹ and JA Cidlowski PhD¹.

¹Natl Inst of Environmental Hlth Scis/NIH Research Triangle Park, NC.

Glucocorticoids are primary stress response hormones that are produced and released from the adrenal cortex. These hormones exert many actions through their cognate receptor, the glucocorticoid receptor (GR). Glucocorticoids can modulate gene expression and are often used to treat immunological and inflammatory diseases. However, not all patients respond equally to glucocorticoid treatment. This has led to the classification of patients as steroid sensitive or steroid resistant, and in turn, to the identification of single nucleotide polymorphisms (SNPs) in the GR gene. One SNP that has been linked to glucocorticoid resistance and coronary artery disease is located in the 3'UTR of the alternatively spliced GR isoform, GR β . This GR β SNP, A3669G in exon 9 β , alters an ATTTA motif which is known to destabilize GR mRNA. *In vitro* assays performed in our laboratory demonstrated that that GR9 β A3669G (GTTTA instead of ATTTA) stabilized the GR β mRNA leading to increased GR β mRNA and protein expression. This increased expression of the GR β isoform may result in inhibition of GR α transcriptional activity and/or change the expression pattern of genes that are regulated by GR β . The GR9 β A3669G SNP has been extensively studied in the clinical setting; however, gene regulation by GR9 β A3669G has not been reported. To determine if there are any differences in gene regulation by GR9 β A3669G, we first generated U-2 OS (human osteosarcoma) stable cell lines expressing either GR β 3'UTR or GR9 β A3669G. Expression of the GR9 β A3669G SNP in the U-2 OS cells stabilized the mRNA and led to increased GR β mRNA and protein expression supporting our previous observations. Utilizing a list of genes that were previously determined to be regulated by GR β and also implicated in cardiovascular disease, we compared the GR β 3'UTR cell line to the GR9 β A3669G cell line using quantitative RT-PCR. This analysis revealed differential regulation of genes by the GR9 β A3669G SNP when compared to the GR β 3'UTR wild type. These genes included interleukin 1-beta (IL1B) an important mediator of the inflammatory response and vascular endothelial growth factor C (VEGFC) which is active in angiogenesis. Genome-wide analysis was employed to identify genes that are activated or repressed by this receptor variant. Together, these studies should provide a molecular understanding for the role of this hGR β polymorphism in human disease.

Nothing to Disclose: CMJ, JAC

P1-27

Anti-Apoptotic Actions of Glucocorticoid in Cardiomyocytes.

R Ren MD, PhD¹ and JA Cidlowski PhD¹.

¹Natl Inst of Environmental Hlth Scis Research Triangle Park, NC.

Apoptosis contributes to the death of cardiomyocytes under several pathological conditions including heart failure, cardiomyopathy, and ischemia/reperfusion. Glucocorticoids have been recognized to have either pro- or anti-apoptotic activity in a cell-type dependent fashion. To explore the role of glucocorticoid signaling in apoptosis of cardiomyocytes, we utilized rat embryonic cardiomyocytes (H9C2 cells) as a model system. Apoptosis was induced in these cells by serum deprivation alone or with the potent apoptotic stimulator Tumor necrosis factor alpha (TNF α) for 24 or 48 hours. Apoptosis of cells was measured by western blot assessment of cleaved caspases and by flow cytometric analysis of propidium iodide staining. Dexamethasone treatment caused a significant decrease in the percentage of apoptotic cells following either serum starvation or TNF α treatment. Interestingly, dexamethasone induced anti-apoptotic gene Bcl-xl at both mRNA and protein levels. The glucocorticoid antagonist RU486 alone did not alter apoptosis but inhibited the anti-apoptotic effects induced by dexamethasone. To verify the necessity of the glucocorticoid receptor in this anti-apoptotic process, H9C2 cardiomyocytes were targeted for the stable knock-down of glucocorticoid receptor by a lentiviral-derived ShRNA system. In contrast to the non-specific knock-down controls, cells depleted of glucocorticoid receptor had increased apoptosis with either serum starvation alone or TNF α cotreatment. Moreover, these cells also failed to show an anti-apoptotic effect and the upregulation of Bcl-xl in response to dexamethasone. Together, these data indicate an essential role for the glucocorticoid receptor in dexamethasone-dependent cardiomyocyte survival and implicate the glucocorticoid signaling pathway as a potential therapeutic target in ischemic heart diseases.

Nothing to Disclose: RR, JAC

P1-28

HDAC Inhibitors and Their Effects on GR-Mediated Hepatic Gene Expression.

V Kadiyala B.Pharm¹ and CL Smith PhD¹.

¹Univ of Arizona Tucson, AZ.

Histone deacetylases (HDACs) associate with glucocorticoid receptor (GR) in various transcription complexes and are involved in the activation and or repression of gene expression. Valproic acid (VPA) is a HDAC inhibitor (HDI) used in the treatment of epilepsy, bipolar disorder, migraine and other mood disorders. The long term usage of VPA results in certain metabolic disorders such as weight gain, fatty liver disease, dyslipidemia and hyperinsulinemia. These are similar to the symptoms of excess secretion of glucocorticoids in the liver, raising the possibility that GR requires HDAC activity to properly regulate expression of metabolic genes. The aim of this study is to determine how HDACs and GR cooperate to regulate transcription, which in turn might also reveal the mechanisms by which VPA causes metabolic disequilibrium.

Our first goal was to determine if HDIs affect the GR-regulated hepatic gene expression. We have up to date performed real time polymerase chain reaction (RT-PCR) on a number of hepatic GR target genes and have found that most of them are affected by VPA. They can be classified into four groups: 1.) Genes at which glucocorticoid-induced activation is impaired by HDAC inhibition (AmpD3, Sgk1, Tgm2 Glu1) 2.) Genes at which glucocorticoids and VPA have an additive effect (MT2). 3.) Genes at which VPA mimicked glucocorticoids (Per1, Suox1, Edn1). 4.) Genes at which HDAC inhibition has no effect on regulation by glucocorticoids (Lcn2). This data suggests that HDACs affect GR-regulated gene expression in a variety of ways. Our next goal was to determine whether the effects of HDIs on GR-regulated hepatic gene expression were due to HDAC involvement in GR-regulated transcription or GR processing with chaperones. By performing immunoprecipitation assays, we have found that VPA does not disrupt GR-HSP90 interaction, strongly indicating that the effects of HDAC inhibition on GR-regulated gene expression cannot be explained by defective GR processing. Studies to determine the mechanism by which GR and HDACs cooperate to induce transcription are ongoing. Our work shows that inhibition of HDACs has a significant impact on glucocorticoid signaling in the hepatic environment, most likely due to direct effects on transcription.

Sources of Research Support: South West Environmental Health Sciences Center(SWEHSC), Arizona Biomedical Research Commission(ABRC), BIO5 Institute.

Nothing to Disclose: VK, CLS

P1-29

Inhibition of Neural Progenitor Cell Proliferation and Gap Junction Intercellular Communication Occurs upon Rapid and Transient Activation of the Glucocorticoid Receptor.

R Samarasinghe BS¹, M Lewis BS² and DB DeFranco PhD^{1,2}.

¹Univ of Pittsburgh Pittsburgh, PA and ²Univ of Pittsburgh Sch of Med Pittsburgh, PA.

Hypothesis: Glucocorticoids (GCs) are widely used to treat pregnant women at risk for preterm delivery given their beneficial effects on neonatal pulmonary function and in the treatment of fetuses with congenital adrenal hyperplasia. However, exposure of fetuses to GCs during development may trigger delayed, adverse neurological side effects mediated in part by reduced neural progenitor cell (NPC) proliferation. While many established cell cycle regulators impact NPC proliferation, other signaling molecules such as the gap junction proteins connexin 43 (Cx43) and connexin 26 (Cx26) appear to also influence proliferation although their precise role and mechanism of regulation remain unresolved. Gap junction function is influenced by GCs in some cells, but hormone effects on this form of intercellular communication in NPCs and resulting functional consequences have not been examined.

Methods: Tertiary neurospheres derived from embryonic (E14) mouse cortices were subject to a 1h exposure to the glucocorticoid receptor (GR) agonist dexamethasone (DEX) and/or the GR antagonist RU486. Whole cell lysates were subject to Western blot analysis to detect Cx43, phospho-Cx43, and total Cx26 protein. Cell proliferation was assayed using BrdU incorporation and Ki67 staining. Cells were also subject to live, single cell, fluorescence recovery after photobleaching (FRAP) with the gap junction permeable dye, Calcein AM, to measure gap junction intercellular communication (GJIC).

Results: Treatment of intact neurospheres with Dex for 1 hr led to increased Cx43 phosphorylation, decreased GJIC and a reduction in NPC proliferation. These effects of Dex were block by a continuous co-administration with RU486. Interestingly, a 1 hr delay in the addition of RU486 following Dex exposure did not reverse the effects of hormone, suggesting that rapid, transient actions of GR are responsible for Dex-dependent inhibition of NPC proliferation and GJIC. **Conclusion:** These findings indicate unique, potentially non-genomic, effects of GCs on regulation of GJIC and subsequent proliferation in NPCs. In addition, these findings suggest that a transient exposure to GCs that limits GJIC, most likely through increased Cx43 phosphorylation, may be sufficient to trigger decreased NPC proliferation.

Sources of Research Support: NIH Grant DK078394 to DBD.

Nothing to Disclose: RS, ML, DBD

P1-30

Increased MR Activity in Aged Rat VSMCs Induces a Proinflammatory Phenotype.

AW Krug MD¹, L Allenhoefer¹, R Monticone¹, G Spinetti MD¹, M Gekle MD², M Wang MD¹ and E Lakatta MD¹.

¹Natl Inst on Aging Baltimore, MD and ²Martin-Luther-Univ of Halle-Wittenberg Halle-Wittenberg, Germany.

Arterial aging is a predominant risk factor for the onset of cardiovascular disease such as hypertension or myocardial infarction, and aging is associated with increased vascular stiffness, intravascular RAS activation and a proinflammatory phenotype. We hypothesized that mineralocorticoid receptor activation and aldosterone might contribute to and possibly accelerate the arterial aging process.

MR expression is increased in whole aortae and early passage aortic vascular smooth muscle cells (VSMCs) from aged (30 months) compared to adult (8 months) F344XBN rats. MR blockade and ERK1/2 MAPK inhibition prevent age-associated increase of proinflammatory marker proteins TGF- β , ICAM-1 and pro-collagen-1. Aldosterone increases expression of proinflammatory marker proteins, shifting the phenotype of adult VSMCs towards the proinflammatory phenotype of aged rats. Moreover, expression of epidermal growth factor receptor (EGFR), a crucial factor in aldosterone and angiotensin II-induced vascular damage, is increased with age and by aldosterone, and inhibition of EGFR tyrosine kinase decreases age-associated proinflammatory marker expression.

In summary, our data support the hypothesis that increased constitutive MR signalling may promote and amplify age-associated inflammation that accompanies arterial aging through increased MR and EGFR expression.

Nothing to Disclose: AWK, LA, RM, GS, MG, MW, EL

P1-31

The Mineralocorticoid Receptor: Exploring Ligand-Specificity Using Peptide Phage Display.

J Yang¹, CD Clyne¹, R Safi², CY Chang², PJ Fuller¹, DP McDonnell² and MJ Young¹.

¹Prince Henry's Inst of Med Res Clayton, Australia and ²Duke Univ Med Ctr Durham, NC.

Introduction

The mineralocorticoid receptor (MR) is a member of the nuclear receptor superfamily. Pathological activation of the MR causes cardiac fibrosis and heart failure, but clinical use of MR antagonists is limited by the renal side effect of hyperkalemia. The glucocorticoid cortisol binds the MR with equivalent affinity to that of the mineralocorticoids aldosterone and deoxycorticosterone. In non-epithelial tissues including the myocardium which do not express the cortisol-inactivating enzyme 11 β hydroxysteroid dehydrogenase 2, cortisol has been implicated in the activation of MR. The mechanisms for ligand- and tissue-specific actions of the MR are undefined. Over the past decade, it has become clear that coregulator proteins are critical for nuclear receptor mediated gene expression. A subset of these coregulators may confer specificity to MR-mediated responses.

Hypothesis

We hypothesize that different physiological ligands can induce distinct MR conformations to allow differential coregulator recruitment and ligand-specific gene expression.

Methods

We used phage display to screen 10¹⁰ 19mer peptides for their interaction with the MR in the presence of aldosterone, cortisol or deoxycorticosterone. The isolated peptides (n = 165) were assessed for a functional interaction with the MR in mammalian two-hybrid and transactivation assays in CV-1 cells. The peptides which interacted with the MR in a ligand-inducible manner were further evaluated for their ability to bind other nuclear receptors.

Results

We identified MR-interacting peptides with partial selectivity for aldosterone and cortisol in mammalian two-hybrid assays, as well as a unique motif, MPxLxxLL, in peptides that strongly bind the MR. However none of the peptides isolated to date showed ligand-selective inhibition of MR transactivation. We also demonstrated that two peptides were selective for the MR, displaying minimal interaction with other nuclear receptors.

Conclusions

Identification of different cohorts of peptides for each ligand supports the concept of ligand-specific MR conformations and provides proof of concept that phage display can identify ligand-specific MR-interacting partners. The ultimate goal is to identify cohorts of ligand- and tissue-specific MR binding proteins which may be utilized in the rational design of a selective MR modulator for the treatment of heart failure without side effects such as hyperkalemia.

Nothing to Disclose: JY, CDC, RS, CYC, PJF, DPM, MJY

P1-32

A Physiological Role for Non-Genomic Androgen Actions in the Absence of Androgen Receptor DNA Binding Activity.

TPS Pang BSc(Hons)¹, RA Davey PhD¹ and HE MacLean PhD¹.

¹Univ of Melbourne Heidelberg, Australia.

We are investigating the role of non-genomic (non-DNA binding-dependent) androgen receptor (AR) actions using our genomic-AR knockout (ARKO) male mice, which have an in-frame deletion of the 2nd zinc finger of the DNA binding domain, causing a sex-reversed female phenotype. Using cultured fibroblasts from genomic-ARKO and wildtype (WT) males, we demonstrated that the mutant AR has normal ligand binding affinity (ARKO vs WT K_d : 0.90 ± 0.36 vs 0.67 ± 0.36 nM (mean \pm SEM)), but reduced nuclear localization. 10 nM DHT treatment increased ERK-1/2 phosphorylation after 1 min in both WT and ARKO fibroblasts (25% increase in phosphorylated:total ERK in DHT vs vehicle, $p < 0.05$), which was blocked by the AR antagonist bicalutamide. In genomic-ARKO mice, AR mRNA and protein were expressed at normal levels in kidney, adipose tissue and bone, and higher than normal levels in testis and skeletal muscle ($p < 0.05$). The genomic AR target gene *Odc1* (1) had 5.6-fold lower expression in genomic-ARKO kidney vs WT ($p < 0.001$), confirming the abolition of genomic-AR actions. Expression of the non-genomic target gene *Mmp-13*, which is transrepressed by the AR via indirect tethering of the Ets-related transcription factor (2), was normal in genomic-ARKO bone; but another non-genomic target, *Ngfr* (3), repressed via AP-1 tethering, was up-regulated 57-fold in testis vs WT ($p < 0.001$). Baseline ERK-1/2 was normal in genomic-ARKO males, but CREB phosphorylation was increased 3.3-fold in ARKO adipose tissue ($p < 0.05$) and Akt phosphorylation increased 1.5-fold in muscle ($p < 0.05$). Genomic-ARKO males aged 3 weeks were treated for 6 weeks with subcutaneous implants containing either testosterone or the non-aromatizable androgen DHT. Androgen treatment had no effect on body weight, testis, kidney, spleen, heart or skeletal muscle mass, all of which are dependent on genomic AR signaling. However, DHT but not testosterone treatment suppressed cortical bone growth (6% decrease in periosteal and 7% decrease medullary circumference vs untreated genomic-ARKOs, $p < 0.05$), opposing the known genomic actions of androgens in bone (4). We are currently investigating the effects of combined orchidectomy and androgen treatment in genomic-ARKO mice. In conclusion, AR lacking the DNA-binding domain can activate non-genomic signaling pathways including ERK-1/2 phosphorylation and target gene transrepression, and these non-genomic androgen actions oppose the genomic action that stimulates bone formation *in vivo*.

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Sources of Research Support: National Health & Medical Research Council (Australia) Project Grant, Endocrine Society of Australia Postgraduate Scholarship (TPSP).

Nothing to Disclose: TPSP, RAD, HEM

P1-33

Mutations in the PPAR- γ DNA Binding Domain Have Differential Effects on Adipocyte Development and Gene Expression.

A R Sidhaye MD¹ and F E Wondisford MD¹.

¹Johns Hopkins Univ Sch of Med Baltimore, MD.

Studies of human patients with partial lipodystrophy bearing mutations in the DNA-binding domain (DBD) of PPAR- γ suggest that PPAR- γ DNA binding is critical for the development of adipocytes. PPAR- γ DBD mutants are reported to affect the adipocyte by either a dominant-negative or haplo-insufficiency mechanism. Our laboratories are interested in understanding the role of the PPAR- γ -DBD in gene regulation. First, we tested whether two mutations in the first zinc finger of PPAR- γ (γ CS and γ GS) could support differentiation of fibroblasts into fat cells. Using a retroviral system we transduced flag-tagged γ CS, γ GS, and wild-type PPAR γ 2 (γ wt) transgenes into PPAR- γ $-/-$ or $+/-$ MEFs and compared these cells to cells transduced with control virus (expressing GFP, MSCV-EV). Introduction of γ CS or γ GS into either MEF type did not allow for differentiation as demonstrated by the absence of oil-red-O staining. Furthermore, while γ wt expressing cells had dramatic increases in the expression of genes specific for adipocyte phenotype (*Fatp4*, *Lpl*, *Scd1*), such increases were not apparent in cells over-expressing γ CS or γ GS. We verified equivalent expression of the transgenes by western blotting for the flag epitope. Further, using ChIP assay we determined that the mutant PPAR constructs had negligible binding to the known *Fatp4* and *Lpl* PPREs as compared to γ wt. Next, we were interested to know whether the PPAR- γ mutants would have a dominant negative effect in a homologous cell system. Thus we over-expressed γ CS and γ GS in 3T3-L1 pre-adipocytes. Using semi-quantitative western blots we verified that cells transduced with the transgene expressed \sim 2-3X more PPAR- γ . Differentiation of 3T3-L1s was not affected. Second, as compared to cells transduced with EV, the PPAR- γ mutants had negligible effects on the gene expression of several genes except for one, *Ob*, where the baseline level of gene expression was altered. Interestingly, the baseline level of *Ob* was increased (3-4X) in cells over-expressing γ CS but slightly reduced in cells expressing γ GS. Rosiglitazone induced down- or up-regulation was generally maintained for all genes. In summary, while an intact first zinger of the PPAR- γ -DBD is required for fat cell differentiation, mutations in this domain have differential effects on gene expression. Whether this is due to difference in DNA-binding selectivity or due to other potential structural changes in PPAR- γ requires further study.

Nothing to Disclose: ARS, FEW

P1-34

Homeostatic Levels of SRC-2 and SRC-3 Promote Early Human Adipogenesis.

SM Hartig Ph.D.¹, B He Ph.D.¹, BM Buerher Ph.D.² and MA Mancini Ph.D.¹.

¹Baylor Coll of Med Houston, TX and ²Zen-Bio, Inc Research Triangle Park, NC.

Adipocyte maturation is driven by the hormonal induction of the nuclear receptor (NR) PPAR γ (PPAR). Recent studies have shown that both the p160 coregulators (CoRs) SRC-2 and SRC-3 coordinate a circuit of interacting factors to drive adipogenesis. While the roles of PPAR and SRC-2/-3 are established at the level of cell-free and organismal models, little information exists that describes their role at the level of individual cells. Consequently, we have utilized a customized, image-based approach to quantify links between PPAR and CoRs with changes in early human adipogenesis using lipid accumulation as the central variable. This high throughput approach established 4-fold and 9-fold increases in mean nuclear PPAR and lipid accretion, respectively, without changes in SRC-2 or -3 during the first 96h of hormonal induction. Importantly, cell-by-cell analysis of PPAR and lipid indicated a highly heterogeneous differentiation response after hormonal induction. Routinely, PPAR and lipid varied 200- and 1000-fold, respectively, during early (<96h) differentiation. Consistent with SRC-2/-3 levels detected by qPCR and imaging, simultaneous detection of SRC-2/-3 or PPAR indicated that cells exhibiting a highly differentiated state occurred without changes in p160s within 4 distinct subpopulations: PPAR^{hi}/Lipid^{hi}, PPAR^{hi}/Lipid^{lo}, PPAR^{lo}/Lipid^{hi}, PPAR^{lo}/Lipid^{lo}. Hence, we hypothesized that SRC-2/-3 comprised an adipogenic setpoint. To probe this setpoint, siRNA was used to downregulate SRC-2 and/or -3 while simultaneously monitoring SRC-2/-3 and PPAR. p160 knockdowns (individually or jointly) inhibited lipid accumulation by preventing the engagement of lipogenic genes, but did not effect PPAR protein levels. Surprisingly, SRC-2/-3 knockdown increased the proportion of cells in a PPAR^{hi}/lipid^{lo} state (+19% after dual SRC-2/-3 siRNA). We then postulated that this population evolved through increased PPAR phosphorylation at S114, found previously to inhibit PPAR-dependent gene transcription and block adipogenesis. In agreement with this hypothesis, p160 depletion decreased adipogenesis by synergistically increasing the level of phospho-PPAR. Together these studies demonstrate that SRC-2 and SRC-3 play non-redundant and uniquely essential roles in the promotion of lipogenesis by attenuating phosphoPPAR at S114. Further, and a less appreciated component of adipogenesis, SRC-2/-3 can modulate PPAR heterogeneity during the early phases of adipocyte differentiation.

Nothing to Disclose: SMH, BH, BMB, MAM

P1-35

Gene Expression Profile of Nuclear Receptors and Coregulators in Adipose Tissues in Morbid Obesity and after Profound Fat Loss.

T Hoang PhD^{1,2}, SN Dankel^{1,2}, DJ Fadnes^{2,3}, VL Veum^{1,2}, V Vage³, VM Steen^{1,2}, JV Sagen^{1,2} and G Mellgren MD, PhD^{1,2}.

¹Univ of Bergen Bergen, Norway ; ²Haukeland Univ Hosp Bergen, Norway and ³Førde Central Hosp Førde, Norway.

Nuclear receptors (NRs) and coregulators (coactivators and corepressors) are transcriptional regulators that play key roles in metabolism. Obesity is characterised by increased fat mass and a significant change in the metabolic profile. The condition also confers substantial risk of developing cardiovascular and metabolic disorders. To gain insight into the functional importance of the nuclear receptor-signalling network in human obesity, we compared the gene expression profiles of NRs and associated coregulators in subcutaneous adipose tissue from obese patients (n=16) before (average BMI = 53.3 kg/m²) and one year after (average BMI = 33.1 kg/m²) bariatric surgery (biliopancreatic diversion with duodenal switch, BPD/DS), and from healthy control individuals (n=13, average BMI = 23.0 kg/m²). To this end we performed a microarray analysis. We found that the expression of approximately 25% of the NRs was significantly changed in the obese patients one year after surgery and about 10% for the coregulators. Among the NRs and coactivators that were up-regulated after fat loss, we found many members that are involved in fatty acid and steroid-mediated signalling (including LXR α , RXR α , COUP-TF2, ER α , GR, and TR α). In contrast, several genes that are associated with developmental processes, immune defense and stress responses were down-regulated after fat loss. The expression of most of these regulated NRs and coregulators in the obese group one year after surgery became comparable to that seen in the control group. Moreover, we observed that NRs and coregulators that are involved in cell proliferation and differentiation were both differentially expressed in the obese relative to the control subjects and showed little change in their expression level after fat loss in the obese. In conclusion, our results emphasise key roles for NRs in stress response and inflammation associated with obesity. The data also reveal a set of NRs involved in the steroid signalling pathway that may promote restoration of adipose tissue function.

Sources of Research Support: RCN, The Norwegian Cancer Society, Samarbeidsorganet Helse Vest RHF, Meltzerfondet and Programstyret for ernæring at the University of Bergen.

Nothing to Disclose: TH, SND, DJF, VLV, VV, VMS, JVS, GM

P1-36

Effects of Dexamethasone and the Type of Diet on Basal Energy Expenditure and Brown Adipose Tissue Thermogenesis in Mice.

R Poggioli MD¹, M Castillo MD¹ and AC Bianco MD PhD¹.

¹Univ of Miami Miller Sch of Med Miami, FL.

Chronic treatment with steroids results in body weight gain and obesity, which could be explained by the known inhibitory effects that steroids have on brown adipose tissue (BAT) UCP1 expression. To test this hypothesis, we studied selected parameters of energy expenditure in mice treated with dexamethasone (dexa); we also looked at whether feeding on a high fat diet (HF, 42% fat) could interfere with this process. Animals (n=6, C57BL/6) were treated with 1 mg/kg dexa i.p. every other day for 2 weeks. Analyses were performed during admission for 48 h to a comprehensive monitoring system at different times to determine energy expenditure (EE), VO₂ consumption and CO₂ production, respiratory quotient (RQ), movements and food consumption. Brown adipose tissue (BAT)-related thermogenesis was assessed for 4 h after administration of the β_3 adrenergic receptor selective agonist CL 316243 (1 mg/kg sc). Treatment with dexamethasone arrested body weight gain (0.2 vs. 0.0 g/day), reduced night EE (0.48 \pm 0.01 vs 0.45 \pm 0.01 KCal/hr, p<0.05), VO₂ consumption (3809 \pm 59 vs 3423 \pm 70 ml/Kg/h, p<0.0001) and RQ (1.0 \pm 0.01 vs 0.95 \pm 0.01, p<0.05) in the animals kept on chow diet. Notably, dexa increased the response to CL 316243 as assessed by EE (1.4 vs 1.8 fold, p<0.0001) and VO₂ (1.4 vs 2.0 fold, p<0.0001). While the body weight gain was also reduced by dexa in the animals kept on the HF diet (0.3 vs. 0.1 g/day), treatment with dexa increased night EE (0.46 \pm 0.01 vs 0.52 \pm 0.01 KCal/hr, p<0.0001) but the response to CL 316243 was unaffected (1.40 vs 1.36 fold, p=0.16). Food consumption and movements were not affected by dexa treatment in either group. In conclusion, treatment with dexa decreased basal EE and increased BAT thermogenesis; feeding a HF diet interfered with this process by increasing basal EE without affecting the BAT thermogenesis. Thus, the effects of dexa are highly sensitive to the type of diet being fed to the animals.

Nothing to Disclose: RP, MC, ACB

P1-37

Effect of Androstenedione on Adipogenesis in an In-Vitro Bioassay Using C3H 10T1/2 Mouse Mesenchymal Multipotent Cells.

P. Ramaraj Ph.D.¹, JN. Artaza Ph.D.¹, I. Sinha-Hikim Ph.D.¹ and WE. Taylor Ph.D.².

¹AT Still Univ of Hlth Scis Kirksville, MO and ²Charles Drew Univ Los Angeles, CA.

The androgenic steroid hormone Androstenedione (AD) is the immediate precursor of testosterone in the androgen synthetic pathway. AD was formerly sold as a dietary supplement, but recently the FDA restricted its over-the-counter sale. Androstenedione's purported performance enhancing effect and easy availability made it popular among bodybuilders. Our previous study using C3H10T1/2 cells *in vitro*, showed that AD promoted myogenic differentiation, mediated through androgen receptor (AR). Administration of 1500 mg of AD daily to hypogonadal men was associated with increases in fat-free mass and muscle strength. However, AD was not associated with decrease in whole body fat mass, even though testosterone decreases adipose mass *in vivo* and inhibits adipogenesis *in vitro*. So, we investigated the effect of AD on adipogenesis *in vitro* using mesenchymal multipotent C3H10T1/2 cells pretreated with azacytidine to induce differentiation, then grown with or without increasing doses of AD.

Our results showed a 35% decrease in fat cells stained by Oil redO as AD dose increased up to 1 μ M, but at higher concentration of 10 μ M, adipogenesis was increased back to control levels. Adipogenic marker C/EBP α expression was inhibited at 300 nM to 1 μ M AD concentrations, as seen by RT-PCR and western blot. When 300 nM AD was incubated simultaneously with AR antagonist bicalutamide, which blocks AD binding to AR, this increased the number of fat cells and also reversed inhibition of C/EBP α expression. We verified that stimulation of myogenesis by high 10 μ M AD concentrations was also blocked by bicalutamide as shown by immunohistochemical staining and western blot for myosin heavy chain IIb. Treatment of a different preadipocyte cell line 3T3-L1 with increasing dose of AD showed the same pattern as observed for C3H10T1/2 cells, namely decreased adipogenesis up to 1 μ M and an increase back to control levels at 10 μ M AD, as measured by colorimetry of Oil redO stain. A possible role of estrogen in increasing adipogenesis was ruled out by coincubating with aromatase inhibitor (AI), which blocks formation of estrogen from testosterone. There was no change in adipogenesis or myogenesis in AD or AI or AD + AI incubated cultures. It is interesting that androstenedione, which is a biologically weak androgen, was not able to suppress adipogenesis at high 10 μ M concentrations *in vitro* as *in vivo*, even though it stimulates myogenesis just like strong androgens such as Testosterone and DHT.

Nothing to Disclose: PR, JNA, IS-H, WET

P1-38

The Implication of GRP1, a Novel PPAR γ Coregulator, in Adipogenesis.

A Emond B.Sc.¹ and MF Langlois M.D.¹.

¹Univ of Sherbrooke Sherbrooke, Canada.

Many studies have shown that peroxisome-proliferator-activated receptor γ (PPAR γ) plays an important role in adipose tissue formation by activating genes implicated in adipogenesis. PPAR γ heterodimerises with retinoid X receptor α (RXR α), in the presence of ligand, on PPAR response elements (PPREs) in the promoter of target genes involved in adipocyte differentiation. PPARs are activated by thiazolidinediones, like rosiglitazone (rosi). These molecules increase insulin sensitivity and are used in type 2 diabetes mellitus (T2DM) treatment. A better understanding of adipogenesis involves identification and characterization of PPAR coregulators. General receptor for phosphoinositides 1 (GRP1) is a corepressor of thyroid hormone receptors (TRs), a nuclear receptor like PPAR γ . GRP1 decreases TRs transcriptional activity by lowering dimerisation on DNA.

Since PPARs and TRs have important structural similarities and that GRP1 interacts with PPARs *in vitro*, we hypothesized that GRP1 could be a coregulator of PPARs and, thus be implicated in adipogenesis.

To better understand GRP1's effect on PPAR γ 2 transcriptional activity, we transfected HEK 293A cells with PPAR γ 2, RXR α , GRP1 and a luciferase reporter gene containing an idealized PPRE. These assays show that increasing concentrations of GRP1 decrease the transcriptional activity of PPAR γ 2 in the presence of rosiglitazone. We also studied GRP1 expression by Western blots (WB) of total protein extracts from 3T3-L1 cells at different times during differentiation: GRP1 is present in 3T3-L1 preadipocytes and its expression varies during adipogenesis. Then, protein expression of GRP1 was lowered using shRNAs lentiviral particles, and the effects on differentiation of 3T3-L1 cells were further investigated by microscopy and WB. Interestingly, GRP1 knock down before inducing 3T3-L1 differentiation, almost completely abolishes adipogenesis.

Our results thus suggest that GRP1 is a PPAR γ 2 corepressor that could be implicated in the regulation of genes linked to adipogenesis. Future experiments will allow us to explore the effects of GRP1 overexpression or knock down at different times during the process of adipocyte differentiation. This will help to better understand the role of GRP1 in adipogenesis and, eventually, comorbidities linked to obesity like cardiovascular diseases and T2DM.

Sources of Research Support: Fonds de recherche en santé du Québec and the Heart & Stroke Foundation of Quebec.

Nothing to Disclose: AE, MFL

P1-39

The Glucocorticoid Receptor and Its Direct Target Gene MuRF1 Are Required for Dexamethasone-Induced Skeletal Muscle Atrophy.

JD Furlow PhD¹, ML Watson BS¹, LM Baehr BS¹, E Karbassi BS¹, HM Reichardt PhD², JP Tuckermann PhD³ and SC Bodine PhD¹.

¹Univ of California Davis, CA ; ²Univ of Göttingen Med Sch Göttingen, Germany and ³Leibniz Inst for Age Res Jena, Germany.

Skeletal muscle atrophy occurs as the result of disuse, such as after denervation or unweighting, and in catabolic states associated with elevated glucocorticoid levels such as fasting and diabetes. Muscle atrophy is also a serious side effect of prolonged anti-inflammatory glucocorticoid treatment, such as dexamethasone (DEX). We have previously demonstrated that the E3 ubiquitin ligase, MuRF1, is induced in several atrophy inducing conditions, including DEX treatment, and is a direct glucocorticoid receptor (GR) target gene (1,2). Here, we show that muscle specific deletion of the GR gene (muGRKO) prevents both muscle atrophy and MuRF1 expression induced by DEX, but not repression of fibroblast specific genes such collagen 1A1. Furthermore, mice lacking the MuRF1 gene are nearly as resistant to DEX induced atrophy as the muGRKO mice, and did not show reduced ability to generate force observed in DEX treated wild-type mice. Based on these findings, synthetic GR ligands that maintain anti-inflammatory activity but do not significantly up-regulate MuRF1 would be therapeutically desirable. Therefore, we screened a series of newly developed non-steroidal compounds that have previously been shown to show selective GR activation in other cell types (3). The NSR-1 compound, in particular, repressed LPS-induced IL-6 induction but did not significantly up-regulate MuRF1 in cultured myotubes over all concentrations tested. In summary, our results implicate MuRF1 as a key target gene downstream of the GR responsible for glucocorticoid induced muscle atrophy, and strategies to prevent MuRF1 induction or function should reduce muscle atrophy that accompanies long term glucocorticoid-based therapies.

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Sources of Research Support: NIH RO1 DK75801 awarded to JDF and SCB; NIH Training Grant 2T32GM007377.

Nothing to Disclose: JDF, MLW, LMB, EK, HMR, JPT, SCB

P1-40

Testosterone-Induced Hypertrophy of L6 Myoblasts Is Dependent upon mTOR and Erk 1/2.

Y Wu MD¹, WA Bauman MD^{1,2}, R Blitzer PhD² and C Cardozo MD^{1,2}.

¹James J Peters VA Med Ctr, Bronx NY ; ²James J Peters VA Med Ctr Bronx, NY ; ³Mount Sinai Sch of Med New York, NY and ⁴Mount Sinai Sch of Med New York, NY.

Testosterone increases the size and strength of skeletal muscle and increases protein content of C2C12 and L6 myotubes that stably express the androgen receptor (AR). In L6 myotubes, hypertrophy is blocked by the mTOR inhibitor rapamycin. This study aimed to further characterize the molecular mechanisms responsible for the anabolic effects of testosterone. Testosterone did not induce hypertrophy in L6 cells lacking the AR-expressing transgene or in L6.AR cells treated with the AR antagonist bicalutamide. IGF-1 is upregulated by testosterone, and IGF-1 stimulates hypertrophy of myotubes and skeletal muscle through pathways that require mTOR. To test for activation of IGF-1 receptor signaling upon incubation of L6 myotubes with testosterone, autophosphorylation of the tyrosine kinase domain of the receptor was determined by immunoprecipitation and Western blotting. Autophosphorylation in L6.AR cells was robustly increased by a 20 min exposure to IGF-1, but, of note, was not altered by incubation overnight with testosterone, suggesting that mechanisms independent of activation of IGF-1/PI3K/Akt signaling were involved in the initiation of its anabolic effects. Because testosterone has been reported to rapidly activate Erk1/2, and because Erk1/2 modulates mTOR activity through phosphorylation and inactivation of the mTOR inhibitory complex of tuberlin and hamartin, the effects of testosterone on Erk1/2 phosphorylation were determined. Overnight incubation of differentiated L6.AR cells significantly increased Erk1/2 phosphorylation without altering total levels of Erk1/2, indicating that testosterone stimulated prolonged activation of Erk1/2. Addition of a MAPK inhibitor which blocks Erk1/2 activation (PD98059) significantly reduced protein content for cells incubated with this agent alone, and prevented testosterone-induced hypertrophy. The findings indicate that testosterone stimulates a lasting activation of Erk1/2 and induces hypertrophy dependent upon its binding to the AR and functional of mTOR and Erk1/2.

Nothing to Disclose: YW, WAB, RB, CC

P1-41

Nandrolone-Induced Nuclear Translocation of Myo-D in Denervated Rat Skeletal Muscle Associated with Induction of Wnt- and Inhibition of Notch-Signaling Pathways.

Xin-Hua Liu Ph.D., M.D.^{1,2}, Jiangping Pan B.S.¹, Yong Wu M.D.¹, Weiping Qin Ph.D., M.D.¹, William A. Bauman M.D.^{1,2} and Christopher Cardozo M.D.^{1,2}.

¹James J Peter VA Med Ctr Bronx, NY and ²Mount Sinai Sch of Med New York, NY.

Nandrolone, an anabolic steroid, slows denervation-atrophy in rat muscle. The molecular mechanisms responsible for this effect are not well understood. Androgens and anabolic steroids upregulate the myogenic differentiation factor D (MyoD), which plays an important role in skeleton muscle growth and repair. During these processes, upregulation of MyoD is stimulated by the Wnt signaling activity. In addition, temporally appropriate activation of Notch and Wnt signaling have been shown to be required for satellite cell proliferation and differentiation after muscle injury, and for muscle repair. With these considerations in mind, we investigated the effects of nandrolone on MyoD, Wnt and Notch signaling in rat gastrocnemius muscle collected at 7, 35, and 56d after denervation by quantifying mRNA and protein levels for MyoD, and key molecules in Wnt and Notch signaling using real time PCR and Western blotting. Nandrolone was begun either at day 0 (7 d group) or day 29 (35 and 56 d groups). Denervation significantly induced MyoD protein and mRNA expression. Although nandrolone did not alter MyoD mRNA levels, it resulted in a significant increase in nuclear MyoD and a decrease in cytosolic levels of this protein. The peak effect was observed at 56 d after denervation, suggesting that nandrolone promoted nuclear translocation of MyoD. Nandrolone increased Wnt activity and prevented denervation-induced upregulation of Notch signaling. These effects peaked at 35 d, and thus preceded the peak in nuclear accumulation of MyoD. Nandrolone increased nuclear levels of b-catenin protein and decreased mRNA and protein levels for two naturally occurring Wnt antagonists, Dkk1 and soluble frizzled related protein 1 (sFRP1). Furthermore, nandrolone enhanced nuclear expression of two direct target genes of Wnt, Runx2 and Pits2. Nandrolone also reduced nuclear content of cleaved Notch protein, and upregulated mRNA and protein for Numb, which targets Notch for degradation. These data suggest that protection against denervation atrophy by nandrolone may be associated with induction of Wnt and inhibition of Notch signaling pathways and subsequent nuclear accumulation of MyoD.

Sources of Research Support: Department of Veteran Affairs.

Nothing to Disclose: X-HL, JP, YW, WQ, WAB, CC

P1-42

***Odc1* Is a Potential Mediator of Androgen Actions in Skeletal Muscle.**

NKL Lee BBSc(Hons)¹, JD Zajac MBBS,PhD¹ and HE MacLean PhD¹.

¹Univ of Melbourne Melbourne, Australia.

We are investigating AR target genes in skeletal muscle. We have used our global AR knockout (ARKO) male mice, which have a 20% decrease in muscle mass [1], to identify two genes with altered expression in ARKO gastrocnemius muscle: *Odc1* (2.6-fold decrease in ARKO vs WT male), and *Tceal7* (2.4-fold increase in ARKO), with a similar pattern of expression in muscle-specific ARKO muscle. *Odc1* encodes ornithine decarboxylase, a regulator of proliferation [2], and *Tceal7*, an ovarian tumor suppressor [3].

We determined the developmental pattern of *Odc1* and *Tceal7* gene expression in muscle of pre-pubertal (4 wk) male and female, and adult (12 wk) male and female mice (n=5-12/grp). *Odc1* expression is increased 4-fold in adult vs pre-pubertal males (p=0.001) and is higher in adult males than females (p<0.001). *Tceal7* expression is decreased 5-fold in adult vs pre-pubertal males (p<0.01). This suggests that *Odc1* and *Tceal7* are physiologically regulated by androgens in pre-pubertal vs adult males and males vs females. We also measured expression in C2C12 proliferating myoblasts and differentiated myotubes (n=6-8). *Odc1* expression is high in myoblasts, and decreased 50-fold (p<0.01) in myotubes. *Tceal7* expression is low in myoblasts and increased >3000-fold (p<0.001) in myotubes. This is consistent with the proposed roles of *Odc1* as a regulator of proliferation and *Tceal7* as an inducer of differentiation. To investigate *Odc1* gene function *in vitro*, C2C12 myoblasts were stably transfected with an *Odc1* overexpression vector or treated with an ODC1 inhibitor, α -difluoromethylornithine (DFMO). Cell proliferation is increased in C2C12 myoblasts overexpressing *Odc1* vs control myoblasts (p<0.001); whereas DFMO treatment inhibits myoblast growth by >75% vs vehicle. These results demonstrate the role of *Odc1* as a positive regulator of myoblast proliferation. Our data demonstrate that *Odc1* and *Tceal7* are developmentally regulated by androgens in adult skeletal muscle, and this occurs directly via the AR in skeletal muscle. We suggest *Odc1* and *Tceal7* play opposing roles in muscle, with *Odc1* expression high in myoblasts to increase proliferation, and *Tceal7* expression high in myotubes to induce differentiation. Androgens up-regulate *Odc1* and down-regulate *Tceal7*. We propose that androgens increase muscle mass in part by maintaining myoblasts in the proliferative state, by inducing *Odc1* and suppressing *Tceal7* expression.

[1] HE MacLean et al. FASEB J. 22: 2676, 2008

[2] JA Nilsson et al. Cancer Cell 7: 433, 2005.

[3] J Chien et al. Oncogene 24: 5089, 2005

Nothing to Disclose: NKLL, JDZ, HEM

P1-43

Differential Response of Levator-Ani, Diaphragm and Gastrocnemius Satellite Cells after Testosterone Treatment: Possible Role of Androgen Receptor and Follistatin/TGF-beta Axis.

M Braga BS¹, S Bhasin MD, PhD², R Jasuja PhD², S Pervin PhD^{1,3} and R Singh PhD^{1,3}.

¹Charles Drew Univ of Med and Sci Los Angeles, CA ; ²Boston Univ Med Ctr Boston, MA and ³David Geffen Sch of Med, UCLA Los Angeles, CA.

Introduction: We have recently demonstrated that testosterone (T)-induced myogenesis in multipotent C3H 10T1/2 cells was associated with up-regulation of follistatin (Fst) expression and modification of TGF- β signaling via androgen-receptor/ β -catenin mediated pathway (Singh et. al. *Endocrinology*:2009). The objective of the current study was to study the differential regulation of myogenic differentiation in androgen receptor (AR) responsive and non-responsive muscle tissues using primary cultures of satellite cells (SCs).

Methods: SC primary cultures were isolated from three different muscle tissues from C57 BL6J6 mice using standard enzymatic digestion in collagenase as described previously. Immuno-cytochemistry, single and double immunofluorescence were performed to identify key satellite cell markers, and their possible co-localization. Quantitative image analysis was performed to analyze the expression levels of different myogenic proteins and Smad3 phosphorylation. Real-time quantitative PCR was performed to analyze the mRNA expression levels of CD34, Pax3/7, MHCIIb, Fst and Mst after various treatments.

Results and conclusions: Primary cultures of satellite cells isolated from gastrocnemius, levator ani, and diaphragm muscles express Pax7 and CD34, AR, Mst and Fst. AR was found to be co-localized with Fst and CD34/Pax7. Mst was detected to be co-localized in the satellite cells with Pax7. These data suggest a possible link between Fst/Mst and AR during muscle differentiation in response to T. 100nM T was able to promote myogenic differentiation (MHCII) in both male (31+/-2.6 vs 49.2+/- 4.6) and female (22.4+/- 1.8 vs 32.2+/-1.8) SCs in diaphragm muscle, and anti-Fst antibody blocked this effect. The myogenic effect of T and recombinant Fst in SCs isolated from levator-ani muscle were significantly higher compared to that from gastrocnemius muscles. There was a significant increase in the mRNA expression of CD34, Pax3 and Pax7 as well as MHCII, Fst and Smad7 genes in the levator-ani SCs after T treatment. There was no significant decrease in the Mst gene expression in these cells after T-treatment, however, TGF- β -induced increase in Mst gene expression was significantly inhibited in the presence of T. We conclude that the myogenic effects of T on various muscles are regulated at the level of their endogenous AR expression, and Fst/Mst and AR interaction may be an important determinant in regulating the overall muscle mass during T treatment.

Nothing to Disclose: MB, SB, RJ, SP, RS

P1-44

The Role of Growth Hormone and Insulin-Like Growth Factor-1 in Mediating Anabolic Effects of Testosterone on the Muscle.

C Serra PhD¹, R Jasuja PhD¹, C Morris PhD², F Tangherlini¹, ER Barton PhD³ and S Bhasin MD¹.

¹Boston Med Ctr Boston, MA ; ²Pfizer Res Cambridge, MA and ³Univ of Pennsylvania Philadelphia, PA.

Testosterone supplementation increases skeletal muscle mass and strength. While insulin-like growth factor-1 (Igf-1) and androgen signaling have been shown to interact, it is not clear if the growth hormone (GH)/Igf-1 signaling is essential for mediating testosterone's effects on the skeletal muscle. In this study, we investigated if testosterone's effects on skeletal muscle are mediated by GH/Igf-1 signaling. Accordingly, we randomly assigned 2 month-old male rats as follows: hypophysectomized (Hypox); castrated (Cas); Hypox/Cas and intact (Cont). Six rats/group were treated with testosterone enanthate (TE) in sesame oil for two weeks and six rats/group with vehicle alone. GH-deficient Hypox and Hypox/Cas rats had lower body weights and levator ani (LA) muscle mass, and lower serum Igf-1 levels than controls. TE supplementation increased body weight in Hypox and Hypox/Cas rats, improved LA mass, and induced Igf-1 and mechano growth factor gene expression in the LA of Hypox, Cas and Hypox/Cas rats. These data suggest that circulating GH and Igf-1 are not essential for mediating testosterone's rescue on skeletal muscle mass. To determine whether intramuscular Igf-1 is essential for mediating testosterone's effects on the muscle, we used MKR mice that express a dominant negative form of Igf-1 receptor (Igf-1R) in muscle fibers. 2 month-old MKR or WT male mice were treated (n=6/group) as follows: saline-treated; mice treated with a GnRH antagonist acyline (Ac); mice treated with Ac plus 0.2 mg/d TE (Ac-TE). Ac treatment reduced body weight of WT mice and the LA mass in both WT and MKR mice. Additionally, TE treatment restored body weight in WT mice and rescued LA mass in both groups, suggesting that testosterone's anabolic effects on the muscle do not require intact Igf-1 signaling *in vivo*. In parallel experiments, we evaluated testosterone's effect on the proliferation and fusion of human primary myoblasts (hPM) *in vitro*. Testosterone treatment induced hPM proliferation and the formation of bigger myotubes in differentiation conditions. Transfection with siRNA for the human IGF-1R inhibited myogenic differentiation of hPM, and testosterone supplementation partially rescued this inhibitory effect. In conclusion, we suggest that intact Igf-1 signaling is not obligatory for mediating the pro-myogenic effects of testosterone on skeletal muscle growth *in vivo* and muscle cell differentiation *in vitro*.

Nothing to Disclose: CS, RJ, CM, FT, ERB, SB

P1-45

Analysis of the Effects of Androgens and Training on Myostatin Propeptide and Follistatin Concentrations in Blood and Skeletal Muscle Using Highly Sensitive Immuno PCR.

Patrick Diel PhD¹, Thorsten Schiffer MD¹, Stefan Geisler¹, Torsten Hertrampf PhD¹, Stefanie Mosler¹, Sven Schulz², Flogian Wintgens PhD³ and Michael Adler PhD².

¹German Sports Univ Cologne Cologne, Germany ; ²Chimera Biotech GmbH Dortmund, Germany and ³Immunodiagnostik AG Benzheim, Germany.

Myostatin propeptide (MYOPRO) and follistatin (FOLLI) are potent myostatin inhibitors. In this study we analysed long-term training effects and effects of androgens on MYOPRO and FOLLI concentrations in blood and skeletal muscle using highly sensitive Immuno PCR. To study training effects in humans young healthy males did either a 3-month endurance-training or a strength-training. Blood and biopsy samples were analysed. Our data revealed that training did not significantly affect MYOPRO and FOLLI concentrations in serum and muscle. To investigate whether total skeletal muscle mass may affect circulating MYOPRO and FOLLI levels, blood samples of tetraplegic patients, untrained volunteers and bodybuilders were analysed. Interestingly, serum levels of MYOPRO were significantly increased in the bodybuilder group. To investigate the effects of androgens on MYOPRO and FOLLI levels, orchiectomised rats were treated with Testosterone. MYOPRO was increased in blood and muscle. In summary Immuno PCR analysis demonstrate that moderate training does not affect the concentrations of MYOPRO to FOLLI. In contrast androgen treatment results in a significant increase of MYOPRO in the skeletal muscle and also in serum. This observation provides insights into the molecular mechanisms of anabolic activity of androgens. In addition it could serve as a basis for a test system to detect abuse of myostatin inhibitors.

Sources of Research Support: World Anti Doping Agency WADA.

Nothing to Disclose: PD, TS, SG, TH, SM, SS, FW, MA

P1-46

Phosphorylation Status of Estrogen Receptor Alpha and Endocrine Resistance in Breast Cancer.

K Kim¹ and BS Katzenellenbogen Ph. D¹.

¹Univ of Illinois Urbana, IL.

The estrogen receptor alpha (ER) contains multiple serine residues capable of undergoing post-translational modification by phosphorylation. In order to understand the role of phosphorylation status in affecting the response of receptor to the natural hormone estradiol, the selective estrogen receptor modulator, tamoxifen, and the selective estrogen receptor down-regulator, ICI182,780 (Fulvestrant), we generated multiple combinations of ER phospho-mutants, at residues serine 104, 106, 118, 167, 236, and 305, and examined their impact on receptor half-life, the agonist and antagonist balance of SERMs and SERDs, the regulation of ER transcriptional activity, and stimulation of proliferation in response to estradiol and SERMs/SERD. ER α mutants were generated by substituting serine residues with either alanine or glutamic acid, and of the sixteen mutants screened, half were selected for further analysis. The mutant receptors were generated into U2OS osteosarcoma-tetracycline regulated- ER α stable cell lines for characterization. These phospho-ER mutant receptors were also expressed in MCF-7 breast cancer cells with concomitant knock-down of endogenous ER α . Receptors with changes at Ser-118 and Ser-167 showed altered responses to the antiestrogens tamoxifen and ICI182,780, i.e. strong agonistic stimulation and weak estrogen antagonistic activity of tamoxifen and ICI182,780 on gene regulation and differential stimulation of cell proliferation. Other mutant ERs showed increased protein stability in the presence of estradiol or ICI182,780. Hence, changes in ER α affecting the phosphorylation status of the receptor greatly impact receptor function and differential SERM and SERD modulated cellular responses that could contribute to resistance to endocrine therapies in breast cancer.

Sources of Research Support: Grants from the Breast Cancer Research Foundation and NIH.

Nothing to Disclose: KK, BSK

P1-47

A Clinically Relevant Phosphorylation Code for Estrogen Receptor alpha (ER α) in Human Breast Cancer.

LC Murphy PhD^{1,2}, GP Skliris PhD^{1,2}, ZJ Nugent PhD², BG Rowan PhD³, CR Penner² and PH Watson MD⁴.

¹Univ of Manitoba Winnipeg, Canada ; ²CancerCare Manitoba and Univ of Manitoba Winnipeg, Canada ; ³Tulane Univ Sch of Med New Orleans, LA and ⁴British Columbia Cancer Agency Victoria, Canada.

Previously we validated several phospho-specific estrogen receptor (ER α) antibodies for immunohistochemistry (IHC) on formalin-fixed paraffin-embedded human breast tissue microarray (TMA) sections. To determine the relationship of ER α , phosphorylated at multiple sites to clinical outcome of tamoxifen therapy, TMA sections representing over 300 ER+ breast cancers from patients who were treated with surgery +/- radiation and then tamoxifen were used for immunohistochemical determination of total ER α , p-S104/106-ER α , p-S118-ER α , p-S167-ER α , p-S282-ER α , p-S294-ER α , p-T311-ER α and p-S559-ER α . Relationships of phosphorylated ER α to overall survival (OS) from breast cancer specific death and relapse free survival (RFS) from breast cancer death or recurrence were tested using single and multiple predictor statistical models.

Large tumor size, node positivity, high grade, progesterone receptor (PR) negative status, low levels of p-S282-ER α and high levels of p-T311-ER α were significantly associated with reduced OS from breast cancer specific death. Closer examination of hazard ratios for individual phosphorylation sites measured in this study suggested two groups of p-epitopes exist: one associated with a better outcome on tamoxifen and another associated with poor outcome. This led us to hypothesize an ER α phosphorylation code, which more precisely reflects the functional status of ER α regulated events in tumors, might exist. A novel phosphorylation score (P7score) taking into account all p-ER α sites was developed and a P7score ≥ 3 was found to be significantly associated with reduced OS in univariate and multivariate analysis (HR = 2.24, 95% C.I. 1.15-4.34, n = 335; P = 0.0175) along with size and nodal status. As well a high P7score (≥ 3) was significantly associated with reduced RFS in univariate and multivariate analysis (HR = 1.71, 95% C.I. 1.03-2.86, n = 332; P = 0.0392) along with size, nodal status, grade and PR status. Our results demonstrate that a low P7score (reflecting the balance of good over bad sites) is a significant independent predictor of better OS and RFS in breast cancer patients on tamoxifen.

Our results also suggest that the phosphorylation score of ER α is a surrogate marker of the balance of estrogen dependent versus cross-talk dependent receptor activity and therefore a more precise marker of treatment response to endocrine therapies.

Sources of Research Support: Canadian Institutes of Health Research (CIHR), the CancerCare Manitoba Foundation (CCMF) and the Canadian Breast Cancer Foundation (CBCF). This study was also supported by the Manitoba Breast Tumor Bank, a member of the Canadian Tumor Repository Network and is funded in part by CCMF and CIHR.

Nothing to Disclose: LCM, GPS, ZJN, BGR, CRP, PHW

P1-48

Differential Expression of microRNAs in Tamoxifen-Sensitive Versus Tamoxifen-Resistant Human Breast Cancer Cells.

C.M. Klinge Ph.D.¹, T.T. Manavalan M.S.¹, S. Datta Ph.D.² and T.S. Kalbfleisch Ph.D.¹.

¹Univ of Louisville Sch of Med Louisville, KY and ²Univ of Louisville Sch of Public Hlth Louisville, KY.

Select changes in microRNA (miRNA) expression correlate with diagnostic markers used in early stage breast cancer therapies, e.g., estrogen receptor α (ER α). Comparatively little is known about miRNA regulation or how antiestrogens, e.g., tamoxifen (TAM), regulate the expression of miRNAs in breast cancer cells. The goal of our research is to determine the identity and function of miRNAs whose expression is differentially regulated by estradiol (E₂) and TAM in antiestrogen/tamoxifen-sensitive *versus* resistant breast cancer cell lines and to correlate these miRNAs and their gene targets with those dysregulated in human breast tumors, thus offering new biomarkers to be tested in patient prognosis and treatment planning. We used miRNA microarray analysis to identify miRNAs differentially expressed and regulated by E₂ and 4-hydroxytamoxifen (4-OHT) in MCF-7 tamoxifen-sensitive, estrogen-dependent *versus* LY2 tamoxifen/endocrine-resistant human breast cancer cells. Four separate experiments were performed for each treatment group. Bioinformatic analyses to impute the biological significance of the identified miRNAs by identifying their computationally predicted target genes in the human genome using TargetScan, PicTar, and the Sanger miRBase Targets databases was performed. Additionally, we compared global miRNA and mRNA expression patterns in 4-OHT-treated MCF-7 cells to identify key targets. We experimentally confirmed the observation that E₂ reduced miR-21 expression in MCF-7 cells. This repression was inhibited by the antiestrogen ICI 182,780 (Faslodex) and ER α knockdown by siRNA, indicating that the E₂-suppression is ER α -mediated. E₂ increased luciferase activity from reporters containing the miR-21 recognition elements from the 3'-UTRs of miR-21 target genes, corroborating that E₂ represses miR-21 expression resulting in a loss of target gene suppression. The E₂-mediated decrease in miR-21 correlated with increased protein expression of endogenous miR-21-targets Pcd4, PTEN, and Bcl-2. We are currently performing quantitative RT-PCR (Q-PCR) assessment of the miRNAs identified by microarray and examining changes in target protein expression by western blot analyses.

Nothing to Disclose: CMK, TTM, SD, TSK

P1-49

Breast Cancer Outcome in Peri- vs. Postmenopausal Women with or without Hormone Therapy Prior to Diagnosis.

VR Seifert-Klauss MD, PhD¹, A Hauesler¹, AK Baumgaertner MD¹, U Schwarz-Boeger MD¹ and M Kiechle MD, PhD¹.

¹Frauenklinik Rechts der Isar der Technischen Univ Muenchen Muenchen, Germany.

Introduction: Use of hormone therapy (HT) has been associated with higher incidence of hormone receptor positive breast cancer in postmenopausal women. However, it is unclear if breast cancers developing after HT use are different in course of disease and prognosis from tumors growing without prior exogenous hormone use. Perimenopause has been called a "window of risk" for estrogen-dependent disease (1), due to endogenous ovarian stimulation resulting from rising FSH. Also, HT is often initiated for symptomatic perimenopause. This is the first study differentiating data on perimenopausal women from information on postmenopausal women with or without prior HT in diagnosed breast cancer patients.

Patients and Methods: Women with hormone receptor positive breast cancer diagnosed between 1984 and 2006 at the Frauenklinik TUM (n=1247) were analyzed in a single-center retrospective trial. Perimenopause was defined by natural LMP \leq 12 months (or FSH/ estradiol in perimenopausal range for women after hysterectomy). Postmenopausal state was defined as natural LMP >12 months preceding diagnosis, high FSH and low estradiol in hysterectomized patients or by surgical menopause.

Outcome data comparing overall survival, local and distant recurrence were extracted from the hospital cancer data base and the breast cancer registry for the greater Munich area, as well as from primary care-giving gynecologists and by review of patient charts. (Cases were stratified according to hormone therapy use before breast cancer diagnosis).

Results: More than 50% of invasive cases (n=1192) were women between 45 and 63 years of age. Information on former HT use was available for 977 women with non metastasized invasive breast cancer. 576 patients (59%) had never used HT, including 204 premenopausal patients. Out of 401 (41%) former HT users, 66 (16.5%) were perimenopausal and 335 (83.5%) were postmenopausal at first diagnosis. At the time of diagnosis, postmenopausal women had similar rates of early stage breast cancer (T1, T2), but increased rates of locally advanced disease (T4) compared with perimenopausal patients. T3 and T4 stages were less frequent in postmenopausal patients with prior hormone therapy compared with postmenopausal patients without prior hormone therapy. Median follow-up was 83 months for all patients. Detailed outcome information on the different patient groups will be presented.

(1) Hale G et al., JCEM 2002; 87(1):3-15

Nothing to Disclose: VRS-K, AH, AKB, US-B, MK

P1-50

Protein Biomarker Expression in Molecular Subtypes of Breast Cancer Identifies Patients with Increased Likelihood of Recurrence.

JL Wittliff PhD, MD hc¹, SA Andres PhD¹, TL Kruer BS¹ and DA Kerr II BA¹.

¹Univ of Louisville Louisville, KY.

Assessment of prognostic factors by immunohistochemistry may be altered notably by conditions of tissue fixation, affinity & specificity of antibody and operator interpretation of immunostaining (e.g. J Clin Oncol 25:118, 2007), which may contribute to inaccurate prediction of prognosis and therapy selection in some patients. To ascertain individual and collective contributions of quantitative expression levels of HER-2/neu oncoprotein (HER2), estrogen (ER) and progesterin receptors (PR) to prediction of clinical outcome of breast cancer subtypes (e.g., triple-negative), biomarkers were determined in cytosols with FDA approved kits. De-identified frozen biopsies, from an IRB-approved biorepository, were used to quantify ER and PR by radio-ligand binding (cut-off level = 10 fmol/mg protein) and by EIA (cut-off level = 15 fmol/mg) while HER-2/neu protein was measured by ELISA (cut-off = 800 HNU/mg). Kaplan-Meier plots of disease-free survival (DFS, n = 936) and overall survival (OS, n = 1158) were significantly different at 5 yr (DFS 87% vs 69% & OS 83% vs 60%) and 10 yr (DFS 76% vs 68% & OS 74% vs 57%) between ER+/PR+ and ER-/PR- subtypes, with intermediate results for ER+/PR- and ER-/PR+ subtypes. Neither [ER] nor [PR] influenced HER2 expression levels. Confounding these results was the observation from 9642 biopsies that menopausal status greatly influenced subtype expression, e.g., 27-38% of biopsies of premenopausal patients exhibited the ER-/PR- subtype decreasing to 12-20% in postmenopausal patients. Further [ER] in biopsies increased with patient age and increased [PR]. Kaplan-Meier plots of DFS (n = 143) and OS (n = 187) separated HER2- and HER2+ subtypes with significant differences at 5 yr (DFS 83% vs 67% & OS 77% vs 64%) and 10 yr (DFS 75% vs 64% & OS 71% vs 51%). Although [HER2] in biopsies did not vary as a function of patient age, [ER] or [PR], status of HER2 notably separated prognostic groups only in postmenopausal patients. Kaplan-Meier plots of DFS (n = 84) and OS (n = 104) separated ER+/HER2- vs ER+/HER2+ subtypes with significant differences at 5 yr (DFS 87% vs 63% & OS 91% vs 61%) and 10 yr (DFS 75% vs 59% & OS 80% vs 45%). No difference was observed in either DFS or OS of ER- cancer regardless of HER2 status. Collectively, results suggest quantitative HER2 status was only of value as a predictor of prognosis in ER+ breast cancer compared to ER- lesions, suggesting the molecular characteristics of subtypes should be examined further.

Sources of Research Support: In part by grants from Phi Beta Psi Charity Trust Fund & a DOD BCRP Predoctoral Traineeship Award W81WXWH-08-0775 to support TLK.

Nothing to Disclose: JLW, SAA, TLK, DAK

P1-51

Regulation of ERbeta1 Transcriptional Activity by Acetylation.

MT Lee M.Phil¹, YK Leung Ph.D¹, I Chung¹ and SM Ho Ph.D¹.

¹Univ of Cincinnati Cincinnati, OH.

Post-translational modification plays a critical role in modulating nuclear receptors (NRs) activity. In this study, we identified TIP60 isoform 3 (TIP60beta) as an interacting partner of ERbeta in a yeast two-hybrid screening with prostate cDNA library. The interaction was confirmed by both in vitro and mammalian co-immunoprecipitation. TIP60 has been shown to acetylate lysine residues of class I nuclear receptor (NR) and promote NR transactivation but information regarding the function of TIP60beta is still limited. While TIP60beta has the deletion of exon 5, which encodes a proline-rich region, it may have different functions as compared with TIP60alpha. The aim of this study is to characterize the role of this newer member of lysine-specific acetylase, TIP60beta in ERbeta1 function. Ectopic expression of TIP60beta in HEK293 cells resulted in reduction of transactivation activities of ERbeta1, ERalpha and AR in different reporters in a ligand-independent manner, suggesting its corepressor role by nature. Its repression property in NR transactivation was conserved in prostate and breast cancer cell backgrounds. Such reduction of ERbeta1 activity could be due to direct acetylation by TIP60beta as it contains an active acetyltransferase domain. This is the first report to reveal that the function of ERβ1 can be repressed by acetylation. Interaction domain(s) between ERbeta1 and TIP60beta will be mapped to illustrate whether the physical interaction is crucial for such modification and thereby down-regulating its activity. Further study will focus on the identification of the downstream target genes of the ERbeta1-TIP60beta interaction and the significance of this interaction in the progression of prostate and breast cancers.

Sources of Research Support: CA112570;ES006096;ES015584;CA1577; CA112532.

Nothing to Disclose: MTL, YKL, IC, SMH

P1-52

The Role of Estrogen Receptor Signaling in mTORC Activity.

BA Hall PhD¹ and SD Conzen MD¹.

¹The Univ of Chicago Chicago, IL.

Serum and glucocorticoid-regulated kinase 1 (SGK1) is a highly conserved, immediate early stress response gene encoding a serine/threonine kinase implicated in breast cancer cell survival and resistance to apoptotic stress. Like Akt, SGK1 is activated downstream of PI3K signaling via the PDK1-dependent phosphorylation of a conserved threonine residue (Thr308) and the mammalian Target of Rapamycin Complex (mTORC)-dependent phosphorylation of a conserved serine residue at SGK1's Ser422. Recent studies have alternatively suggested that either mTORC1 or mTORC2 mediates SGK1 Ser422 phosphorylation, while Akt has consistently been found to be exclusively downstream of mTORC2. Using a variety of conditions (insulin stimulation, endoplasmic reticular stress and amino acid starvation/refeeding) in estrogen receptor (ER)-positive and ER-negative cell lines, we found that both mTORC1 and mTORC2 appear to be required for SGK1 Ser422 phosphorylation in ER-positive cell lines. In contrast, mTORC2, and not mTORC1, is required for phosphorylating SGK1 at Ser422 in ER-negative breast cancer cell lines. We are therefore currently investigating the role of ER expression and ER activation in mTORC1-mediated phosphorylation and activation of SGK1. Preliminary data suggest that rapid estradiol-mediated activation of the ER may be required for robust mTORC1-dependent phosphorylation of SGK1. These experiments illustrate the potential crosstalk between the ER and the mTORC1 and mTORC2 pathways as well as the differential sensitivity of Akt versus SGK1 activity following rapamycin treatment. The clinical implications of rapamycin-mediated inhibition of mTOR/SGK1 activity in ER-positive breast cancers will be discussed.

Nothing to Disclose: BAH, SDC

P1-53

Interactions between Insulin-Like Growth Factor-I, Estrogen Receptor- α (ER α) and ER β in Regulating Growth/Apoptosis of Human Breast Cancer Cells.

Sylvia M Mejia Student¹, Marlene I Enriquez Student¹, Emily E Moody Student¹, Rhone A Mendoza Student¹ and Gudmundur Thordarson Ph.D.¹.

¹Texas Tech Univ Hlth Scis Ctr, Paul L Foster Sch of Med El Paso, TX.

Estradiol (E2) and insulin-like growth factor-I (IGF-I) interact closely to regulate growth of estrogen receptor (ER)-positive breast cancers (1). However, the understanding of these interactions is still incomplete. We have generated several cloned cell lines derived from the MCF-7 breast cancer cells with suppressed expression of the IGF-I receptor (IGF-IR) termed IGF-IR-low cells by stably transfecting the cells with expression vector generating small interfering RNA (siRNA) corresponding to a 21 nucleotide (nt) sequence in the human IGF-IR sequence. Vector for transfecting control cells carried sequence generating non-interfering, 21 nt RNA. Concomitant with reduction in the IGF-IR levels, the IGF-IR-low cells also showed a reduction in ER α and progesterone receptor expressions and a significant elevation in the expression of ER β . The overall growth rate of the IGF-IR-low cells over 10-day period was significantly reduced in response to IGF-I and E2 was even less effective growth stimulator compared to controls, but their growth rate was not diminished when the cells were treated with growth hormone plus epidermal growth factor. The short-term proliferation rate of the IGF-IR-low cells was only reduced in response to E2 compared to controls, whereas their apoptosis rate was increased both at the basal level and after hormonal stimulation. The phosphorylation of p38 mitogen activated protein kinase (p38 MAPK), a kinase associated with apoptosis (2), was significantly increased in the IGF-IR-low cells when they were treated with E2, and the elevation of this phosphorylation occurred within 15 min of treatment, but E2 treatment did not increase the phosphorylation state of p38 MAPK in the control cells. Further, phosphorylation of the tumor suppressor protein p53, a substrate for p38 MAPK (3), was significantly elevated in the IGF-IR-low cells compared to the controls. In summary, suppressing the IGF-IR expression decreased the level of ER α but increased the level of ER β . Concomitantly, the overall growth rate of the IGF-IR-low cells was reduced mostly through an increase in apoptosis rate without affecting proliferation substantially. We conclude that decrease in the ER α :ER β ratio triggered a rapid phosphorylation of p38 MAPK which in turn phosphorylated the p53 tumor suppressor and accelerated apoptosis rate of the cells.

(1) Fagan DH, Yee D J *Mammary Gland Biol Neoplasia* 2008; 13:423

(2) Xia Z et al., *Science* 1995; 270:1326

(3) Bulavin DV, Fornace AJ Jr. *Adv Cancer Res* 2004; 92:95

Sources of Research Support: Grants from Laura W. Bush Institute for Women's Health-Permian Basin and NIH/NCI RO3CA128067-01A1S1 awarded to GT.

Nothing to Disclose: SMM, MIE, EEM, RAM, GT

P1-54

MiRNAs that Define Breast Cancer Phenotypes.

DR Cochrane PhD¹, EN Howe BS¹, EL McKinsey BS¹, BM Jacobsen PhD¹, SM Anderson PhD¹ and JR Richer PhD¹.

¹Univ of Colorado Denver Aurora, CO.

To identify miRNAs associated with estrogen receptor alpha (ER) status in breast cancer, we performed miRNA profiling of ER+ luminal A (MCF7 and T47D) versus ER- triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and BT549). Members of the miR-200 family show the highest fold difference, with miR-200c being 53 fold more abundant in Luminal A type cells. MiR-200c has been shown to be critical to the epithelial phenotype and is always associated with ER positivity. We find that miR-200c represses a program of mesenchymal and neuronal genes which are not normally expressed in epithelial cells, but are aberrantly expressed in carcinoma cells that have undergone epithelial to mesenchymal transition. These include fibronectin, moesin, NTRK2 and class III beta-tubulin, which are involved in migration, invasion, resistance to anoikis, and taxane resistance respectively. Additional miRNAs more abundant in luminal A cells control key genes involved in metabolism. Aggressive breast tumors overexpress the glucose transporter GLUT1, resulting in increased glucose uptake. They also rely on *de novo* fatty acid synthesis and often overexpress fatty acid synthase (FASN). We demonstrate that miR-193b, miR-15b and miR-103/107 directly target FASN and miR-301 and miR-148a directly target GLUT1. Restoration of these miRNAs to TNBC cells dramatically reduces FASN and GLUT1 protein levels and reduction in FASN leads to apoptosis. Although the majority of differentially expressed miRNAs are higher in luminal A cells, some are higher in TNBC. For instance, miR-222/221 levels are over 90 fold higher in TNBC cell lines and are only expressed in ER negative clinical specimens. Interestingly miR-222/221 and miR-29 (the top miRNAs more abundant in TNBC cells) directly target Dicer itself. Using mimics and inhibitors of these miRNAs we can modulate Dicer protein levels. Lower Dicer levels could explain why most miRNAs are less abundant in TNBC. We also identify miRNAs regulated by estradiol and progesterone. MiR-7 is upregulated by E2 and higher in ER+ cells, and when we increase its levels in TNBC cells, it represses EGFR, IGF1Rbeta and IRS2. Collectively, our data indicate that specific miRNAs control distinguishing characteristics of luminal A versus TNBC and may influence their aggressive clinical behavior. Manipulation of these miRNAs may have potential as a treatment for TNBC, for which there are currently no optimal therapeutics.

Nothing to Disclose: DRC, ENH, ELM, BMJ, SMA, JRR

P1-55

Selective Down-Regulation of Vitamin D Receptor Expression Is Associated with Snail-Induced Epithelial to Mesenchymal Transition in Breast Cancer Stem-Like Cells.

M Braga BS¹, M Hewison PhD², S Pervin PhD^{1,2} and R Singh PhD^{1,2}.

¹Charles Drew Univ of Med and Sci Los Angeles, CA and ²David Geffen Sch of Med, UCLA Los Angeles, CA.

Introduction: Vitamin D has been shown to have potent anti-proliferative effects on breast cancer cells. The expression of vitamin D receptor (VDR) is an important determinant in breast tumor cell's response to vitamin D. Many breast cancer lose sensitivity to vitamin D due to loss of VDR expression. Snail has been reported to inhibit VDR expression and activity by binding to its promoter region. We propose that vitamin D resistance occurs in breast cancer stem cells due to the down-regulation of the vitamin D signaling pathway associated with the induction of snail, a key component of the epithelial-mesenchymal transition.

Methods: Breast cancer stem-like cells isolated from breast cancer cell lines MCF-7 and SKBR3 were grown under non adherent culture conditions. The mammospheres were characterized for embryonic stem cell markers Sox2, Oct4, Klf4, c-myc, and nanog, by immunofluorescence and real time quantitative PCR (qPCR). They were also analyzed for the expression of CD44+, ESA+ and CD24-/absent, key breast cancer initiating markers, and for their ability to differentiate into various lineages by analyzing specific cell-surface antigens, such as CK14 and CK18. Expression levels VDR target genes were analyzed by qPCR and western blot analysis. Cells grown on high attachment plates and as mammospheres were treated with various doses of 1,25(OH)₂ Vitamin D (0-100nM) for 0-7 days. In some cases cells were simultaneously treated with 1mM DETA-NONOate. The size and number of mammospheres and total number of cells were analyzed.

Results and conclusions: Breast cancer stem cells which expressed CD44+, ESA+, and CD24-/low exhibited at least a 2-fold induction of key stem cell markers. VDR protein (~75%) and mRNA (55+/- 6 %) levels were down regulated in breast cancer-like stem cells. The protein levels of downstream VDR targets such as RXR- α and PAI-1 were significantly down regulated in comparison to the cells grown on high attachment plates. Expression levels e-cadherin was down regulated (~68%) and the expression of Snail was up regulated by 2.2 fold. Unlike cells grown on high attachment plates, stem-like cells were resistant to vitamin D treatment, had increased expression of anti-apoptotic proteins and expressed stable nuclear phospho-ERK. A combination of DETA-NONOate, a NO-donor, and Vitamin D was highly effective in reducing the number and size of these stem-like cells, suggesting that combination therapy should be explored in Vitamin D resistant breast cancer.

Nothing to Disclose: MB, MH, SP, RS

P1-56

Breast Tumor Kinase (Brk/PTK6) Mediates HGF-Induced Met Receptor Signaling to Cell Migration in Keratinocytes and Breast Cancer Cells.

NE Castro M.S.¹ and CA Lange Ph.D.¹.

¹Univ of Minnesota, Masonic Cancer Ctr Minneapolis, MN.

Breast tumor kinase (Brk/PTK6) is a non-receptor, soluble, tyrosine kinase that was cloned from a human metastatic breast tumor. Brk is overexpressed in numerous types of cancer, including the majority of breast tumors and breast cancer cell lines, but is not found in normal breast tissues. Previously, our lab demonstrated the importance of the ErbB ligand, heregulin- β 1, in activating Brk kinase activity downstream of rac-1 and upstream of ERK5 and p38 MAP kinases. Herein, we show that hepatocyte growth factor (HGF) and macrophage stimulating factor (MSP), peptide ligands specific for c-Met and Ron receptors, respectively, activate Brk kinase activity in Brk+ keratinocytes (HaCat cells) and breast cancer cell lines (MDA-MB-231 and T47D cells). Brk gene silencing studies revealed that HGF, but not MSP, induced robust Brk-dependent cell migration. Brk and ERK5 associated in HGF-induced protein complexes in both cell types; Brk/ERK5 complexes formed independently of Brk kinase activity in COS cells. ERK5 was required for breast cancer cell, but not keratinocyte cell migration, which became ERK1/2-dependent upon ERK5 knock-down. Notably, the protein tyrosine kinase activity of Brk was not required for HGF-induced cell migration, as indicated by rescue experiments. Further, expression of either wt or kinase-inactive Brk in Brk-null MDA-MB-435 cells activated ERK5 and conferred increased HGF-induced cell migration. These results have identified Brk and ERK5 as important downstream effectors of c-Met signaling to cell migration. MET receptors are emerging as important therapeutic targets for advanced breast cancer. Targeting downstream ERK5 kinase activity or inhibiting the formation of Brk/ERK5 complexes may provide an additional means of blocking cell migration associated with breast cancer progression towards metastasis.

Sources of Research Support: NIH Grants R01 CA107547-01A1 (to CAL), and NIH/NCI; Univ of Minnesota Masonic Cancer Ctr Training Grant CA009138 (to NEC).

Nothing to Disclose: NEC, CAL

P1-57

Novel ALK5 Inhibitor Inhibits Breast Tumor Metastasis and Increases the Interactions of SnoN and Ski to the Promoters of TGF β Target Genes.

Jee Y Son BS¹, Eun J Yoon BS¹, Chul Y Park Ph.D¹, Dae-Kee Kim Ph.D¹ and Yhun Y Sheen Ph.D¹.

¹Ewha Womans Univ Seoul, Republic of Korea.

We have synthesized novel ALK5 inhibitors and investigated their effects on breast cancer metastasis MDA-MD-231 cells as well as tumor bearing MMTV/cNeu transgenic mice and orthotopically injected mice. The treatment of ALK5 inhibitors inhibited lung metastasis in breast tumor bearing MMTV/cNeu transgenic mice and orthotopically injected mice compared to that of untreated animal. ALK5 inhibitor decreased the number of metastatic nodules in the lung of the breast tumor bearing MMTV/cNeu transgenic mice and orthotopically injected mice compared to that of untreated animal. In these animals as well as MDA-MB-231 breast cancer cells, ALK5 inhibitors increased SnoN and Ski proteins. These effects of ALK5 inhibitors on SnoN and Ski proteins seemed to be mediated through blocking the proteosomal degradation of SnoN and Ski based on MG132 treatment that nullified the effect of ALK5 inhibitor. Furthermore, ALK5 inhibitors decreased the expression of Arkadia, E3 ubiquitin ligase which is known to be involved in TGF β mediated SnoN and Ski protein degradation. The results of the ChIP analysis of the promoters of TGF β target genes such as PAI-1, Smad7, and ID1 confirmed ALK5 inhibitor stimulated the interactions of SnoN, and Ski to these promoters as co-repressors. ALK5 inhibitors blocked the TGF- β stimulated phosphorylation of smad2/3 based from the western blot analysis and confocal microscopic analysis. And also, ALK5 inhibitors showed strong inhibition of TGF β induced wound healing and invasion of MDA-MB-231 cells. This data strongly suggested that ALK5 inhibitors have anti-metastasis effects via inhibiting ALK5 activity in vivo and in vitro.

Nothing to Disclose: JYS, EJY, CYP, D-KK, Y-YS

P1-58

Androgen Receptor Is a Tumor Suppressor in DMBA-Induced Mammary Tumors.

Ulla Simanainen PhD¹, Kirsty A Walters PhD¹, Geoff Watson MB BS², Yan R Gao BSc¹ and David J Handelsman MB BS, PhD¹.

¹ANZAC Res Inst, Univ of Sydney Sydney, Australia and ²RPA Hosp Sydney, Australia.

Hormones, notably estrogens, are pivotal in the origins of breast cancer but androgenic effects remain controversial. To determine the role of the androgen receptor (AR) in experimental mammary tumorigenesis we generated homozygous androgen receptor knockout (ARKO) female mice that are androgen resistant due to Cre/LoxP recombination producing in-frame excision of exon 3 of the Ar gene (ArΔEx3). ArΔEx3 translates mutant AR lacking the second zinc finger and thereby unable to bind DNA and activate genomic AR signalling. To induce mammary tumors, wild-type (WT) and ARKO female mice were treated with 1mg 7,12-dimethylbenz[a]anthracene (DMBA) in 100μl of peanut oil by gavage, weekly for 6 weeks. The onset of palpable mammary tumors was significantly faster in ARKO females (median 20 (9-31) weeks [95%CI], n=17) than in WT females (34 [31-37] weeks, n=16; p=0.003; Kaplan-Meier survival analysis), leading to a higher cumulative incidence in the ARKO females at 9 months (79±11% [mean ± SD] vs. 62±17%). By contrast, the development of skin cancer had a significantly lower cumulative incidence in ARKO females (13±12% vs. 90±10%, p=0.048), demonstrating a tissue specific role of AR inactivation on DMBA-induced tumorigenesis.

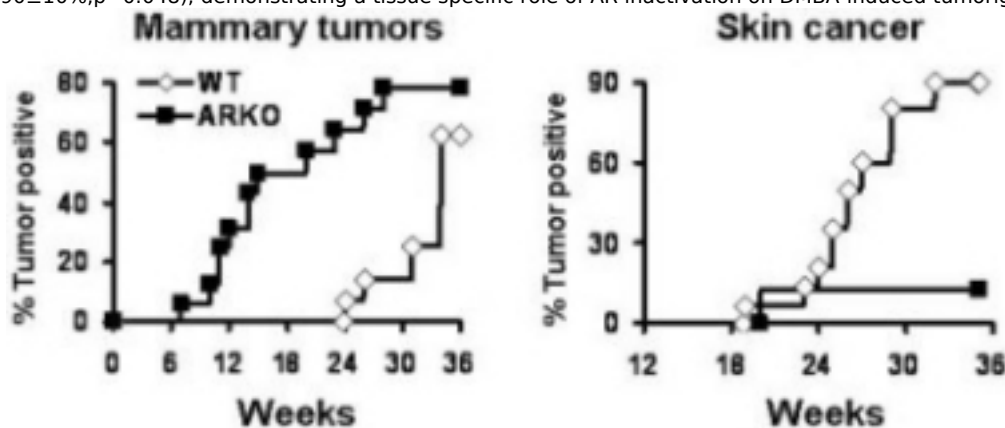


Figure: Proportion of ARKO and WT female mice with palpable mammary and skin tumors. To explore the mechanisms involved, intact mammary glands from adult WT and ARKO virgin females were compared. ARKO mammary glands displayed normal structural and functional development, normal progesterone and estrogen receptor levels, but altered cell cycle signaling compared with WT mammary glands, indicating that the increased ARKO susceptibility to DMBA-induced mammary tumorigenesis was due to altered molecular mechanisms rather than structural changes in mammary gland development or morphology. Our results provide the first definitive evidence for AR-mediated androgen actions as tumor suppressors in experimental breast cancer. These findings support further evaluation for targeting AR to improve endocrine therapy at all stages of human breast cancer.

Nothing to Disclose: US, KAW, GW, YRG, DJH

P1-59

Tissue-Specific Crosstalk between Estrogen and Growth Hormone Contributes to Mammary Gland Growth and Development in the Spontaneous Dwarf Rat.

DL Felice PhD¹, Q Shen¹, D Lantvit¹, SM Swanson PhD¹, TG Unterman PhD¹ and JM Frasor PhD¹.

¹Univ of Illinois at Chicago Chicago, IL.

Estrogen (E2) and growth hormone (GH) play important roles in promoting growth and development of the breast, as well as initiation and growth of hormone-dependent breast tumors. Studies in Spontaneous Dwarf Rats (SDR), which have undetectable levels of GH, demonstrate that GH is required for N-methyl-N-nitrosourea-induced mammary carcinogenesis (1, 2). Approximately 90% of tumors in the SDR express the estrogen receptor (ER) and are dependent upon both E2 and GH for continued growth (1-3). To better understand the interactions between E2 and GH in the breast, we examined the tissue-specific crosstalk between these hormones in SDR and a human breast cancer model. E2 and GH have opposing effects on liver IGF-I production and body weight, where GH-treated SDR have higher body weights than animals treated with E2+GH, consistent with previous studies (4). There is no crosstalk between E2 and GH in the uterus, with GH having no effect on E2-stimulated uterine weight and progesterone receptor expression. In contrast, positive crosstalk between E2 and GH occurs in both the mammary gland (MG) of SDR and in T47D breast cancer cells as demonstrated by enhanced cell proliferation by the combination of E2+GH in both models. One mechanism underlying this positive crosstalk involves GH potentiation of E2 action. GH increases ER α expression in the MG and enhances the transcription of ER target genes in response to E2. A second mechanism involves potentiation of GH action by E2 via effects on Stat5 signaling. GH and E2 both increase Stat5 phosphorylation in mammary epithelial cells as determined by immuno-histochemistry and increased expression of Elf5, a known Stat5 target gene. This enhanced Stat5 activation occurs despite the fact that E2 up-regulates SOCS2 and GH up-regulates SOCS1, both negative regulators of the Jak/Stat pathway. In summary, E2 and GH demonstrate positive crosstalk specifically in the mammary gland, which occurs at the level of increased ER and Stat5 transcriptional activity. A deeper understanding of E2/GH crosstalk may help to reveal underlying mechanisms in both normal MG development and the initiation and progression of cancer specific to the breast. In turn, this may lead to drug design or combination therapies with improved efficacy and fewer off-target effects.

(1) Swanson and Unterman, *Carcinogenesis* 2002; 23:977

(2) Thordarson et al., *Breast Cancer Res Treat* 2004; 87:277

(3) Martin et al., *Cancer Invest* 1997; 15:8

(4) Borski et al., *Endocrinology* 1996; 137:3253

Sources of Research Support: NIH RO1 CA130932-01A2 (JMF); NIH Training Grant HL07692-20 (DLF).

Nothing to Disclose: DLF, QS, DL, SMS, TGU, JMF

P1-60

Rapid Activation of cPLA₂α Induced by Estrogen in ER-Positive and ER-Negative Breast Cancer Cell Lines.

F Caiazza¹, W Thomas PhD¹ and BJ Harvey PhD¹.

¹RCSI Dublin, Ireland.

The cytosolic phospholipase A2 (cPLA₂α) catalyzes the hydrolysis of membrane glycerophospholipids to release arachidonic acid, which is converted to bioactive eicosanoid lipid mediators, including prostaglandins (like PGE₂) produced through cyclooxygenases (COX), promoting activation of downstream proliferative cell signalling pathways. The eicosanoid signalling contributes to cell proliferation in breast cancer, as demonstrated by numerous studies outlining a crucial role of COX-2 and PGE₂ in breast carcinoma progression. The specific role of cPLA₂α however, is not established. Recent work from our group demonstrated that 17β-estradiol (E2) rapidly activated cPLA₂α in the breast cancer-derived MCF-7 cell line (1), leading to the hypothesis that the rapid release of bioactive lipids may play a role in the proliferative signalling responses stimulated by E2 in breast cancer cells (2). We have used model breast cancer cell lines differentially expressing the estrogen receptor (ER) to study the molecular mechanism involved in the E2-induced rapid activation of cPLA₂α, and we show here that this was dependent on specific ER-mediated trans-activation of EGFR/HER2 heterodimers and downstream signalling through ERK1/2 MAPK to phosphorylate cPLA₂α on Ser505. E2 also promoted cPLA₂α trafficking to perinuclear membranes, and this effect was subsequent to, and dependent on, MAPK-induced phosphorylation on Ser505. The endocrine-resistant SKBR3 cell line, which over-expresses EGFR and HER2 and is ER-negative, showed elevated cPLA₂α expression and activity compared to MCF-7 (3). E2 promoted rapid activation of cPLA₂α in this endocrine-resistant cell line through EGFR/HER2 trans-activation, and this effect was mediated by the G protein-coupled receptor GPR30. Inhibition of cPLA₂α with a specific antagonist or by siRNA-mediated gene silencing suppressed the growth of both cell lines, by reducing E2-induced proliferation and by stimulating cellular apoptosis and necrosis. cPLA₂α is likely to play a key role in regulating the already established growth-promoting effects of estrogen and COX-2 in breast cancer, balancing the cytotoxic effects of free arachidonic acid with the proliferative effects of prostaglandins. This study highlights a role for cPLA₂α in the E2-induced growth of both endocrine-sensitive and endocrine-resistant breast cancer cells.

(1)Thomas W et al., *Steroids* 2006; 71(3):256-265

(2)Thomas W et al., *Front Biosci* 2008; 13:2604-13

(3)Caiazza F et al., *Eur J Cancer Supplements* 2009; 7(3):34LBA

Sources of Research Support: Higher Education Authority of Ireland; RCSI Research Committee.

Nothing to Disclose: FC, WT, BJH

P1-61

Role of Wnt Signaling Pathway in Adrenal Tumorigenesis: Mutations of β -Catenin and APC in Adrenocortical Tumors.

Y Lee MD¹, HY Cho MD¹, SH Kwak MD¹, HY Ahn MD¹, HS Jung MD, PhD¹, CS Shin MD, PhD¹, SY Kim MD, PhD¹, YA Kim MD, PhD¹ and SW Kim MD, PhD¹.

¹Seoul Natl Univ Coll of Med Seoul, Korea.

Backgrounds: Most sporadic adrenal tumors are monoclonal, suggesting that a somatic genetic defect occurs early during tumorigenesis. To date, somatic mutations of TP53, PRKARIA and IGF-II have been reported. Activating mutations of the Wnt signaling pathway have been observed in more frequent cancers. The genetic alterations of Wnt signaling pathway identified to date in adrenocortical tumors are limited. We investigated whether Wnt pathway activation is involved in adrenocortical tumorigenesis.

Methods: Patients included in the study were investigated for a sporadic adrenocortical adenomas in the Endocrinology department of Seoul National University Hospital January 2001 to April 2008. For mutation analysis, exon 3 sequences of the β -catenin gene and exon 16 sequences of the APC gene were amplified by PCR from tumoral DNA.

Results: Enrolled 38 cases (29 females) of adrenocortical adenomas were consisted of cortisol producing adenoma (N=11), aldosterone producing adenoma (N=35) and non-functioning adenoma (N=3). All cases are sporadic adrenocortical tumor. None presented features of any adrenocortical tumor-predisposing syndromes (Beckwith-Wiedemann, Carney complex, McCune Albright, Multiple Endocrine Neoplasia type 1, or Li-Fraumani syndrome). All of the patients had localized tumors (McFarlane stage I). Two somatic mutations of β -catenin gene were observed in 1 case of 38 cases (2.6%) which was cortisol producing adenoma. The mutations were point mutations altering the Q76R and A80T of exon 3. Six genetic alterations of the APC gene were observed in 5 cases of 34 adrenocortical tumors (14.7%). All of the mutations were point mutations; one case is E1345K (aldosterone producing tumor), two cases were S1421I (aldosterone producing tumors), two cases were L1423F (aldosterone producing tumors), and other one case was P1372P (cortisol producing tumor). The 26.3% of aldosterone producing tumors had point mutation of APC gene.

Summary: Genetic mutations of genes involving Wnt signaling pathway in adrenocortical adenomas were observed frequently. Especially, aldosterone producing tumor showed frequent APC gene mutation. This finding may contribute to understanding of tumorigenesis of adrenocortical tumors.

Nothing to Disclose: YL, HYC, SHK, HYA, HSJ, CSS, SYK, YAK, SWK

P1-62

Loss of Imprinting in the Mouse Adrenal Cortex: A Potential Role in Adrenocortical Carcinoma.

FM Barlaskar BS¹ and GD Hammer MD PhD¹.

¹Univ of Michigan Med Sch Ann Arbor, MI.

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with a poor clinical prognosis and a lack of efficacious therapeutic regimens. However, data is emerging regarding the molecular determinants involved in its initiation and/or progression. We recently published a preclinical study targeting the IGF signaling pathway in adrenocortical carcinoma (ACC) as IGF2 was the single highest expressed gene in over 90% of these cancers. Closer examination of the 11p15.5 chromosomal region revealed the majority of ACCs possess a remarkably similar expression profile observed in Beckwith-Wiedemann Syndrome, thus suggesting Loss of Imprinting (LOI) may be a common pathomechanism employed by both sporadic and hereditary forms of ACC. Here, we present data comparing pyrosequenced DNA derived from normal human adrenal cortices, adrenal adenomas and ACCs. Several critical CpG islands contained throughout the IGF2 genomic region were differentially methylated in tumors when compared to normal tissues, giving additional credence to dysregulated imprinting. Additionally, we modeled 11p15.5-specific LOI through a Cre recombinase-mediated deletion of a differentially methylated domain (DMD), leading to IGF2 overexpression in the mouse adrenal cortex. First, we examined IGF2 expression and observed a two-fold increase above IGF2 transcript levels observed in control adrenal tissues. Also, immunoblotting of adrenal lysates revealed increased phospho-Akt, indicative of active IGF-mediated signaling. Histologic examination of adrenals at 15 weeks revealed evidence of cortical enlargement and, in some cases, displaced medullar tissue. Intriguingly, β -catenin immunohistochemistry revealed a stronger subcapsular signal in DMD knockout adrenals. This observation was confirmed with immunoblotting for total β -catenin and unphosphorylated β -catenin, a marker of active canonical Wnt signaling. Conversely, examination of 30 week adrenals revealed evidence of cortical failure, suggesting exhaustion of tissue-specific progenitors. Our group and others have previously shown active canonical Wnt signaling in the mouse cortex results in dysplastic tissue that progresses to adenomas and, in certain cases, adrenocortical carcinoma. These findings posit a novel role of IGF-II in mediating activation of canonical Wnt signaling and merits further experimentation to determine a possible mechanism and functional consequence of this crosstalk.

Nothing to Disclose: FMB, GDH

P1-63

The Targeted Cytotoxic Somatostatin Analog AN-162 Has Pronounced Growth Inhibitory and Apoptotic Effects on SW-13 Human Adrenal Carcinoma Cells in Culture.

M. Moreno M.D.^{1,2}, J.W. Brown Ph.D.^{1,2}, C. Perez-Stable Ph.D.^{1,2}, A.V. Schally Ph.D., M.D.(hc)^{1,2} and L.M. Fishman M.D.^{1,2}.

¹VA Med Ctr Miami, FL and ²Univ of Miami Miller Sch of Med Miami, FL.

Somatostatin and its octapeptide analogs are known to exert cytostatic effects on a number of malignancies through specific membrane receptor subtypes (SSTR) which are often significantly over-expressed in highly malignant tumors. In previous studies, we have shown that SW-13 human adrenal carcinoma cells strongly express SSTR 2 and weakly express SSTR subtypes 1, 4 and 5. In this study we demonstrate that the SSTR 2 targeted, doxorubicin-linked somatostatin analog AN-162 not only strongly inhibits the growth of SW-13 cells in culture but triggers apoptosis to an extent greater than that seen with doxorubicin alone. SW-13 cells were incubated in Dulbecco's Modified Eagle's Medium supplemented with 10 % heat-inactivated fetal calf serum, as well as penicillin, streptomycin and amphotericin B. When cultures reached confluence, they were washed and new medium was added containing either AN-162, doxorubicin (each 10^{-6} M) or control vehicle (0.1 % v/v DMSO). Cultures were incubated for 2 - 6 days. Cell cycle analysis studies by flow cytometer showed that treatment of cells with 1 micromolar AN-162 for as little as two days resulted in a substantial accumulation of sub-G1 nuclei, indicating unspecified toxicity. Subsequent Western blot studies of the apoptosis marker, cleaved PARP (poly-ADP-ribose polymerase) levels clearly demonstrated that this toxicity is in part the result of increased apoptosis. Furthermore, cleaved PARP levels were increased more strongly in AN-162 treated cells than in cultured cells treated with doxorubicin alone. Six day incubations with either AN-162 or with doxorubicin by itself at different doses, however, yielded similar EC50s (approximately 10^{-8} M for both compounds). These *in vitro* data suggest that *in vivo* studies of targeted cytotoxic somatostatin agonists such as AN-162 may be warranted in an effort to develop more effective therapies for human adrenal cortical carcinoma.

Sources of Research Support: U.S. Department of Veterans Affairs and the South Florida Veterans Affairs Foundation for Research & Education.

Nothing to Disclose: MM, JWB, CP-S, AVS, LMF

P1-64

Angiogenic Factors in Adrenal Tumors and Response of the Adrenal Cells to a Multikinase and mTOR Inhibitors.

B Mariniello¹, B Rubin¹, A Patalano¹, MV Cicala¹, R Pezzani¹, I Finco¹, M Iacobone² and F Mantero¹.

¹Div of Endocrinology Padova, Italy and ²Div of Endocrine Surgery Padova, Italy.

VEGF overexpression in adrenocortical carcinomas (AC) has been recently shown. The founding of VEGF receptors (VEGFR1 and VEGFR2) on adrenal cancer cells suggests a possible autocrine VEGF effect on cell growth. Sorafenib, a multikinase inhibitor, inhibits VEGF-stimulated phosphorylation of VEGFR2 and induces complete tumor stasis in vivo in several tumors. RAD001 inhibits mTOR (Mammalian Target Of Rapamycin), which has a key role in regulating basic cellular function, among which proliferation, survival and angiogenesis.

The aim of this study was: to examine the VEGF, VEGFR1-2 expression in 15 AC compared to 29 aldosterone producing adenomas (APA) and 7 normal adrenals (NA) analyzed by real-time PCR and immunohistochemistry; to examine the effects of Sorafenib and RAD001, alone or in combination, on cell viability (MTT-test) and apoptosis (TUNEL-system by flow cytometry) of tumoral adrenal cells (SW13, H295R).

The expression of VEGF and its receptors was confirmed in our adrenal samples; these genes were particularly over-expressed in some AC. A dose-dependent (0.1nM to 1microM) inhibition of cell viability was observed particularly in SW13 after 24h of treatment with both drugs. Besides, this combination induced a marked inhibition of cell viability in SW13. Finally, the TUNEL-assay showed an increase of apoptosis and a cell cycle alteration in the adrenal cells treated with Sorafenib and RAD001.

Our data suggest that an autocrine VEGF loop may exist within AC. Furthermore, a combination of molecular targeted agents may have both antiangiogenic and direct anti-tumoral effects and could represent a new therapeutic tool for treatment of AC.

Sources of Research Support: AIRC.

Nothing to Disclose: BM, BR, AP, MVC, RP, IF, MI, FM

P1-65

Neuropeptide Hormone Receptor Expression in Human Adrenal Tumors and Cell Lines: Antiproliferative Effects of Peptide Analogues.

CG Ziegler Dr.¹, JW Brown Prof. Dr.², AV Schally Prof. Dr.², A Erler¹, L Gebauer¹, G Eisenhofer Prof. Dr.¹, M Ehrhart-Bornstein Dr.¹ and SR Bornstein Prof. Dr.¹.

¹Univ Hosp Carl Gustav Carus Dresden, Germany and ²Univ of Miami Miller Sch of Med Miami, FL.

Anti-tumor therapy based on receptor-targeted radiotherapy or gene transfer methods have been successfully employed in various cancers. In this study we evaluated specific receptor-targeted chemotherapeutic peptide antagonists and agonists for potential future use in antineoplastic therapy of adrenal tumors. Based on our own microarray data which revealed a significant and differential expression of somatostatin-type-2 receptor (sst2), growth hormone releasing hormone (GHRH) receptor and luteinizing hormone releasing hormone (LHRH) receptor in human pheochromocytoma subtypes, we comprehensively analysed expression of these receptors in adrenal tumors of both the medulla and cortex on mRNA and protein level. Furthermore, we tested antiproliferative effects of peptide analogues targeting these receptors. Cytotoxic derivatives of somatostatin AN-238 and to a lesser extent AN-162 reduced cell numbers of uninduced and NGF-induced adrenomedullary PC-12 pheochromocytoma cells. Ultrastructural analysis confirms a pro-apoptotic mode of cell death induced by AN-238. Adrenocortical SW-13 tumor cells were found to additionally express receptors for GHRH and LHRH. The non-cytotoxic somatostatin receptor agonist RC-160, the cytotoxic GHRH receptor antagonist MZ-4-71 and the cytotoxic LHRH receptor antagonist Cetrorelix all significantly reduced cell growth of SW-13 cells. In conclusion, in this study we could demonstrate a significant antiproliferative effect of peptide analogues targeting certain neuropeptide hormone receptors, being overexpressed on adrenal tumors. Our results raise hope for improved targeted treatment strategies for rare but frequently lethal adrenal cancers.

Nothing to Disclose: CGZ, JWB, AVS, AE, LG, GE, ME-B, SRB

P1-66

Interactive Effects of Epidermal Growth Factor Receptor (EGFR) and Na⁺/Glucose Transporter 1 (SGLT-1) Inhibitors on Viability of Adrenocortical Tumor Cells.

Takafumi Taguchi¹, Yasumasa Iwasaki¹, Mitsuru Nishiyama¹, Makoto Tsugita¹, Yoshinori Taniguchi¹, Mizuho Okazaki¹, Shuichi Nakayama¹, Toshihiro Takao¹, Fumio Otsuka² and Yoshio Terada¹.

¹Kochi Med Sch, Kochi Univ Kohasu, Oko-cho, Nankoku, Japan and ²Okayama Univ Graduate Sch of Med Okayama, Japan.

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy that sometimes presents with symptoms of hormone excess, including hypertension and diabetes, and carries an extremely poor prognosis. Traditional medications seldom afford long-term control. EGFR kinase inhibitors provide targeted oncologic treatment and have, in recent years, been reported to be partly effective for advanced adrenal cancer. EGFR facilitates glucose transport into cells by associating with and stabilizing SGLT-1. In this study, we evaluated the effect of a kinase inhibitor (AG1478) in combination with a SGLT-1 inhibitor (phlorizin) on the viability of two human adrenocortical carcinoma cell lines, H295R and SW13. We confirmed the expression of SGLT homologs, EGFR and other key genes involved in glucose metabolism in these cells and in an ACC tissue sample obtained from a patient with diabetes and hypertension. In the APOPercentage Apoptosis Assay, AG1478 alone induced concentration-dependent tumor cell death, whereas phlorizin alone had no effect. AG1478 in combination with phlorizin exerted an additive inhibitory effect on ACC cell growth. PCR-array gene expression profiling suggested the involvement of some genes associated with glucose metabolism in these additive inhibitory effects. These results demonstrate that the combined use of EGFR and SGLT-1 inhibitors impair glucose metabolism in tumor cells and inhibit ACC cell growth.

Nothing to Disclose: TT, YI, MN, MT, YT, MO, SN, TT, FO, YT

P1-67

2-Methoxyestradiol and ENMD-1198 Have Strong Growth Inhibitory and Differential Apoptotic Effects on SW-13 Human Adrenal Carcinoma Cells in Culture.

S. Casula M.D.^{1,2}, J.W. Brown Ph.D.^{1,2}, C. Perez-Stable Ph.D.^{1,2}, R. Parrondo¹ and L.M. Fishman M.D.^{1,2}.

¹Univ of Miami Miller Sch of Med Miami, FL and ²VA Med Ctr Miami, FL.

Metastatic adrenal cortical carcinoma is a highly aggressive and almost universally fatal malignancy. While surgical resection is the primary treatment in early disease, it has significant long-term effectiveness only in individuals with non-metastatic cancer. There are a number of established pharmacologic options for treatment of metastatic disease but all of these have limited benefit, as well as significant adverse side-effects. The 2-methoxy derivative of estradiol (2-MeE2) is an experimental drug which is currently undergoing clinical trials for the treatment of prostate cancer. Although this compound has little estrogenic effect, it is known to be a microtubule-altering agent and is capable of inhibiting cell division through this mechanism. We tested 2-MeE2, as well as a related analog, ENMD-1198, for cell growth effects as well as for direct effects on apoptosis over a six day period of time in SW-13 human adrenal carcinoma cells in culture. Treatment of SW-13 cells for six days with 1.0 uM of either 2-MeE2 or ENMD-1198 in Dulbecco's Modified Eagles Medium containing 10 % fetal calf serum (with medium and drug replacement every two days) resulted in dramatic declines in cell survival, which were seen as early as two days in culture. Incubation with 1.0 uM 2-MeE2 for two days reduced the number of cells in each tissue culture dish to 16.3 ± 1.7 % of control values (Mean \pm SE, N =4 and $p < 0.001$ for this and all other comparisons); exposure to 10 uM 2-MeE2 for six days reduced cell number to 0.2 ± 0.03 % of control incubations. ENMD-1198 had a similar but more potent effect at a low dose, reducing cell number to 0.2 ± 0.05 % of control after 1.0 uM incubation for six days. The number of cells per dish remained at approximately this value after incubation with 10 uM ENMD-1198 for the same period. Western blot studies of cleaved PARP levels (an activated caspase 3 marker) indicate that ENMD-1198 acts, in part, through the stimulation of apoptosis at the low dose (1.0 uM) but at this same concentration 2-MeE2 showed very little effect on PARP levels. From our earlier studies using flow cytometry, 2-MeE2's cytotoxic effect at low dose in SW-13 cells appears to be mediated by a G2/M blockade. Collectively, these data indicate that ENMD-1198 is more cytotoxic to SW-13 human adrenal carcinoma cells than 2-MeE2.

Sources of Research Support: U.S. Department of Veterans Affairs and the South Florida Veterans Foundation for Research and Education.

Nothing to Disclose: SC, JWB, CP-S, RP, LMF

P1-68

Nutrients Promote Pancreatic Cancer Stem Cell Metastasis.

Haibo Liu Ph.D.¹, Danshan Huang MD¹ and Anthony Heaney MD, Ph.D.¹.

¹David Geffen Sch of Med at UCLA Los Angeles, CA.

There is emerging evidence that cancer growth and therapy resistance is dependent on a small subset of cancer stem cells (CSC). Pancreatic cancer is a highly lethal disease that is usually diagnosed late and for which there are few effective therapies. Recently, CD24+, CD44+ and CD133+ were identified as surface markers for pancreatic cancer stem cells. Aldehyde dehydrogenase1 (ALDH1) oxidizes retinal to retinoic acid plays a key role in early stem cell differentiation and has been used to isolate breast and colon cancer stem cells. Life style factors including diet can significantly affect cancer susceptibility and growth and recently increased refined fructose consumption has been highlighted as conferring greater pancreatic cancer risk than other sugars. We have demonstrated that the metabolism of fructose and glucose in pancreatic cancer is very different with fructose preferentially providing carbons for nucleic acid synthesis.

Given its role in early stem cell differentiation, we used ALDH1 antibodies and flow activated cell sorting (FACS) to isolate high ALDH1 expressing pancreatic cancer (Panc-I) cells. ALDH1 expressing cells also expressed high CD44 and moderate CD133 and formed 10-fold larger colonies in soft agar assays. To further characterize this subpopulation of ALDH1 positive pancreatic CSC, we used quantitative real-time PCR to measure several genes implicated in pancreatic cancer growth. ALDH1 positive cells exhibited higher expression of CXCR4, a chemokine receptor that promotes angiogenesis and metastasis. Treatment (96h) of the pancreatic cancer stem cells with fructose (5.5 mM, or 0.55 mM) significantly increased CXCR4 expression in comparison to glucose (0.55 mM to 20 mM). In contrast ALDH1 positive HPDE6, normal pancreatic ductal cells did not exhibit increased CXCR-4 expression at baseline or following fructose treatment. These results demonstrated ALDH1 expression delineates a highly invasive pancreatic cancer stem cell that expresses high levels of the cytokine receptor CXCR4 which has been previously shown to correlate with pancreatic cancer metastasis. Importantly, for the first time, we demonstrate that environmental nutrients, in this case, the carbohydrate fructose can specifically induce pancreatic cancer stem cell transcription. These findings have important implications for nutrition in patients with pancreatic and potentially other cancers.

Sources of Research Support: Jonsson Cancer Center.

Nothing to Disclose: HL, DH, AH

P1-69

Parathyroid Hormone Related Protein Expression Increases in Squamous Cell Carcinoma of the Bladder.

LJ Deftos MD¹, DW Burton MS¹, CK Ghaffary BS¹, J Wang-Rodriguez MD¹, WM Bazzi MD¹ and TM Downs MD¹.

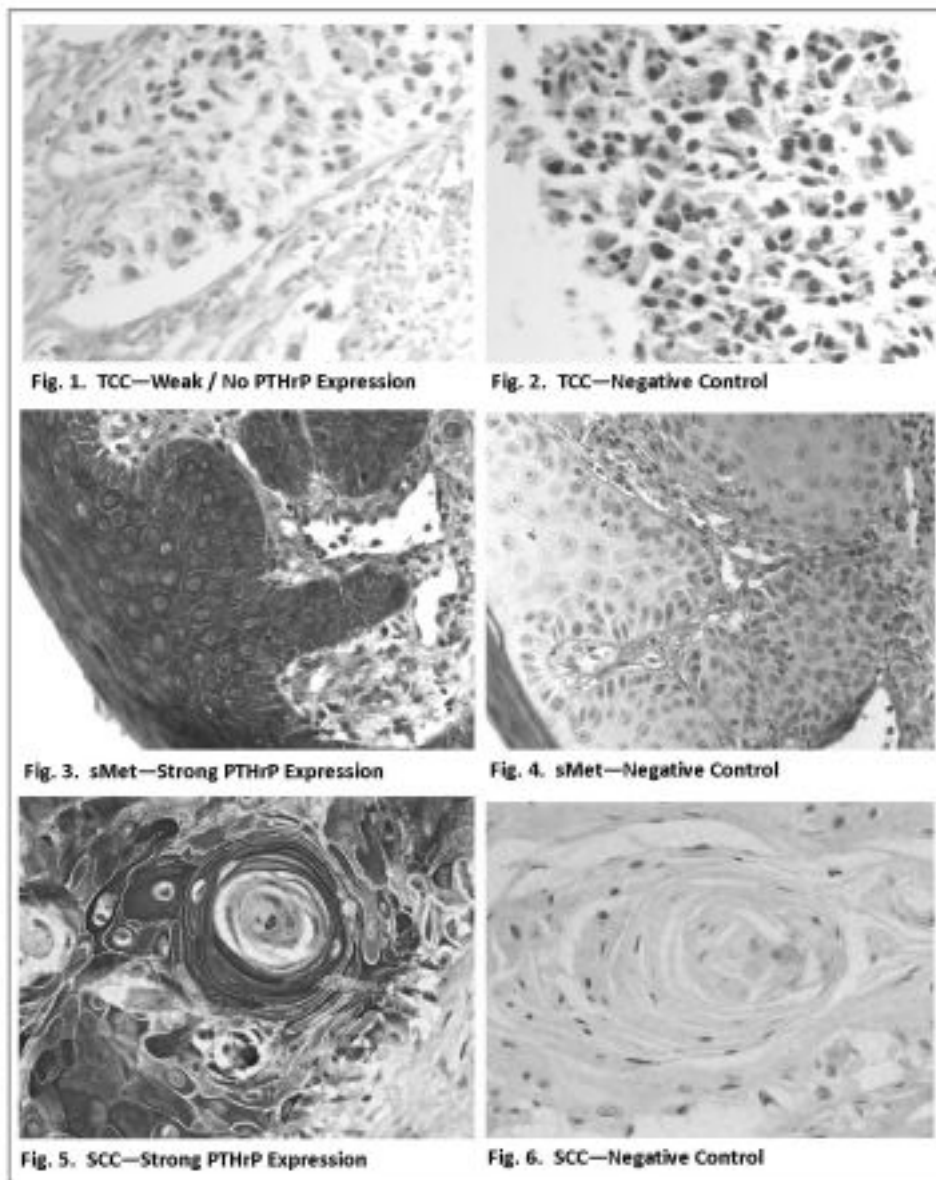
¹Univ of California and Veterans Affairs Healthcare Syst San Diego, CA.

Background: Parathyroid hormone related protein (PTHrP) has been demonstrated to be a product of many malignant tissues where it can regulate growth in vitro and in vivo, but its role in bladder cancer is not defined. Investigators have connected PTHrP with hypercalcemic in squamous cell carcinoma (SCC) patients, as the amino-terminus of PTHrP has sufficient homology to PTH to activate the PTH receptor and produce many of the biological effects of native PTH, including hypercalcemia. In this study, we assessed the level of PTHrP expression in bladder urothelial cells during the progression from normal bladder epithelial cell to SCC.

Methods: Retrospective immunostaining analyses for PTHrP expression in paraffin embedded- formaldehyde fixed specimens from patients who underwent cystectomy at the San Diego Veterans Affairs Hospital from 1993-2009. The number of cases were as follows: SCC (10), Transitional Cell Carcinoma (TCC) (10), Squamous Metaplasia (SMet) (6), and concurrent SCC with TCC (2). We utilized a streptavidin-biotin-DAB immunostaining method with a validated PTHrP mouse monoclonal antibody (9H7) to localize PTHrP and compared the PTHrP staining to serial sections stained with standard H&E.

Results: PTHrP immunostaining was highly selective for squamous differentiation of urothelial tissue in SCC and SMet. The TCC cases had no positive PTHrP immunostaining and adjacent non-neoplastic or no-metaplastic normal bladder transitional cell epithelium did not demonstrate any staining for PTHrP.

Conclusions: PTHrP staining can be an additional tool in the identification of squamous differentiation of urothelial tissue. More studies are needed to understand the role of PTHrP in the progression of bladder cancer.



Nothing to Disclose: LJD, DWB, CKG, JW-R, WMB, TMD

P1-70

Functional Mechanism of Pituitary Tumor Transforming Gene (PTTG) through Regulation of Integrin $\alpha\beta3$ in EMT Induction.

PP Shah Ph.D.¹ and SS Kakar Ph.D.¹.

¹Univ of Louisville Louisville, KY.

Epithelial-mesenchymal transition (EMT) involves changes in the expression, distribution and function of a number of proteins that play critical roles in extracellular matrix (ECM) remodeling or in cell-cell adhesion, such as activation of matrix metalloproteinases (MMPs), E-cadherin, and integrins. The role of EMT has been postulated as an absolute requirement for tumor invasion and metastasis. Several inducers of EMT are transcription factors that repress E-cadherin expression, such as Twist, Snail and Slug. PTTG participates in several key cellular events such as sister chromatid separation, expression and secretion of various growth and angiogenic factors including bFGF, VEGF and IL-8. PTTG levels correlate with the tumor development, growth and metastasis and serve as a marker of malignancy in several cancer types. Several pathways related to oncogenic signaling, epithelial-mesenchymal transition (EMT) and metastasis have been discovered. However the role of EMT in metastasis remains controversial. We hypothesize that PTTG plays important role in EMT induction through the activation of $\alpha\beta3$ integrins in EMT through FAK pathway. To define the molecular importance of PTTG in the induction of EMT, we used HEK293 cells transfected with PTTG cDNA. Using Real Time PCR, we observed that overexpression of PTTG in HEK293 cells resulted in a significant increase in the expression of integrins αv and $\beta 3$ compared to untransfected cells. These results were confirmed by the down-regulation of PTTG expression in ovarian epithelial cancer cell (A2780) with adenovirus expressing PTTG-specific siRNA that showed significant decrease in the expression of integrins αv and $\beta 3$. These results were confirmed by performing FACS analysis and immunostaining for $\alpha\beta3$. Analysis of downstream signaling genes such as Paxillin, Metavinculin and Rac 1 by quantitative real time PCR analysis showed positive relationship between levels of PTTG and these genes. The Vinculin and Phalloidin are responsible for the functional link of FAK to the actin cytoskeleton. Actin cytoskeleton disruption during EMT was confirmed with immunostaining. Based on our data, we conclude that PTTG plays a key role in inducing EMT through the regulation of αv and $\beta 3$ integrins leading to change in Focal Adhesion Kinase (FAK) pathway.

Sources of Research Support: NCI124630.

Nothing to Disclose: PPS, SSK

P1-71

A Glycoprotein from *Laminaria japonica* Induces Cell Cycle Arrest and Apoptosis in HT-29 Colon Cancer Cells.

H Go M.A¹, IH Kim PhD¹, HJ Hwang PhD¹ and TJ Nam PhD¹.

¹Pukyong Natl Univ Busan, Korea.

We isolated a novel glycoprotein from the brown alga *Laminaria japonica* that has antiproliferative effects in HT-29 colon cancer cells. We also identified the mechanism by which this glycoprotein, named LJGP, induces apoptosis. The results of MTS assays showed that LJGP inhibited the proliferation of several cancer cell lines (AGS, HepG2, HT-29) in a dose dependent manner. In HT-29 cells, proliferation was significantly decreased. LJGP treatment on HT-29 cells displayed several apoptotic features, such as, DNA fragmentation, G1 arrest, caspase-3 activation, and PARP degradation. Consistent with G1 arrest, LJGP decreased the expression of Cdk2, cyclinE1, E2F1, and phosphorylated Rb. We also determined that LJGP-induced apoptosis causes via a death-induced signaling complex (DISC) formation of FAS and FADD and procaspase-8. Furthermore, LJGP induced the loss of mitochondrial membrane potential with activation of Bcl-2 family proteins and caspase-9. These findings suggest that LJGP inhibits HT-29 cell proliferation by inducing apoptosis, which may be mediated via both Fas signaling pathway, mitochondrial pathway and cell-cycle arrest. LJGP can be a useful treatment option for colon cancer in humans.

Nothing to Disclose: HG, IHK, HJH, TJN

P1-72

Induction of Apoptosis by a Glycoprotein from *Hizikia fulverscens* in HepG2 Cells.

JA Ryu MA¹, SJ Park MA¹, IH Kim PhD¹, HJ Hwang PhD¹ and TJ Nam PhD¹.

¹Pukyong Natl Univ Busan, Korea.

Hizikia fusiformis is an edible brown alga that is widely consumed in Korea, Japan, and China and possesses a number of potentially beneficial compounds, including antioxidants and anticoagulants. In this study, we extracted a biological glycoprotein from *Hizikia fulverscens* (HFGP) which has anticancer effect against HepG2 cells, and determined the mechanism related with its activity in hepatocyte. In the results of SDS-PAGE, we confirmed that the extract from *H. fulverscens* was a glycoprotein (HFGP) and named it to HFGP. Using the MTS assay and H33342 staining, we obtained HFGP induced cell death in a dose-dependent manner. To determine the mechanism related with the effect of HFGP, cells were treated HFGP and examined expression of several proteins related with apoptosis by Western blotting. We obtained HFGP activated Fas signaling pathway, that is, Fas expression and the formation of Fas and FADD complex. Furthermore, it stimulated the activation of caspase-cascade and PARP cleavage, which is a substrate of caspase-3. Therefore, we suggest that HFGP could be a potential source of bio-functional food to have anticancer effect in hepatocyte.

Nothing to Disclose: JAR, SJP, IHK, HJH, TJN

P1-73

Wnt Signalling in Oestrogen-Induced Lactotroph Proliferation.

AA Giles¹, F Madec¹, S Friedrichsen¹, K Featherstone¹, T Chambers¹, J Resch², G Brabant² and JRE Davis¹.

¹Univ of Manchester Manchester, UK and ²The Christie Hosp Manchester, UK.

Oestrogen has long been known to exert a proliferative effect on pituitary lactotrophs, though the mechanism remains elusive. We previously demonstrated that Wnt-4, a developmental protein known to play a role in pituitary cell-fate determination, is upregulated during oestrogen-induced lactotroph hyperplasia in the Fischer 344 rat. We show here using dual-immunofluorescence staining that Wnt-4 is expressed in all of the endocrine cell types in the anterior pituitary. Wnt-4 was also detected in the intermediate lobe and the marginal zone, a region adjacent to the Rathke's cleft thought to harbour progenitor cells, and Wnt-4 expression in the marginal zone was markedly increased during lactation or after diethylstilbestrol treatment.

The effects of oestrogen on the Wnt canonical signalling pathway were assessed using the TopFlash TCF-dependent transcriptional reporter system. Oestrogen, Wnt-4, LiCl and Wnt-3A were unable to induce expression of luciferase in somatolactotroph GH3 cells, whereas LiCl and Wnt-3A induced robust signals in HEK-293 cells (27-fold and 5-fold respectively). Furthermore, a constitutively active form of β -Catenin was unable to induce luciferase expression in GH3 cells, while it strongly induced TopFlash reporter expression in HEK-293 cells, suggesting that the canonical pathway does not function in GH3 cells. Oestrogen was also unable to induce nuclear translocation of β -Catenin in GH3 cells, primary cultures of dispersed pituitary cells or pituitary tissue from rats treated *in vivo* with oestrogen. Together, these data suggest that the canonical signalling pathway is not activated by oestrogen in the pituitary.

In summary, expression of Wnt-4 in the adult pituitary is subject to hormonal regulation, indicating that this developmental factor is involved in pituitary plasticity. However, it does not appear to activate the canonical signalling pathway, suggesting that Wnt-4 may be acting through β -catenin-independent pathways.

Sources of Research Support: BBSRC PhD Studentship to AAG and the Wellcome Trust (Grant project number - 082794).

Nothing to Disclose: AAG, FM, SF, KF, TC, JR, GB, JRED

P1-74

Assessing Genomic Deregulation in Human Pituitary Tumors To Identify Markers of Disease Pathogenesis.

AJ Knox PhD¹, M Xu MS^{1,2}, MG Edwards PhD¹, BK Demasters MD¹, KO Lillehei MD¹, M Geraci MD¹ and ME Wierman MD^{1,2}.

¹Univ of Colorado Denver-Anschutz Med Campus Aurora, CO and ²Veterans Affairs Med Ctr Denver, CO.

Patients with gonadotrope pituitary tumors expressing components of FSH and/or LH present clinically with hormone imbalances, headaches and visual disturbances progressing to blindness. No medical therapy is available. Surgery remains the treatment of choice, but tumors are locally invasive and recur. The genetic and molecular mechanisms underlying their pathogenesis are unknown and few markers predict aggressiveness or recurrence. To identify new candidates involved in tumorigenesis or progression, microarray expression analysis was performed comparing fourteen individual human gonadotrope pituitary tumors and nine normal pituitaries (due to the lack of a pure population of normal human gonadotropes). A one-way analysis of variance (ANOVA) model was used to generate p-values for all present genes (38,932 of 54,675 transcripts probed on the Affymetrix U133 2.0 array) with a false discovery rate (FDR) of 5% to control for multiple testing. Differentially expressed genes were analyzed using the Ingenuity Pathway Analysis software identifying numerous pathways that are significantly different (Fisher Exact Test, $p < 0.05$) in tumor vs normal, including p53 ($p = 1 \times 10^{-4}$) and neuregulin ($p = 2 \times 10^{-5}$) signaling pathways. The altered pathways predict increased angiogenesis, cell cycle progression, cell movement and proliferation with decreased cell cycle arrest and apoptosis. A gene-based array CGH survey to detect copy number variation (CNV) in ten tumors showed that 394 genes have both an amplification/deletion in at least half of the pituitary tumor samples and differential expression ($FDR < 0.05$) in the tumor vs normal pituitary gene expression study. Thirty-seven Gene Ontology canonical pathways contained more than the expected number of genes (Fisher Exact Test, $P < 0.05$) differentially expressed between tumor and normal and also contained CNV. Four signaling pathways associated with other types of cancer (acute myeloid leukemia, endometrial cancer, hereditary breast cancer, and renal cell) were detected as well as the p53 (gene expression $p = 1 \times 10^{-4}$; CNV $p = 0.03$) and neuregulin (gene expression $p = 2 \times 10^{-5}$; CNV $p = 0.02$) pathways. This analysis also extends the understanding of single genes known to promote tumorigenesis to include knowledge of changes in their pathways and interaction partners. Further analysis of these pathways will provide insights into novel candidates involved in tumorigenesis to identify new treatment options for our patients.

Sources of Research Support: VA Merit Review to MEW supported by UCD Cancer Center and Genomics Core.

Nothing to Disclose: AJK, MX, MGE, BKD, KOL, MG, MEW

P1-75

Differentiation of Sphere-Forming Cells from Human Pituitary Adenomas.

Maya Tobita¹, Chie Inomoto², Johbu Itoh², Robert Y. Osamura², Katsuyoshi Tojo¹ and Shozo Yamada³.

¹The Jikei Univ Sch of Med Tokyo, Japan ; ²Tokai Univ Sch of Med Kanagawa, Japan and ³Toranomon Hosp Tokyo, Japan.

BACKGROUND:

Pituitary adenomas are not uncommon among the brain tumors. Recently, it has been reported that stem-like cells with sphere-forming nature which initiate tumors are present in pituitary adenomas as well as in the other brain tumors. They are considered to remain to be undifferentiated. In this study, we cultured sphere-forming cells from pituitary adenomas and analyzed the time-dependent changes of differentiation for pituitary hormones.

METHODS:

The tumor cells were from a human silent ACTH adenoma obtained at surgery. We cultured them in two different types of assay: neurosphere assay from day 1 to 6 and differentiation assay from day 7 to 11. Then, the obtained sphere-forming cells were fixed at day 4, day 7 and day 11. We performed immunofluorescence for pituitary hormones and S-100 protein as a marker for folliculo-stellate cell in all stages of sphere-forming cells.

RESULTS:

In addition to ACTH, the sphere-forming cells expressed S100 which represented the retained undifferentiated property. The expressions of the pituitary hormones were observed according to the time course of cell culture. Especially, the expression of α SU became obvious after they were cultured in differentiation assay. In contrast, some hormones remained as the same expression pattern.

Table1. Expression patterns of pituitary hormones in sphere-forming cells.

| Hormone expression length of culture | ACTH | LH | FSH | α SU | PRL | GH | TSH |
|--------------------------------------|------|----|-----|-------------|-----|----|-----|
| Day4 | + | - | - | + | + | + | - |
| Day7 | ++ | ± | ± | + | + | + | - |
| Day11 | +++ | + | - | +++ | + | + | - |

CONCLUSION:

It should be emphasized that the sphere-forming cells from ACTH producing adenoma showed the apparent additional expression of α SU. ACTH and α SU are two earliest hormones of among pituitary hormones during development and differentiation. The presence of S100 protein+ cells may indicate the presence of stem-cell nature in the tumor. This sphere-forming experiment is expected to serve as a good system to pursue the stem-cell analysis in the pituitary adenomas.

Nothing to Disclose: MT, CI, JI, RYO, KT, SY

P1-76

Stem Cell Markers Gene Expression in Corticotroph Pituitary Adenomas.

Ricardo V. Araujo PhD¹, Ibero C. Soares MD¹, Maria Candida B.V. Fragoso MD, PhD¹, Marcello D. Bronstein MD, PhD¹, Berenice B Mendonca MD, PhD¹ and Luciani R Carvalho MD, PhD¹.

¹Clin Hosp Med Sch Sao Paulo, Brazil.

Human pituitary adenomas are benign neoplasm of epithelial origin. They are grouped according to plasma hormone levels or immunohistochemical staining in three lineages, GH-PRL-TSH, ACTH and FSH/LH. Pituitary adenomas are considered benign tumor, but may be deleterious due to hormone hypersecretion and/or mass effect. Reports have been published on the identification of tumor cells with stem cells characteristics in different types of tumors, including brain tumors. Studies on the stem cells in human pituitary adenomas, particularly corticotroph tumor are scanty. The aim of this study is to identify the presence of stem cells in the human pituitary adenomas that produce ACTH. Fourteen ACTH pituitary adenomas obtained at surgery and 8 non-neoplastic pituitary tissues surrounding adenomas were disposed on a tissue microarrays (TMA) of 1 mm cores. They were Immunoperoxidase stained for stem cell markers OCT4 and NANOG. Total RNA of corticotroph pituitary adenomas were extracted and samples were used for the synthesis of cDNA. Human Pituitary Gland - Poly A + RNA (Clontech) was used as pituitary normal control. Real time PCR analysis was performed by SYBR green based assay using Actin as endogenous gene. TMA immunostaining results have shown that NANOG and OCT4 are present in corticotrophs and non-neoplastic pituitary tissues. OCT4 immunostaining presents similar pattern on both samples, whereas NANOG immunostaining is more evident in corticotroph pituitary adenomas than in non-neoplastic pituitary tissues. qRT-PCR analysis demonstrate that NANOG and OCT4 are 4,6 and 2,7 fold more expressed in tumor tissue, respectively. These preliminary results runs along with the idea of the existence of an oncogenic stem cell population on corticotroph pituitary adenomas, but its whole in the maintenance of the tumor remains to be investigated.

Nothing to Disclose: RVA, ICS, MCBVF, MDB, BBM, LRC

P1-77

Menin Is Critical for TGF- β Upregulation of *p15^{Ink4b}* and *p21^{Cip1/Waf1}* Genes by Inhibiting *c-Myc* Expression and Myc Transcriptional Activity.

Lucie Canaff Ph.D¹ and Geoffrey N. Hendy Ph.D¹.

¹McGill Univ Montreal, Canada.

Background: Menin, the product of the Multiple Endocrine Neoplasia type 1 (MEN1) tumor suppressor gene, facilitates the cytostatic actions of TGF- β by binding the cell-signaling molecule, Smad3, and facilitating Smad transcriptional activity. The cyclin dependent kinase inhibitor (CDKI) *p15* and *p21* genes are upregulated by TGF- β . Myc activates growth-promoting genes by binding their promoters as a heterodimer with the Max protein. TGF- β down-regulates the *c-Myc* protooncogene promoter via a TGF- β inhibitory element (Tie). The *p15* and *p21* genes are targets of Myc that binds to the core promoter initiator element (Inr) of these genes repressing their activity. Here, we evaluated the role played by menin in the mutual antagonism of TGF- β and Myc with respect to expression of these CDKIs.

Methods: The rat somatolactotrope GH4C1 cells were transiently transfected with *p15* or *p21* or *c-Myc* or Tie-SV40 promoter-reporter constructs with vectors expressing menin (sense or antisense) or Myc or Max followed by luciferase assay. Oligo pulldown assays with biotinylated oligonucleotides assessed specific endogenous protein-DNA binding. Coimmunoprecipitation and GST-pulldown assays evaluated protein-protein interaction. TGF- β (100ng/ml) was added as appropriate.

Results: The TGF- β -stimulated (2.5-4.0-fold) *p15* and *p21* promoter activities were attenuated, in a dose-response fashion, by co-transfection of antisense menin. Transfection of Myc attenuated the responsiveness of *p15* and *p21* promoters to TGF- β but co-transfection of menin restored it. Endogenous menin bound the Smad binding element (SBE) and Inr elements of the *p15* and *p21* promoters upon addition of TGF- β . Menin directly bound Myc and the related factor, Max. The activity of the *c-Myc* promoter was decreased and increased by co-transfection of menin or antisense menin, respectively. The basal activity of a *c-Myc* Tie-SV-40 promoter construct was inhibited with added TGF- β . Co-transfection with menin led to a slight decrease (20%) in basal activity and a further decrease with added TGF- β that was lost with co-transfection of antisense menin. TGF- β stimulated binding of menin to the *c-Myc* Tie element.

Conclusion: Menin plays an integral role in the TGF- β upregulation of *p15* and *p21* gene promoters not only by facilitating Smad activity but also by blocking Myc transcriptional activity. In addition, menin is critical for the downregulation of *c-Myc* gene expression.

Nothing to Disclose: LC, GNH

P1-78

Proliferation of Pancreatic Islet and Anterior Pituitary Neuroendocrine Tumours (NETs) Developing in a Mouse Model of Multiple Endocrine Neoplasia Type 1 (MEN1) Is Consistent with a Third Order Regression Mathematical Model.

GV Walls BA(Hons), MB BS¹, J Jeyabalan BSc(Hons), DPhil¹, AAC Reed BSc(Hons), DPhil¹, NR Hill DPhil, MBCS^{1,2}, B Harding PhD¹ and RV Thakker MD¹.

¹Univ of Oxford Oxford, UK and ²Natl Inst of Hlth Res (NIHR) Oxford, UK.

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disease with the combined occurrence of parathyroid adenomas, and neuroendocrine tumours (NETs) of pancreatic islets and the anterior pituitary. In man, MEN1-associated tumours usually develop between 20 and 40 years of age, and this contrasts with other inherited tumour disorders, such as Wilms' tumours, which develop earlier at a mean age of 3.5 years. This suggests that MEN1 tumours have slow growth kinetics, and we assessed this by measuring *in vivo* proliferation rates in 18 month old *Men1*^{+/-} mice, which develop pancreatic islet and anterior pituitary NETs in association with parathyroid tumours. *Men1*^{+/-} mice and wild type (*Men1*^{+/+}) littermates (n=4 per group) were given continuous 5-bromo-2-deoxyuridine (BrdU), which is incorporated into dividing cells during S-phase, for 1, 4, 8 or 12 weeks, and pancreatic and pituitary tissues were collected for immunostaining with BrdU and appropriate peptide hormone antibodies. In *Men1*^{+/-} mice, the mean daily proliferation rates, expressed as percent (\pm SEM): in pancreatic β cell NETs were 0.39% \pm 0.08 versus 0.06% \pm 0.02 in *Men1*^{+/+} normal islets; and in anterior pituitary NETs were 1.77% \pm 0.16 versus 0.015% \pm 0.002 in *Men1*^{+/+} pituitaries ($P < 0.0001$). A time-course analysis of proliferation rates fitted a second order regression line in *Men1*^{+/+} tissues and a third order regression line in *Men1*^{+/-} NETs ($R^2=0.999$). Design of a mathematical model based on these data, predicted that in pancreatic islets, after loss of a second *Men1* allele in either alpha, pancreatic polypeptide (pp) or delta cells, the development of glucagonomas, ppomas or somatostatinomas would require 942, 831 or 648 days, respectively, compared to 365 days for insulinoma development from β cells. Similarly, for the development of pituitary NETs, somatotrophs and corticotrophs would require 542 and 879 days to grow, respectively, compared to 1 year for prolactinomas. Thus, these results explain the predominant occurrence of pancreatic insulinomas and pituitary somatolactotrophinomas in the *Men1* mouse model, as mice usually live for ~700 days in captivity, and hence tumours that are predicted to arise after this period are unlikely to be observed. In summary, the assessment of *in vivo* proliferation rates in *Men1*^{+/-} mice using long term BrdU has demonstrated slow NET growth with third order kinetics and enabled the design of predictive *in silico* models for pancreatic and pituitary NETs.

Sources of Research Support: Medical Research Council, UK, Clinical Research Training Fellowship (G0501780) awarded to GVW; National Institute of Health Research (NIHR) Grant (A90503), UK awarded to NRH; Medical Research Council Programme Grant (G9825289, 2004), UK awarded to RVT.

Nothing to Disclose: GVW, JJ, AACR, NRH, BH, RVT

P1-79

Rare Metastatic Pancreatic Neuroendocrine Tumor Secreting Both ACTH and PTHrP in a Patient with MEN 1 Syndrome.

LZ Khan MD¹, R Friedman DO² and SM Challinor MD¹.

¹Univ of Pittsburgh Pittsburgh, PA and ²Allegheny Gen Hosp Pittsburgh, PA.

Multiple Endocrine Neoplasia type 1 (MEN 1) is an autosomal dominant disorder characterized by parathyroid hyperplasia, pituitary neoplasias, and pancreatic neuroendocrine tumors (NET)¹. We report a metastatic pancreatic NET secreting both ACTH and PTHrP in a patient with MEN 1. There have been 2 prior case reports of pancreatic NET secreting PTHrP in the context of MEN 1, but none secreting both ACTH and PTHrP².

TP was a 34 y/o female presenting with extreme muscle weakness, facial plethora, confusion, and abdominal distension, without other classic features of Cushing's syndrome. Abdominal MRI showed multiple hypervascular, necrotic lesions in the liver, the largest 8 cm and a similar 5 cm mass in the pancreatic tail invading the splenic hilum and no adrenal mass. Liver biopsy confirmed metastatic pancreatic NET. Twelve years before, she had a history of hyperprolactinemia and parathyroid-related hypercalcemia, but did not undergo routine abdominal imaging to screen for pancreatic NET. Her father, sister, and paternal grandmother had a history of indolent pancreatic tumors and hyperparathyroidism. The patient was not gene tested, but gene testing in her sister revealed heterozygosity in the MEN1 gene. Twenty-four hour urine free cortisol level was markedly elevated at 1250 mcg/vol (20-90) and 8 am serum cortisol was 51 mcg/dl(7-25) with ACTH level of 37 pg/ml (9-46). After 8 mg of dexamethasone at 11 pm, her 8 am cortisol was 43 mcg/dl. Gastrin was normal at 82 pg/ml (<100). Pituitary MRI was normal. Chest CT scan showed no thymic mass and multiple small pulmonary metastases. She had severe hypercalcemia with adjusted calcium of 17.7 mg/dl (8.5-10.5). Intact PTH was 32 pg/mL (10-65) and PTH-rP level was markedly elevated at 461 pg/mL (14-27). She died four months later.

This is the first report of a pancreatic NET secreting both ACTH and PTHrP in a patient with MEN 1. This case illustrates that one should consider PTHrP hypersecretion in a patient with NET and MEN 1 who presents with severe hypercalcemia and disproportionately low PTH. Screening for Ectopic ACTH syndrome in this case was important as this diagnosis might easily have been missed. The absence of hypergastrinemia is also atypical for metastatic NET. This case also underscores the importance of appropriate screening for pancreatic NET in MEN 1 as her rapid demise might have been prevented had this been initiated earlier in her course per current MEN1 NIH guidelines¹.

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Nothing to Disclose: LZK, RF, SMC

P1-80

Acromegaly and Familial Paragangliomas: A New Syndrome?.

P Xekouki MD,PhD¹, MF Azevedo MD,PhD¹, B Pasini Prof², A Lytras MD,PhD³, E Lange¹, M Keil MS,CRNP¹, K Pacak Prof¹, A Horvath PhD¹, G Tolis Prof³ and CA Stratakis MD, DMSci¹.

¹Natl Inst of Hlth (NIH) Bethesda, MD ; ²Univ of Torino Torino, Italy and ³“Hippocrateion” Gen Hosp Athens, Greece.

Background: Acromegaly along with multiple familial extra-adrenal paragangliomas (PGL) due to mutations in succinate dehydrogenase (SDH) mitochondrial complex II has not been described before. There is only one other similar case reported in the literature (1) but no genetic testing was available that time.

Clinical case: We studied a family with inherited PGLs and pheochromocytoma (PHEO). The proband, a 37 year old male, presented with acromegaly due to a pituitary macro-adenoma. GHRH levels were undetectable. During pre-surgical imaging evaluation for trans-sphenoidal surgery (TSS) bilateral carotid body masses were revealed; the lesions had characteristics typical of PGL. Biochemical evaluation showed elevated plasma and urinary levels of catecholamines: plasma norepinephrine: 847 pg/ml (120-350), dopamine: 153 ng/l (30-120); urine normetanephrines: 3506 µg/24h (<500), chromogranin-A: 1087 ng/ml (<98). Further imaging showed multiple PGLs and bilateral PHEOs. However, bilateral adrenalectomy revealed extra-adrenal PGLs in close proximity to the adrenal glands, rather than PHEOs. The mitogenic index Ki67 was 1%, and the Pheochromocytoma of the Adrenal gland Scaled Score (PASS) was 8. During TSS a GH-secreting macroadenoma was excised in part. The patient was placed on somatostatin (SMS) analogue therapy with near-complete remission of his disease. Genetic analysis revealed a novel *SDHD* mutation (c.298_301delACTC), resulting in a frame-shift and a premature stop at codon 133 of the SDHD protein. Loss-of-heterozygosity (LOH) and immunostaining studies are intended to clarify the implication of *SDHD* in the pathogenesis of the pituitary tumor. Interestingly, TSS and SMS treatment led to a 2-fold reduction of catecholamine levels in this patient, suggesting that GH oversecretion may have contributed to progression or increased function of PGLs in this patient.

Conclusion: In this report, we describe the first kindred with germline *SDHD* pathogenic mutation, inherited PGLs and acromegaly due to a GH-producing pituitary adenoma. Given the existence of at least one other report in the literature, it is possible that this combination is not accidental. Interesting implications of increased GH and catecholamine secretion and the role of SDH in pituitary tumorigenesis are discussed

(1)Farhi F et al. Paragangliomatosis associated with multiple endocrine adenomas. Arch Pathol Lab Med. 1976;100:495

Nothing to Disclose: PX, MFA, BP, AL, EL, MK, KP, AH, GT, CAS

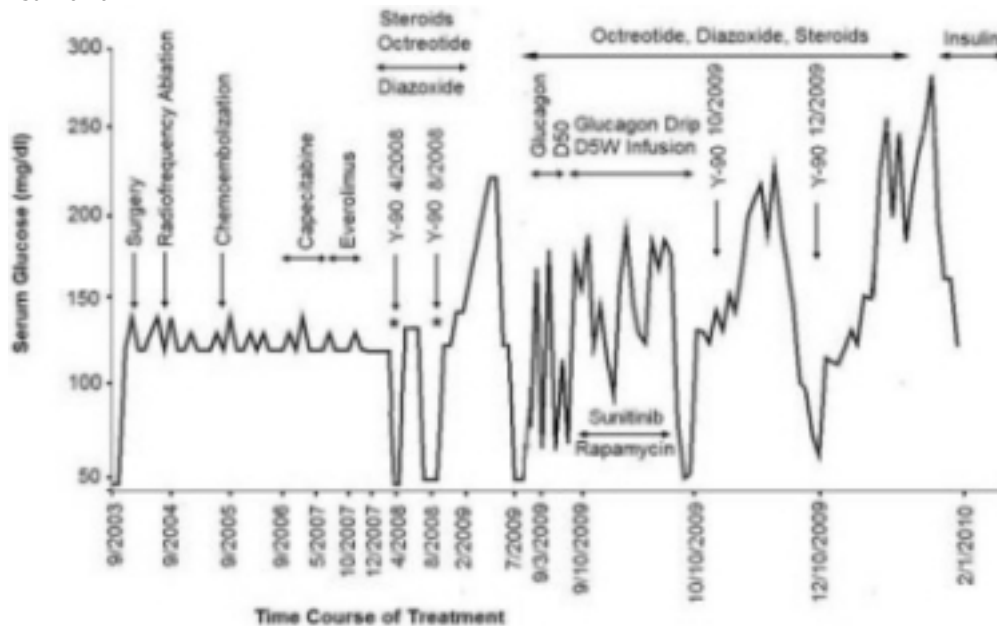
P1-81

Resolution of Intractable Hypoglycemia with Yttrium-90-Labelled Microsphere Radioembolization in a Patient with Malignant Insulinoma.

P Chandra MD, MS¹, SS Yarandi MD¹, N Khazai MD¹, S Jacobs MD¹ and G Umpierrez MD¹.

¹Emory Univ Atlanta, GA.

Background: Yttrium-90-labeled (Y-90) microsphere radioembolization therapy has been useful in treating neuroendocrine tumors but experience with malignant insulinomas is limited. **Clinical Case:** A 56-year-old female with a history of T2DM was diagnosed seven years ago with a malignant insulinoma of pancreas with liver metastasis. Despite undergoing distal pancreatectomy, and ECOG protocol hepatic chemo-embolization followed by systemic chemotherapy tumor progression in liver continued resulting in refractory hypoglycemia. Repeated admissions for hypoglycemia ensued during the subsequent three years despite therapy with sandostatin, diazoxide, and steroids. In April 2008, hypoglycemia (BG=20 mg/dl) recurred with elevated levels of C-peptide (8.8 ng/mL) and Insulin (25.8 mIU/ml). A treatment with Y-90 was injected into the hepatic artery which resulted in resolution of hypoglycemia for 3 months. A second dose of Y-90 was given in August, 2008 which maintained normoglycemia for ten months. In July 2009, she again presented with hypoglycemia. Despite high dose diazoxide (200mg Q 6h), dexamethasone (4mg Q6h), octreotide (300mcg q8h), sunitinib and continuous dextrose infusion she remained hypoglycemic and required continuous glucagon infusion up to 2 mg/hr to prevent hypoglycemia. MRI showed diffuse hepatic metastases that had uptake on SPECT. A repeat treatment of Y-90 was administered selectively into the right liver lobe. Patient was weaned off glucagon and glucose infusions within 2 days. BG ranged between 92 and 268 mg/dL, and she was discharged on dexamethasone, octreotide and diazoxide. Hypoglycemia recurred two months later and patient received another dose of Y-90 this time to left liver lobe. Hypoglycemia resolved yet once again. But two weeks post-treatment patient became hyperglycemic with BGs in 300-400s, which persisted despite stopping diazoxide, steroids and octreotide and had to be ultimately discharged home on rapid acting insulin to keep BG < 200 mg/dl (Figure 1). **Conclusion:** Our study indicates that Y-90 radioembolization therapy shows promising results in treatment of intractable hypoglycemia due to malignant insulinoma.



Nothing to Disclose: PC, SSY, NK, SJ, GU

P1-82

Ectopic CRH Production from Pancreatic Tumors May Mimic Cushing Disease Due to Pituitary Corticotroph Cell Hyperplasia: The First-Ever Cases in Young Children.

F Kamran MD¹, R Cherqaoui MD², C Kemp MD¹, M Crocker MD¹, O Jailan MD¹, M Keil CRNP¹, E Lange RN¹, M Tsokos MD¹, MJ Merino MD¹, M Hughes MD¹, E Kebebew MD¹ and C Stratakis MD¹.

¹Natl Inst of Hlth Bethesda, MD and ²Howard Univ Hosp Washington, DC.

Ectopic CRH-producing tumors are extremely rare. We report the first two cases of ectopic Cushing syndrome (CS) secondary to ACTH and CRH secretion in young children. Case 1: 10 y/o male presented with rapid weight gain, moon face, and headaches. He had crossed height percentiles from the 50th % at age 7 to the 10th % at age 10. The diagnosis of CS was confirmed by loss of circadian rhythmicity of cortisol (12:00 am cortisol of 9 µg/dl) and elevated 24 hr excretion of UFC (64µg/24h, n 2.6-37 µg/24h). Plasma ACTH level was elevated (79.4 pg/ml, n 0-46 pg/ml) suggesting an ACTH-dependent CS. oCRH stimulation test had a partial positive response with an increment of serum cortisol and plasma ACTH of 30% and 21% of basal values respectively. MRI of the sella showed a 3.6mm adenoma. IPSS before and after oCRH stimulation showed no significant central-to-peripheral ACTH gradient (ratio IPSS/P: 1.17 basally and 1.45 at 3 min) supporting an ectopic ACTH syndrome. Adrenal CT showed mild bilateral hyperplasia (HP) but also detected a large lesion at the pancreatic tail; 18F-FDG PET confirmed the presence of a hypermetabolic focus in this area. Hepatic venous sampling (HVS) revealed elevated plasma CRH (Left: 270, Middle: 248; n 2.3-2.7 pg/ml). FNA of the pancreatic masses was performed. Immunohistochemistry confirmed expression of chromogranin A, CD-57, CD-59 and synaptophysin, suggesting a neuroendocrine tumor (NET). Genetic testing ruled out MEN1. Patient underwent distal pancreatectomy with resection of the spleen and omentum. Hepatic micrometastases were identified and biopsied. Metastatic NET was confirmed by staining. Post-op MRI of the sella noted a decrease in size of the pituitary adenoma to 2.6 mm. Post-op CRH levels fell to 2.7pg/ml. Follow-up at 2.5 and 6 months after surgery showed remission of hypercortisolism. Case 2: 11 y/o female presented with clinical and biochemical evidence of CS after a failed transsphenoidal surgery. Histological examination of pituitary tissue showed corticotroph HP. CT scan of the abdomen revealed a 1.4 cm pancreatic tail mass. HVS showed a moderate step up in CRH and ACTH levels. Surgical excision of this lesion is planned. Conclusion: ectopic ACTH and/or CRH production from pancreatic tumors can occur in childhood; diagnosis is difficult because CS in these patients is ACTH-dependent and pituitary lesions may be present due to ACTH-producing cell HP that appears to be reversible after excision of the pancreatic mass.

Disclosures: EK: Study Investigator, Veracyte, Inc.

Nothing to Disclose: FK, RC, CK, MC, OJ, MK, EL, MT, MJM, MH, CS

P1-83

Novel Germline SDHB Mutation in a 35 Year-Old Male with Invasive Bladder Paraganglioma.

MB Heller BA¹, DM Elaraj MD¹, G Aleppo MD¹ and C Sturgeon MD¹.

¹Northwestern Univ Chicago, IL.

Introduction: Up to one-third of pheochromocytomas are associated with one of 6 germline mutations in RET, VHL, NF1, and subunits B, C, or D of succinate dehydrogenase (SDH). SDHB mutations lead to paraganglioma syndrome type 4 and are strongly associated with malignant extraadrenal paragangliomas. Codon specific genotype-phenotype relationships have not been identified for SDHB. Herein is described a case of invasive bladder paraganglioma in a young male with a novel W200R SDHB missense mutation. The pertinent literature is reviewed, and implications for clinical management are discussed.

Clinical Case: A 35 year-old male with a 17 year history of uncontrolled type-1 diabetes and hypertension (HTN) controlled with lisinopril (10mg) developed proteinuria. A 5 cm mass was identified involving the lateral aspect of the bladder. Urine cytology was negative. Cystoscopy and transurethral resection were attempted, but HTN and massive hemorrhage necessitated truncation of the procedure. Immunohistochemistry and histopathology were consistent with invasive paraganglioma. Plasma free normetanephrine and 24 hour urine normetanephrine levels were >5 times the upper limit of normal. Family history was negative for pheochromocytoma. Cross-sectional imaging and MIBG revealed locoregional disease but no metastases. Alpha blockade was initiated. A novel W200R SDHB germline missense mutation was identified. Formal oncologic resection and familial screening were recommended.

Conclusion: This is the first report of the germline missense mutation W200R in the SDHB gene. This patient presented with an unsuspected aggressive bladder paraganglioma. SDHB mutations have a 50% penetrance by age 35 and approximately one third of paragangliomas are multifocal. SDHB mutations are associated with a 35-85% malignancy rate, and 50-70% of these malignant paragangliomas develop metastases. Based on the aggressive nature of this mutation, we recommend preoperative staging, an aggressive treatment regimen, and intensive screening for recurrence. This case demonstrates the importance of considering the diagnosis of paraganglioma in a young patient with a bladder tumor and HTN. Furthermore, the aggressive behavior of SDHB-associated paragangliomas underscores the importance of germline mutation screening to select appropriate treatment and initiate familial screening. Characterizing the genotype-phenotype relationship for specific SDHB mutations might have meaningful clinical implications.

Nothing to Disclose: MBH, DME, GA, CS

P1-84

Ectopic Cushing's Presenting with Diabetes Insipidus: Pituitary Metastasis vs Glucocorticoid Inhibition?.

Andrea Alvarez MD¹, Navneet Mangat MD¹ and Jan M Bruder MD¹.

¹Univ of Texas Hlth Sci Ctr at San Antonio San Antonio, TX.

Background: Small cell lung carcinoma (SCLC) is a neuroendocrine tumor that progresses rapidly and disseminates early in the course of the illness. Paraneoplastic syndromes from hormone secretion or metastasis are usually the first clinical manifestation of these cancers. Here we describe an unusual case of metastatic SCLC presenting with central diabetes insipidus (CDI) presumed due to excess glucocorticoid inhibition of AVP release

Case: 56 yo woman presents with 2 month history of polyuria, polydypsia, weight loss, proximal muscle weakness, easy bruising, hirsutism, severe hypokalemia and lower extremity edema. Serum Na on admission was 153 with a urine osmolarity of 168. Urine osmolarity increased by 50% after DDAVP and symptoms resolved on a therapeutic dose. 24h urine cortisol was dramatically elevated, 4636 and 4923mcg/d (<45mcg/d) with an AM cortisol of 98.6mcg/dl after 1mg DST. ACTH level was 495 pg/mL (6-58pg/ml) and did not decrease after metyrapone test suggesting ectopic ACTH production. A hypointense T1 signal in the neurohypophysis was seen on sella MRI but was otherwise normal. Pan CT showed bilateral subcentimeter lung nodules with mediastinal lymphadenopathy and multiple liver lesions. FNA of a liver lesion revealed small cell carcinoma positive for chromogranin, TTF-1, AE 1/3 and focally for CD56 suggestive of a lung primary. Maximum dose of ketoconazole failed to decrease cortisol levels. Metyrapone was added with successful control of cortisol excess. DDAVP was discontinued once cortisol levels dropped without further polyuria or polydypsia. After one course of chemotherapy the patient decided to go home on hospice and died 2 weeks after. Request for an autopsy was declined.

Conclusion: CDI is a rare manifestation of metastatic SCLC. Most metastasis to the pituitary gland are asymptomatic and incidentally found on autopsy. It is unclear how small pituitary metastases could cause CDI, since destruction of more than 50% of the hypothalamic-pituitary neurons are needed in order for DI to manifest. Alternatively, the etiology of CDI in this case could have been due to the known inhibitory effect that excess glucocorticoid has on the biosynthesis and release of vasopressin from the hypothalamus, as well as on its action in the kidney.

(1)Kovasc KJ et al.,The Journal of Neuroscience 2000;20:3843

(2)Komninos J et al., JCEM 2004; 89:574

(3)Hershel et al.,The American Journal of physiology 1987; 252:635

Nothing to Disclose: AA, NM, JMB

P1-85

Ectopic Acromegaly due to a Growth Hormone Releasing Hormone Producing Pancreatic NET.

DE Weiss MD¹, BS Lopes MD, PhD² and L Katznelson MD¹.

¹Stanford Univ Sch of Med Stanford, CA and ²Univ of Virginia Hlth Syst Charlottesville, VA.

Background: Over 95% of cases of acromegaly are due to a pituitary somatotroph adenoma. We report a case of a patient with acromegaly who presented with a pituitary adenoma, but was ultimately found to have acromegaly due to ectopic GHRH secretion from a pancreatic neuroendocrine tumor (PNET) in the context of multiple endocrine neoplasia-1 (MEN-1) and a nonfunctioning pituitary adenoma.

Case: A 46 year old woman presented with clinical signs of acromegaly, which was confirmed with a serum IGF-1 1054 ng/mL (nl 49-292 ng/mL) and random GH 43 ng/mL. Family history was notable for nephrolithiasis in her father and peptic ulcer disease in her daughter. Pituitary MRI showed a 1.2 cm sellar mass with suprasellar extension to the chiasm. Following complete resection of the macroadenoma, serum IGF-1 and GH levels remained unchanged. Pathology revealed a nonfunctioning pituitary adenoma (GH stain negative). Repeat pituitary MRI showed no clear residual tumor, despite persistent, increased sellar contents. Search for an ectopic tumor included a negative indium-111 octreoscan and abdominal CT. Octreotide LAR was administered, but IGF-1 remained elevated. Primary hyperparathyroidism was diagnosed, and multigland parathyroid hyperplasia was noted at surgery. GHRH was > 1,000 pg/mL (nl < 300 pg/mL). Repeat abdominal CT two years later revealed a 6.2 cm mass in the tail and body of the pancreas. In retrospect, this was noted on the earlier scan. Distal pancreatectomy revealed a PNET that stained positive for GHRH, Ki-67 antigen, synaptophysin, and chromogranin A, but negatively for GH. Postoperatively, GHRH and IGF-1 normalized. Re-evaluation of the initial pituitary pathology revealed a non secreting adenoma with somatotroph hyperplasia of the adjacent, normal pituitary gland. Genetic testing revealed a novel heterozygous deletion c152_160del9 in the menin gene.

Conclusion: Ectopic GHRH production is a rare cause of acromegaly. In this case, a PNET produced GHRH in the setting of MEN-1. The acromegaly was resistant to somatostatin analog therapy, reflecting the negative octreoscan uptake. In addition, this case is novel because the patient presented with a concomitant nonfunctioning pituitary macroadenoma, which was presumably associated with MEN-1 as well.

Nothing to Disclose: DEW, BSL, LK

P1-86

Malignant Glucagonoma in a Patient with Diabetes Mellitus (DM) and Prader-Willi Syndrome (PWS) Treated with Glargine.

M Noda MD¹, K Yoshii MD¹, K Abe MD¹, Y Naiki MD, PhD¹ and R Horikawa MD, PhD¹.

¹Natl Ctr for Child Hlth and Development Tokyo, Japan.

Glargine is a long-acting recombinant insulin which is also a potent agonist for IGF-I receptor. Recently the occurrence of malignancy was reported among patients with diabetes mellitus (DM) treated with Glargine. Glucagonoma, a rare neuroendocrine tumor, usually occur in pancreas often accompanied by hepatic metastasis, and its prognosis tend to be poor. We report a 35-year-old woman with Prader-Willi syndrome (PWS) with DM treated by Glargine, who developed malignant glucagonoma with liver metastasis. She was diagnosed as having PWS at the age of 4-month by typical clinical features. She has been suffered from DM from 20-year-old. At 22-year-old, insulin treatment has started. From 30 years old, Glargine admimistration at a dose of 50U/day has been necessary to maintain glycemic control, due to hyperphagia. From 34 years old, she was suffered from intractable hypoglycemia. She showed refractory diarrhea for 6 months, hypoalbuminemia (1.8g/dL), steatorrhea and intractable skin eruption. Computed tomography showed one hypervascular lesion in the head of pancreas and multiple hypervascular lesions in the liver. Plasma glucagon level was high at 1700pg/mL. Other hormones, such as VIP, 5-HIAA and gastrin were within normal range. Octreoscan revealed pancreatic tumor and multiple liver metastasis. The combined treatment were performed with octretide LAR, arterial chemotherapy for pancreatic lesion and arterial chemoembolization for liver metasis. A rare combination of PWS and malignant glucagonoma in a patient treated with relatively high-dose Glargine suggested the involvement of this insulin analog for the formation of rare neuroendocrine tumor of pancreatic origin .

Nothing to Disclose: MN, KY, KA, YN, RH

P1-87

Malignant Somatostatinoma Presenting with Diabetic Ketoacidosis.

A Theodoraki MRCP¹, B Khoo MA, PhD, MRCP¹, A Hamda MRCP¹, T Meyer PhD, MD, MRCP¹ and PM Bouloux MD, FRCP¹.

¹Royal Free Hampsted NHS Trust London, UK.

Background. The somatostatinoma inhibitory syndrome consists of mild non- ketotic hyperglycaemia, hypochlorhydria, cholelithiasis, steatorrhoea, anaemia and weight loss¹.

Clinical Case. A previously healthy 30 year old afro-caribbean female presented with diabetic ketoacidosis. She admitted two months of weight loss, abdominal pain and diarrhoea. On examination hepatomegaly and cervical lymphadenopathy were noted. Investigations showed a normocytic anaemia (haemoglobin 9.8 g/dL, n 11.5- 15.5 and MCV 88 fL, n 80-98 fL) and raised alkaline phosphatase (384 U/L, n 35- 129 U/L).

An abdominal US revealed gallstones and multiple liver lesions up to 5cm. CT scanning confirmed the presence of liver deposits and showed bone metastasis and abdominal lymphadenopathy. Histology of the liver lesions sampled under US guidance diagnosed an intermediate grade neuroendocrine tumour with proliferation rate 15%. High somatostatin (>1000 pmol/L, n < 150 pmol/L), chromogranin A (754 pmol/L, n < 60) and chromogranin B (268 pmol/L, n < 150) were found on a fasting plasma gut hormone profile. C peptide was 77 pmol/L, glucagon 8 pmol/L (n < 50). Pancreatic islet cell and GAD antibodies were negative. An upper gastrointestinal endoscopy with duodenal biopsies and a colonoscopy failed to locate the primary tumour. An Octreotide scan revealed no somatostatin receptor positive disease.

The diagnosis of a malignant somatostatinoma arising from an unknown primary tumour was made. The patient was commenced on chemotherapy with FCIST (Folinic acid, 5Fluorouracil, Streptozocin, Cisplatin). The newly diagnosed diabetes was addressed with the use of insulin glargine and insulin aspart.

The presentation of our patient with diabetic ketoacidosis as part of the somatostatinoma inhibitory syndrome is unusual. In these tumours the absence of ketoacidosis has been attributed to the somatostatin induced suppression of the secretion of insulin and glucagon. Diabetic ketoacidosis in somatostatinomas has been reported before only on a few occasions. It is hypothesized that the secretion of larger molecular weight forms of somatostatin from the tumour (Somatostatin-28) may cause a greater suppression of insulin that glucagon secretion with resultant ketogenesis.

Conclusions. Diabetic ketoacidosis may be the presenting feature of a somatostatin secreting tumour. In this report we review the effects of somatostatin on glucose homeostasis and discuss the underlying pathophysiological mechanisms.

(1)Larsson LI, Hirsch MA, Holst JJ et al. Pancreatic somatostatinoma. Clinical features and physiological implications. *Lancet* 1977; 1: 666-668.

Nothing to Disclose: AT, BK, AH, TM, PMB

P1-88

A Rare Case of Multiple Metastases from Recurrent Paraganglioma of the Urinary Bladder: Effectiveness of Repeated ¹³¹I-MIBG Therapy.

SH Ko¹, BY Cha¹, DJ Lim¹, MH Kim¹, S Moon¹, YB Ahn¹, MI Kang¹ and HY Son¹.

¹The Catholic Univ of Korea Seoul, Republic of Korea.

Introduction: Paraganglioma of the urinary bladder is very rare, accounting for less than 1% of all pheochromocytomas and 0.05% of bladder tumors. Of these, only about 10% is malignant. The prognosis of malignant paraganglioma is relatively poor, less than 50% of a 5-year survival rate. Recently, it has been reported that ¹³¹I-MIBG therapy can be an effective treatment to malignant pheochromocytoma and paraganglioma for longstanding palliation and survival prolongation. However, there have been few reports that patient with metastatic paraganglioma of the urinary bladder was effectively managed with repeated ¹³¹I-MIBG therapy.

Clinical case: A 58-year-old man had a history of recurrent paraganglioma of the urinary bladder with multiple metastases. At the age of 33, he underwent twice transurethral resection of bladder tumor (TUR-BT) due to gross hematuria. 19 years later, he presented with painless, gross hematuria for 4 months. There was no clue of pheochromocytoma such as high blood pressure and micturitional attacks consisting of headache, palpitations, or sweating. Cystoscopy demonstrated multiple nodules in the urinary bladder. TUR-BT was performed and the diagnosis of paraganglioma was made by the histopathological examination. Since then, he had received TUR-BT three times for 5 years due to recurrent paraganglioma of the urinary bladder.

In 2007, at the age of 56, he had gross hematuria again. An abdominal CT, bone scan and diagnostic scintigram with ¹³¹I-MIBG demonstrated the multiple polypoid nodules in the bladder, invading left seminal vesicle, prostate gland, right perivesical space and multiple metastases involving pelvis, spines, right 7th rib and mandible. Biochemical analysis showed elevated 24hr urine VMA level with normal 24hr urine metanephrine, normetanephrine and catecholamine levels. He received a total of 600 mCi ¹³¹I-MIBG in three fractions for 28 months without complication. At the last follow-up, VMA is normalized and objective response determined by CT and ¹³¹I-MIBG scan is a stable disease, although 24hr urine norepinephrine is mildly elevated. He remains clinically asymptomatic.

Conclusion: Our case demonstrated that repeated ¹³¹I-MIBG therapy would be an effective palliative treatment for multiple metastases from recurrent paraganglioma of the urinary bladder.

Nothing to Disclose: SHK, BYC, DJL, MHK, SM, YBA, MIK, HYS

P1-89

VIPoma: Octreoscan Imaging Does Not Predict Effectiveness of Therapeutic Response to Octreotide.

Archana Reddy M.D.¹ and Jose Goldman M.D., Ph.D.¹.

¹Michigan State Univ Coll of Human Med East Lansing, MI.

Background: VIPomas are rare neuroendocrine tumors secreting vasoactive intestinal polypeptide (VIP). Clinical symptoms include profuse watery diarrhea, dehydration, hypokalemia, and achlorhydria. Therapy with the Somatostatin (SST) analogue Octreotide frequently allays both symptoms and metabolic abnormalities, and may also have antitumor effects. Positive Octreoscan imaging may localize primary pancreatic and metastatic tumors, and it is reportedly predictive of favorable responses to Octreotide.

Clinical Case: A 42 yr old schizophrenic male presented with diarrhea, dehydration, hypokalemia, metabolic acidosis and weight loss. CT imaging of the abdomen showed metastatic liver disease, the largest mass measuring 10.2 cm at its main dimension. Liver biopsy showed a neuroendocrine tumor with cytokeratin 7, synaptophysin and chromogranin A staining. An octreoscan confirmed three hepatic masses, without uptake in the pancreas or elsewhere. Serum VIP (780 pg/mL) (1049% above normal) and chromogranin A (22 nmol/L) (440% above normal) were elevated, while pancreatic polypeptide, insulin, C-peptide and glucagon levels were normal, consistent with a diagnosis of VIPoma. Baseline tests showed hypokalemia (K^+ 2.7 mEq/L), metabolic acidosis (CO_2 10 mEq/L) and pre-renal azotemia (creatinine 2.0 mg/dL). Octreotide LAR 30 mg IM per month was started, but clinical and metabolic abnormalities remained unabated and serum VIP measured one month later was higher (1420 pg/mL) (1893% above normal). A subsequent CT scan of the abdomen performed 8 months later showed progression of liver metastatic disease with the largest mass now measuring 15.6 cm at its main dimension.

Discussion: This case shows a dichotomy between positive octreoscan imaging and both the clinical and metabolic responses to Octreotide treatment. Octreotide effects are mediated by SST receptors (SSTR) (SSTR2, SSTR3 and SSTR5). These are G-protein coupled receptors in which SST binding activates cAMP and/or Ca^{2+} -dependent signaling pathways. Octreotide anti-tumor effects may be mediated by SSTR coupling to a tyrosine phosphatase, thereby inhibiting epidermal growth factor phosphorylation. Loss of function mutations or repression of gene expression involving either SSTR at areas outside the SST binding domain or post SSTR signaling pathways could explain the lack of predictive value of positive octreoscan imaging with regard to therapeutic responses to Octreotide therapy.

Nothing to Disclose: AR, JG

P1-90

Multi-Modality Treatment in a Patient with Malignant Insulinoma.

C W Ho¹, G Y W Kam¹ and M W Tsang¹.

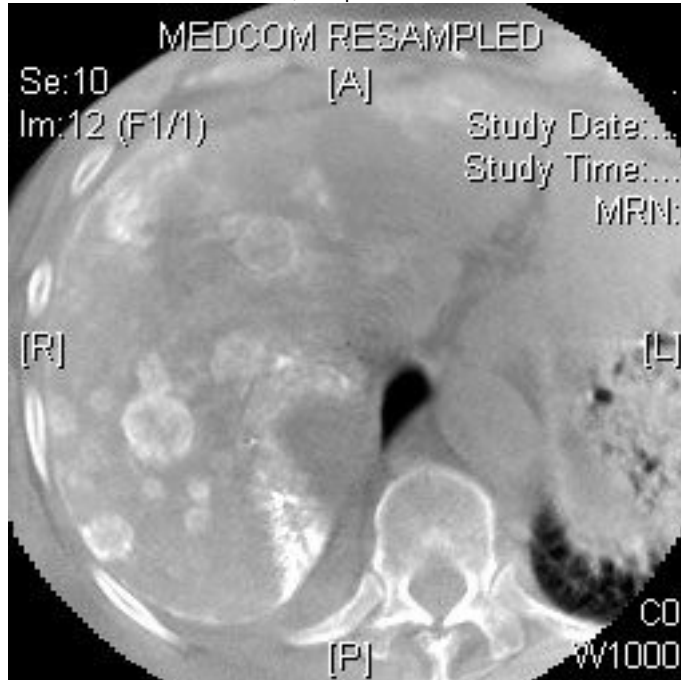
¹United Christian Hosp Hong Kong, Hong Kong.

A 67-year-old man with good past health presented with fasting hypoglycemia and weight gain for three months in July09. His spot glucose was 44mg/dl with paired insulin 48 μ U/ml and c-peptide 2.57nmol/l. Urine toxicology was negative for sulphonylurea. CT abdomen found a hypervascular mass with diameter of 8cm arising from distal body and tail of pancreas, enlarged peripancreatic lymph nodes and more than twenty liver metastases. The diagnosis was malignant insulinoma with liver metastases. Multiple endocrine neoplasia 1 was excluded.

The patient had recurrent hypoglycemia while on multiple drugs including diazoxide, steroid and octreotide. Diazoxide was later stopped because of diazoxide related thrombocytopenia. The patient underwent resection of pancreatic tumor and intra-operative radiofrequency ablation to liver metastases. The histology confirmed neuroendocrine tumor staining positive for insulin.

The patient was still having recurrent hypoglycemia after surgery despite on octreotide 200mg eight-hourly, phenytoin 230mg at bedtime and prednisolone 20mg daily. Continuous dextrose infusion was required.

Sunitinib, a multi-targeted tyrosine kinase inhibitor, was started at 25mg daily since Sept09 for a six-week cycle with four weeks on treatment followed by two weeks off treatment. Other oral drugs were stopped. The patient was still having recurrent hypoglycaemia. The patient underwent transarterial chemoembolization (TACE) using cisplatin mixture in Oct09. After the first TACE, the patient could be weaned off from dextrose infusion.



Second TACE was done four weeks apart with minimal tumor uptake. Positron emission tomography scan showed multiple active metastases at liver, retroperitoneal lymph, left adrenal gland, rib, thoracic spine and right femoral neck. Second and third cycle of sunitinib were increased to 37.5mg daily. The patient developed side effects including nausea, hypertension, stomatitis and hand foot syndrome. Sunitinib was stopped after the third course as patient could not tolerate the side effects. Regular hemoglucostix by patient at home showed normoglycemia. The patient is still on regular follow up as an out-patient.

Nothing to Disclose: CWH, GYWK, MWT

P1-91

Duodenal Somatostatinoma in Neurofibromatosis Type 1.

AR Barnosky DO¹ and GG Fareau MD¹.

¹Med Coll of Wisconsin Milwaukee, WI.

Background: Somatostatin producing tumors are very rare and are frequently associated with a hormonal syndrome (somatostatinoma syndrome) and high malignant potential. Duodenal somatostatinoma is a less common form of the tumor that is strongly associated with neurofibromatosis type-1 (NF-1) and has a more insidious presentation.

Clinical case: A 56 year-old woman with a history of NF-1 and schizophrenia presented with five days of abdominal pain, nausea, vomiting, diarrhea, and fatigue. A complete blood count and basic chemistry panel were normal. Abdominal imaging confirmed cholelithiasis and a 2 cm hypervascular lesion in the duodenal wall. Twenty-four hour urine 5-hydroxyindoleacetic acid and serum chromogranin, human pancreatic polypeptide, glucagon, vasoactive intestinal polypeptide, and gastrin were normal. A somatostatin level was mildly elevated (32 pg/mL, n 10-22 pg/mL). The patient's abdominal pain was attributed to cholelithiasis and she underwent a cholecystectomy with intraoperative biopsy of the duodenal wall lesion. The cytology from the biopsy was consistent with a neuroendocrine malignancy with strongly positive immunohistochemistry for somatostatin and weak immunoreactivity for synaptophysin, chromogranin, CD56, and VIP. Surgery for the tumor is pending initial management of her schizophrenia.

Clinical Lesson: Twenty-five percent of NF-1 patients have an intestinal manifestation of the disease, most of which are neurofibromas; however, neuroendocrine tumors (NETs) are the most common duodenal tumor in NF-1¹. NETs of the duodenum comprise 2 % of all enteropancreatic NETs². The overall prevalence of duodenal somatostatinoma is one in 40 million, and 40 % of all duodenal somatostatinomas are found in NF-1^{1,2}. Somatostatinoma syndrome (hyperglycemia, diarrhea, and cholelithiasis) is common in pancreatic somatostatinomas, but rare in duodenal tumors due to lower levels of somatostatin secretion³. Duodenal somatostatinomas typically present with local mass effects, or are discovered incidentally on imaging or during cholecystectomy. The malignancy rate of duodenal somatostatinoma is approximately half of pancreatic tumors, and the five-year survival is up to 60 % with metastases and approaches 100 % without metastases⁴.

In patients with NF-1, the differential for an incidental duodenal mass should include a somatostatinoma, even in the absence of overt clinical or biochemical hormonal abnormality.

(1)Fendrich V et al.,J Hepatobiliary Pancreat Surg 2004 11:417

(2)Sakorafas GH et al.,JOP 2008; 9(5):633

(3)Green BT et al.,J Clin Gastroenterol 2001;33(5):415

(4)Simon P et al.,Endocrinol Metab Clin North Am 2006; 35:431

Nothing to Disclose: ARB, GGF

P1-92

Localization of Insulinoma by Arterial Stimulation and Venous Sampling (ASVS) and Intraoperative Ultrasonography.

N Hiroi¹, M Sue¹, A Yoshihara¹, R Iga¹, A Yasuyo¹, N Masai¹, R Shigemitsu¹ and G Yoshino¹.

¹Toho Univ Tokyo, Japan.

A 45-year-old woman who had experienced abnormal behavior, such as suddenly standing up, fidgeting, and repeating the same words admitted to our hospital because of evaluation for hypoglycemia. She had gained 15 kg over the past two years (45 kg → 60 kg), and she was unable to tolerate not having 3 full meals a day. Sixty two ng/dL of plasma glucose and 16.4 μU/mL of immunoreactive insulin were observed. Levels of plasma glucose and immunoreactive insulin were 42 mg/dL and 10.4 μU/mL, respectively, in response to 15-hr fast. No mass lesions were detected by any imaging examinations. Because an arterial stimulation with venous sampling (ASVS) showed increased insulin secretion in response to Ca stimulation on the splenic artery, we decided that surgical resection of pancreas body and tail would be necessary. Intraoperative ultrasonography and palpation of the pancreas revealed a clearly demarcated mass measuring approximately 10 mm on the pancreatic body. Four months after the surgery, body weight had decreased to 50 kg and good glycemic control was achieved. Pathological diagnosis was an insulinoma. We presented a case of insulinoma in which it was possible to localize the lesion by ASVS and by intraoperative ultrasonography, even though no mass lesion was observed on the diagnostic imaging examinations.

Nothing to Disclose: NH, MS, AY, RI, AY, NM, RS, GY

P1-93

Discontinuation of Chemotherapy and Continuation of Collaborative Care for a Schizophrenic Patient with Malignant Paraganglioma.

Masayasu Iwabuchi MD PhD¹, Toshihide Tanaka MD¹, Yutaka Oki MD PhD² and Hirotoishi Nakamura MD PhD².

¹Seirei Mikatahara Hosp Hamamatsu, Japan and ²Hamamatsu Univ Sch of Med Hamamatsu, Japan.

BACKGROUND

Malignant paraganglioma is rare, deceptive and deadly. Averbuch reported some success with the CVD regimen that consists of 750 mg/m² of cyclophosphamide on day 1, 1.4 mg/m² of vincristine on day 1, and 600 mg/m² of dacarbazine on days 1 and 2. Prior to treatment, an informed consent should be obtained.

CASE REPORT

We have reported the continuation of collaborative treatment of a schizophrenic patient with malignant paraganglioma. A woman has been treated with CVD therapy since January 2008 under careful observation of psychiatrist. A diagnosis of malignant paraganglioma has been confirmed with the evidence of metastases to rib in her chest wall and lumbar vertebrae in December 2007. After the diagnosis, she made a decision of chemotherapy under the informed consent because she could share all medical information we had. We started the CVD chemotherapy with the collaboration of psychiatrists and other medical staffs. We have continued the treatment in a ward of psychiatry. Decrease in 24-hour urine normetanephrine suggested efficacy of the chemotherapy as shown in figure 1-A and MIBG scanning turned negative. Repeated chemotherapy of CVD had been performed in confirmation of informed consent until her psychiatric condition worsened in March 2009. She could not share any medical information about malignant paraganglioma. From both ethical and medical point of view, we decided to discontinue the chemotherapy because informed consent requires a process in which a person is given important facts about a medical procedure or treatment before deciding whether or not to participate. Since the decision of discontinuation of chemotherapy of CVD was made, careful observation of CT, MIBG and 24-hour urine norepinephrine has been continued as shown in figure 1-B. Repeated MIBG scanning continued negative.

RESULT

We treated a schizophrenic patient with malignant paraganglioma in collaboration with psychiatrists. We had continued chemotherapy in confirmation of informed consent and discontinued because of psychiatric condition. Discontinuation of chemotherapy should be discussed from both ethical and medical point of view.

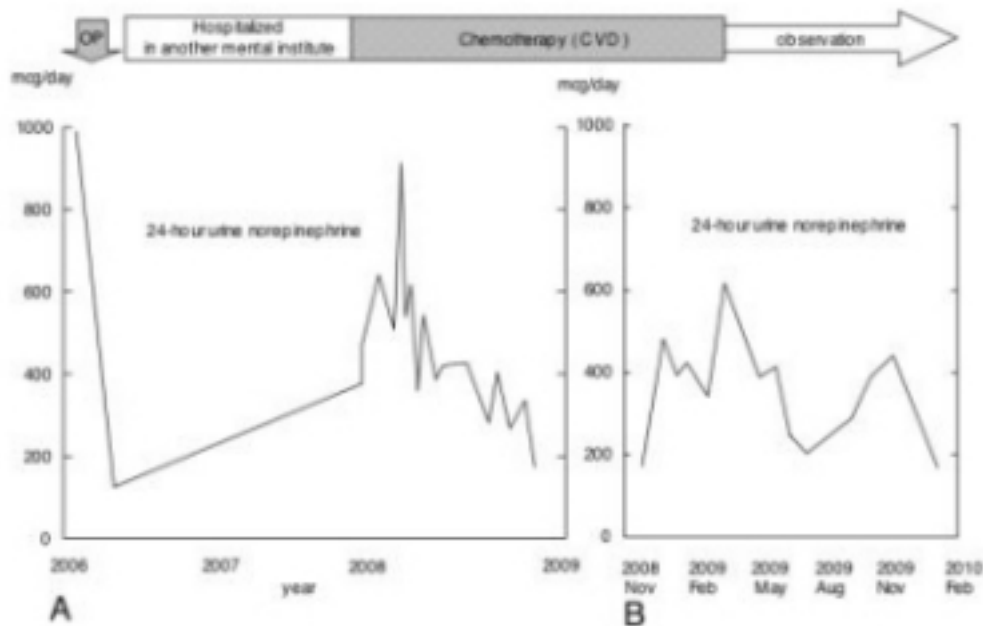


Figure 1-A) Continuous CVD produced a decrease in 24-hour urine norepinephrine excretion. Normal range of 24-hour urine norepinephrine is 26-121 mcg/day.
 Figure 1-B) After discontinuation of CVD because of psychiatric condition, careful observation of CT, 131I-MIBG and 24-hour urine norepinephrine has been continued.

Nothing to Disclose: MI, TT, YO, HN

P1-94

HCG Elevation in a Patient with a Neuroendocrine Tumor of the Gallbladder and Pituitary Adenoma.

VR Marlar MD¹, JA Fratkin MD¹, C Subramony MD¹, GI Uwaifo MD¹ and CA Koch MD¹.

¹Univ of Mississippi Med Ctr Jackson, MS.

Background: Neuroendocrine tumors are heterogeneous, rarely metastasize to the pituitary, and can produce various hormones incl hCG (1).

Clinical Case: A 58-yr AA man was admitted with hypotension, bradycardia and abd pain. CT abd showed diffuse liver lesions suggesting metastatic disease. Further imaging noted a 1.5 cm pit mass with possible hemorrhage. Endocrine was consulted to r/o pit apoplexy. Lab investigation noted: s-cortisol 230 mcg/dL (10 pm), ACTH 630 pg/mL (10-46), PRL 672 ng/mL (4.6-21.4), TSH 0.75, FT4 0.33 ng, IGF-1 40.7 ng, LH 0.1, FSH <0.1, total testosterone 831 ng, estradiol 189 pg (10-50), (intact + beta) hCG 17 mIU/mL (< 5, human, ECLIA, cobas). AFP and DHEAS were normal. History was notable for recent 30 lb wt loss and long-standing ED. Review of chart confirmed a diagnosis of hypogonadism 4 yrs prior though he never received tx. He did not appear Cushingoid but did have bilateral gynecomastia. The testes were nl in size and without masses, testicular US was nl. The pt underwent liver bx but developed acute peritonitis and septic shock following the procedure and died. At autopsy, he was found to have a metastatic undifferentiated gallbladder carcinoma with focal neuroendocrine differentiation. The pit tumor was described as a prolactinoma with focal cystic change and hemorrhage. On IHC, the pit tumor was positive for PRL, ACTH, synaptophysin and NSE but negative for hCG (rabbit polyclonal, Cell Marque). Interestingly, the surrounding normal pituitary was positive for hCG but negative for LH. The gallbladder tumor stained positive for NSE, CD56, cytokeratin and CA 19-9 but negative for ACTH, PRL and hCG. Both testes showed severe atrophy and sclerosis; the adrenal glands were unremarkable.

Conclusions: This patient's presentation was suspicious for an ectopic source of ACTH. However, IHC was consistent with a pituitary source, suggesting a co-secreting ACTH- and prolactin adenoma. The testosterone elevation may have been due to hCG stimulation. HCG secretion occurs in 60% of biliary tract cancers and is frequently observed in neuroendocrine tumors (1). IHC failed to demonstrate hCG(beta) positivity in this case using a nonhuman antibody. False negative IHC could be due to a delay in formalin fixation as this has been described for other biomarkers (2-5). Though hCG production by normal pituitary can occur in menopause and in hypogonadal men, it is not felt to be the cause of this pt's serum elevation and total testosterone level.

- (1) Stenman UH, Alftan H, Hoakainen K. Human chorionic gonadotropin in cancer. *Clin Biochem* 2004;37:549-561
- (2) Khoury T et al. Delay to formalin fixation effect on breast biomarkers. *Modern Pathol* 2009;22(11):1457-1467
- (3) Woltering EA, Knox RD, Javadpour N, Soares T, Chen HC. Detection of human chorionic gonadotropin in fresh and formalin-fixed testicular tumor tissue. Comparison of sensitivity of immunoperoxidase to radioimmunoassay. *Urology*. 1980 Aug;16(2):215-8.
- (4) Jacobsen M, Jacobsen GK. The influence of various fixatives on the immunohistochemical demonstration of a number of plasma proteins and oncofetal proteins in paraffin embedded material. *Acta Pathol Microbiol Immunol Scand A*. 1984 Nov;92(6):461-8.
- (5) Carlinfante G, Lampugnani R, Azzoni C, Aprile MR, Brandi ML, Bordi C. Expression of the alpha- and beta-subunits of human chorionic gonadotropin by subsets of parathyroid cells in states of hyperparathyroidism. *J Pathol*. 1998 Aug;185(4):389-93.
- (6) Hoermann R, Spoettl G, Moncayo R, Mann K. Evidence for the presence of human chorionic gonadotropin (hCG) and free beta-subunit of hCG in the human pituitary. *J Clin Endocrinol Metab* 1990;7:179-186

Disclosures: CAK: Consultant, Novo Nordisk.

Nothing to Disclose: VRM, JAF, CS, GIU

P1-95

EUS Guided Alcohol Ablation in Pancreatic Insulinoma: Is It Really Successful?.

Tasma Harindhanavudhi MD¹, Bruce Redmon MD¹ and Rajeev Attam MD¹.

¹Univ of Minnesota Minneapolis, MN.

Insulinoma is an islet cell tumor that frequently causes symptomatic hypoglycemia. The first line of treatment is surgical resection. However, in a poor surgical candidate, various options have been used to control hypoglycemia. Recently, endoscopic ultrasound (EUS) guided ablation of pancreatic insulinoma has been described⁽¹⁾⁽²⁾.

An 84 year-old male with metastatic colon cancer, renal cell carcinoma and parkinson's disease presented with hypoglycemic symptoms and a blood glucose of 33 mg/dl. Concomitant insulin level was 54 mU/L (0-20), C-peptide 19.3 ng/ml (0.9-6.9) and negative sulfonylurea screen. Abdominal CT was unremarkable. EUS showed an irregular, 3.2 x 2.9 cm mass between the pancreatic neck and body. FNA and cytology confirmed insulinoma. Due to advanced co-morbidities, conservative treatment was elected. Both octreotide and diazoxide were unsuccessful in controlling hypoglycemia. EUS-guided alcohol ablation was attempted as an alternative option. A total of 14 ml 98% ethanol was injected into the tumor. Two hours after the procedure, he became unconscious with blood glucose of 19 mg/dl which resolved after dextrose infusion. Diazoxide was discontinued 3 days after the procedure. Subsequent fasting insulin and C-peptide were 10 mU/L and 4.2 ng/ml respectively with a blood glucose of 108 mg/dl. On repeat EUS at 4 weeks post ablation, the tumor was 2.9 x 1.7 cm. Unfortunately, 6 weeks after ablation he experienced recurrent hypoglycemia with blood glucose of 37 mg/dl. Diazoxide was reinstated. Minimally invasive procedures such as EUS-guided alcohol ablation, radiofrequency ablation and embolization have been reported as alternative therapies for patients who are not surgical candidates. EUS-guided alcohol ablation had been reported to be feasible and effective to eliminate the hypoglycemia. However, there are several interesting concerns pointed out in our case. The immediate post procedure hypoglycemia might be due to insulin release from manipulation and destruction of tumor cells. The subsequent hypoglycemia after an impressive initial response might not reflect long term outcome. Although one report documented euglycemia 34 months post procedure, the number of cases was small⁽¹⁾. Our patient's tumor was larger compared to other cases, which may have been a factor in the outcome. Larger series are needed to better define the efficacy and durability of the procedure as well as clinical characteristics which might predict successful outcome.

(1)Jurgensen C et al.,Gastrointestinal endoscopy 2006;63:1059-1062

(2)Deprez PH et al.,Acta Gastro-Enterologica Belgica 2008;LXXI:333-337

Nothing to Disclose: TH, BR, RA

P1-96

Novel MEN1 Gene Mutation in a Family with Multiple Endocrine Neoplasia Type 1.

M Cho¹, JS Park¹, WH Shim¹, EH Lee¹, SH Beom¹, SA Kim¹, ES Kim¹, JS Yoo¹, JS Nam¹, CW Ahn¹, BS Cha¹, EJ Lee¹, SK Lim¹, KR Kim¹ and HC Lee¹.

¹Yonsei Univ Coll of Med Seoul, Korea.

Background: Multiple Endocrine Hyperplasia Type 1 (MEN1) is an autosomal dominantly inherited tumor syndrome characterized by tumors of parathyroid, anterior pituitary, gastro-intestinal and other endocrine tissues. There are more than 400 mutations proved along the MEN1 gene.

Clinical case: A 42-year old man was confirmed to MEN1 with hyperparathyroidism, prolactin-secretion pituitary adenoma, non-functioning pancreas adenomas and duodenal carcinoid tumor. Thymic carcinoid tumor and non-functioning adrenal tumor was detected additionally. We investigated his family members. His brother and one sister were diagnosed to MEN1 too. And other two sisters had not any tumors. The brother had thymic carcinoid tumor but others had not. Genomic DNA extraction and DNA sequence analysis were performed. A novel mutation in affected three was found in exon 9 and it was not reported previously MEN1 mutation. It was a nonsense mutation at a codon 122 of exon 9 (TGG→TAG) resulting in a stop codon and termination. This mutation was not detected in unaffected two sisters and 100 healthy Korean gene bank.

Conclusion: This is a novel germline mutation in the MEN1 gene and it would play a role for a loss of tumor suppressive activity of menin.

Sources of Research Support: Grant of Korean Endocrine Society (2007).

Nothing to Disclose: MC, JSP, WHS, EHL, SHB, SAK, ESK, JSY, JSN, CWA, BSC, EJL, SKL, KRK, HCL

P1-97

A Novel Mutation in a Patient with MEN-1 Associated with Ectopic ACTH Syndrome and Recurrent Thymic Carcinoid Tumour.

S Samad MB BChir MRCP¹ and P A Price MBBS FRCP¹.

¹Great Western Hosp Swindon, UK.

Background: MEN-1 can commonly cause a number of different endocrine tumours however we report a case of MEN-1 due to a novel mutation causing several endocrine tumours and the rarely described ectopic ACTH syndrome.

A 49 year old male presented in 1993 with hypercalcaemia and a mass on chest x-ray: investigation revealed an anterior mediastinal thymic carcinoid tumour, a parathyroid adenoma, a macroprolactinoma and multiple lipomata, strongly suggesting a clinical diagnosis of Multiple Endocrine Neoplasia Type 1 (MEN-1). The thymic carcinoid and parathyroid adenoma were removed and the hypercalcaemia resolved. The macroprolactinoma was not fully responsive to dopamine agonist therapy and the patient therefore underwent a transsphenoidal adenectomy.

A family history of MEN-1 was discovered in two of his children, his brother and a nephew and niece. Genetic sequencing in the patient and his son with primary hyperparathyroidism revealed that they were heterozygous for a novel missense mutation at codon 375 in the *MEN1* gene.

The patient's thymic carcinoid was found to have recurred in 1998, but he was asymptomatic and further treatment was deferred until 2006 when the tumour was debulked surgically, followed by subsequent radiotherapy to the tumour bed.

Tumour immunostaining for ACTH was negative. In 2007 he was diagnosed with Cushing's syndrome secondary to ectopic ACTH production, although CT scanning did not show clear tumour recurrence. The Cushing's syndrome was treated with metyrapone resulting in normalisation of his cortisol levels and resolution of his clinical state.

He subsequently developed radiation-induced pulmonary fibrosis and multiple pulmonary emboli. In 2008 the patient developed weak legs and a spinal MRI revealed osteosclerotic metastatic deposits at T1/T2: histology showed a poorly-differentiated metastatic neuroendocrine tumour. He was treated with spinal irradiation but deteriorated and died in September 2009.

Conclusion: Malignant thymic tumours associated with ectopic ACTH secretion are a very rare feature of MEN-1, but when present are as malignant and as difficult to treat as when they occur as sporadic cases.

Nothing to Disclose: SS, PAP

P1-98

Bariatric Surgery in MEN1 and Duodenal Carcinoid - An Interesting Challenge.

MSB Huda MRCP¹, DH Lewis MRCP¹, M Koteswara MRCP¹, C Sinclair MRCP¹, D Kariyawasam MRCP¹, AG Patel MS FRCS², L Izatt MRCPATH¹, F Chang MRCPATH¹, J Powrie MD FRCP¹ and B McGowan MRCP PhD¹.

¹Guys and St Thomas's NHS Foundation Trust London, UK and ²King's Coll Hosp London, UK.

A 27 year old lady presented with primary hyperparathyroidism and underwent a 3½ gland parathyroidectomy. Histology showed parathyroid hyperplasia and subsequent genetic testing confirmed MEN1.

She complained of weight gain, putting on 50kg over 5 years and with a present BMI of 56.7. Lifestyle changes and medical management were unsuccessful at achieving weight reduction. Examination was unremarkable. Cushing syndrome was excluded by a low dose dexamethasone suppression test. Baseline pituitary function tests, fasting gut hormones and pituitary MRI were normal.

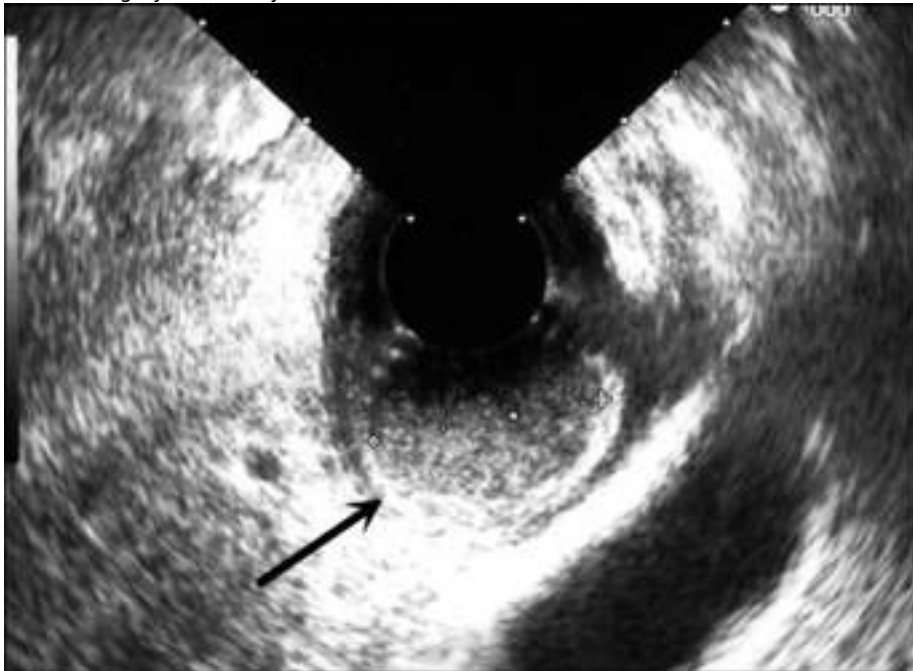
Abdominal MRI did not reveal pancreatic abnormalities but endoscopic ultrasound (EUS) showed a 3.5mm lesion in the uncinata of the pancreas and a 2.5 mm lesion in the duodenum [figure 1].

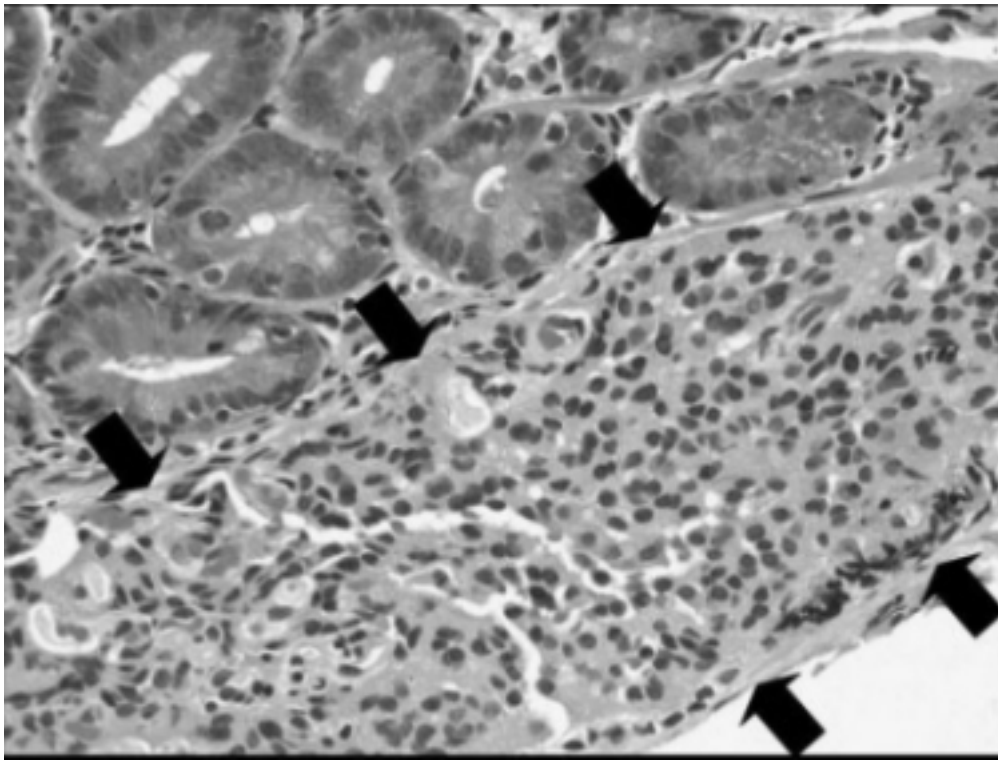
Histology of the duodenal biopsy demonstrated a well differentiated carcinoid tumour staining positive for chromogranin, synaptophysin and pan-cytokeratin (MNFI16) [figure 2]. FNA of the pancreatic lesion was inconclusive and we are monitoring this lesion with repeat EUS and MRI periodically. We are also arranging a 72 hour fast to exclude an insulinoma.

CONCLUSIONS

Our patient wished to undergo bariatric surgery to aid weight loss. Bariatric surgery poses a challenge in the context of a patient with MEN-1, carcinoid, and the possibility of future enteropancreatic and foregut pathology. Duodenal carcinoid has been associated with MEN-1 although uncommonly, and little is known about optimum treatment. It has also been previously reported that insulinoma can be unmasked after bariatric surgery (1) and hence a 72 hour fast has been arranged.

We are currently liaising with the bariatric surgeons to discuss management of the duodenal carcinoid, whether our patient should undergo bariatric surgery, and if so the optimal procedure. Our case illustrates the challenge of planning bariatric surgery in MEN1 syndrome.





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Nothing to Disclose: MSBH, DHL, MK, CS, DK, AGP, LI, FC, JP, BM

P1-99

SOX2 Regulates the *Prop1* Expression in Pituitary Cell Lines.

Akio Ishikawa M^{1,2}, Takao Susa Dr², Saisyu Yosida M¹, Takako Kato PhD¹ and Yukio Kato PhD¹.

¹Meiji Univ Kawasaki, Japan and ²Res Fellow of the Japan Society for the Promotion of Sci Tiyodaku, Japan.

Prop1 (Prophet of PIT1) is first identified as a responsible gene for *Ames* dwarf mice which show inheritable combined pituitary hormone deficiency [Sornson et al, 1996]. PROP1 is known as a factor which participates in the pituitary organogenesis.

Recently, we studied the localization of PROP1 in the rat pituitary by immunohistochemistry [Yoshida et al, 2009]. As a result, PROP1-positive cells consistently expressed *Sox2*, a marker of stem/progenitor cell and did not overlap with any endocrine cell. Hence, we investigated roles of SOX2 in the expression of *Prop1* by transfection assay.

We constructed reporter vectors fused mouse *Prop1* -2994/+21 region and its deletion mutants with pSEAP2 Basic. Culture cells used were CHO (Chinese hamster ovary cells), AtT20 (mouse corticotrope-lineage), LβT2 (mouse gonadotrope-lineage), GH3 (rat somato/lactotrope-lineage) and Tpit/F1 (mouse folliculostellate cell-lineage).

Transfection assay demonstrated that *Prop1* -2994/+21 has low promoter activity except for LβT2 in which it promoted 2-fold. When *Sox2* was coexpressed, promoter activity of *Prop1* -2994/+21 was increased in CHO, GH3 and Tpit/F1 cells but decreased in LβT2 and AtT20 cells. These effects were high in LβT2 and Tpit/F1 cells. Serial deletion mutants of *Prop1* reporter vector revealed that the responsibility to SOX2 is located in the upstream region over -772 in all cell types examined. In LβT2 cells, another repressive effect of SOX2 was observed within -154/+21.

This cell-type specific bidirectional effect of SOX2 suggests existence of interactive factors. Therefore we tested synergy between SOX2 and 10 transcription factors on *Prop1* -2994/+21 in CHO cells. 10 factors used were LHX3, HESX1, PROP1, PIT1, HES1, BRN4, PITX2, PITX1, PRX2 and MSX1.

As a result, LHX3, HESX1, PROP1, PIT1, HES1 and BRN4 increased the promoter activity of *Prop1* in the existence of SOX2. Only PITX2 decreased the activity when SOX2 was coexpressed. PITX1, PRX2 and MSX1 didn't show any effects. Thus, this study demonstrated that *Prop1* -2994/+21 region has a promoter activity and SOX2 regulates the expression of *Prop1* positively or negatively in a cell-type specific manner under the existence of interactive factor(s).

Nothing to Disclose: AI, TS, SY, TK, YK

P1-100

FoxJ1 Is an Essential Co-Factor in the Regulation of Growth Hormone and Pituitary Development.

S.R. Venugopalan DDS¹, S. Florez BS¹, F. Pinho BS¹, H. Cao BS¹, J. Wang Ph.D.¹ and B.A. Amendt Ph.D.¹.

¹Texas A&M Hlth Sci Ctr, Inst of Bioscis and Technology Houston, TX.

Pituitary development is a complex process and provides an excellent model to study the combinatorial transcriptional regulation of gene expression. FoxJ1 is a member of the fork-head family of transcription factors and is expressed during pituitary development. FoxJ1^{-/-} mice exhibit a hypoplastic pituitary and stunted growth. Pitx2 is a major player during pituitary development and is co-expressed with FoxJ1 during pituitary development. PITX2 binds to the FoxJ1 promoter and activates FoxJ1 expression. Co-Immunoprecipitation assays demonstrate that Pitx2 physically interacts with FoxJ1. Chromatin immunoprecipitation assays demonstrate that Pitx2 endogenously binds to the growth hormone (GH) promoter and FoxJ1 and Pitx2 form a complex on the growth hormone promoter. Pitx2 activates the GH promoter and in concert with FoxJ1 synergistically activate the GH promoter. Moreover FoxJ1, Pitx2, Lef-1 and β -catenin form a complex on the GH promoter and dramatically increase activation of the GH promoter. These data demonstrate that FoxJ1 is an essential co-factor in regulating growth hormone expression. Immunohistochemistry experiments collaborate these data and reveal that growth hormone levels are significantly reduced in the FoxJ1^{-/-} mouse pituitary. Our data suggests a new role for FoxJ1 in pituitary development and it is an essential co-factor in regulating the transcriptional control of growth hormone.

Sources of Research Support: Grant DE018885 from the National Institute of Dental and Craniofacial Research.

Nothing to Disclose: SRV, SF, FP, HC, JW, BAA

P1-101

Multiple *Cis*-Acting Enhancers Regulate Temporal and Spatial Expression of the Human *LHX3* Gene in the Developing Endocrine and Nervous Systems.

RD Mullen¹, S Park¹, J Kusche MD², J Klammt MD², RW Pfaeffle MD² and SJ Rhodes PhD¹.

¹Indiana Univ Sch of Med Indianapolis, IN and ²Univ of Leipzig Leipzig, Germany.

LHX3 is a LIM homeodomain transcription factor necessary for proper development of the pituitary and central nervous system. Our laboratory and others have shown that mutations in coding regions of *LHX3* cause combined pituitary hormone deficiency (CPHD) with a clinical phenotype of short stature, hypothyroidism, and hypogonadism. The majority of LHX3-deficient patients also have a rigid cervical spine and limited neck rotation. Previous studies from our group and others have identified promoter and intronic elements of *LHX3* that are important for basal gene expression *in vitro*. The key regulatory elements necessary for *in vivo* expression are unknown. Using transgenic mouse models and bioinformatic approaches, we have mapped novel tissue-specific enhancer regions. Bioinformatic searches of the DNA sequences surrounding the human *LHX3* gene revealed non-coding regions conserved in multiple vertebrate species. Such conserved non-coding sequences often have regulatory function. Using a *beta galactosidase* reporter gene system in transgenic animals, we have demonstrated that conserved regulatory regions direct spatial and temporal expression to the developing pituitary gland, brain, and spinal cord *in vivo*. Ongoing experiments include identification of transcription factors regulating *LHX3* expression. In addition, to test our hypothesis that mutations in key regulatory regions may result in loss of LHX3 protein expression and cause CPHD disease, we are screening candidate patients for variations in the *LHX3* promoters and enhancer regions. Any potential mutations identified will be further assessed *in vivo* using the transgenic mouse model. Characterization of novel genetic defects will facilitate patient treatment and enable genetic counseling.

Sources of Research Support: NIH HD42024 to SJR.

Nothing to Disclose: RDM, SP, JK, JK, RWP, SJR

P1-102

Lin28A Transgenic Mice Manifest Size and Pubertal Phenotypes Identified in Human Genetic Association Studies.

H Zhu MD¹, S Shah¹, N Shyh-Chang BS¹, G Shinoda¹, WS Einhorn¹, SR Viswanathan BS¹, A Takeuchi BS¹, C Grasemann PhD², JN Hirschhorn MD, PhD¹, MR Palmert MD, PhD² and GQ Daley MD, PhD¹.

¹Children's Hosp Boston Boston, MA and ²The Hosp for Sick Children and Dept of Paediatrics Toronto, Canada.

The regulation of an organism's size and developmental timing are fundamental biological processes. Recently, genome-wide association studies (GWAS) have linked the Lin28B locus to both human height and timing of menarche. Lin28B and its homologue Lin28 (hereafter, Lin28A) are functionally redundant RNA-binding proteins that block let-7 microRNA (miRNA) biogenesis. Lin28 and let-7 were originally discovered in *C. elegans* as heterochronic genes that regulate larval and vulval development, but more recently have been implicated in cancer, stem cell aging, and pluripotency. The let-7 targets c-Myc, K-ras, IMP1/2 and HMGA2 play important roles in mammalian body size regulation and metabolism. In an effort to understand the role that Lin28A plays in tumorigenesis, we engineered doxycycline inducible Lin28A transgenic (Tg) mice. To explore the role of the Lin28A/let-7 pathway in the recent GWAS phenotypes of growth and sexual maturation, we examined crown-rump length, body size and pubertal timing in these mice. In Lin28A Tg mice, we observed significant increases in body weight (38.3g vs. 30.9g in 8 week old males, $p = 0.010$) and length (10.3 cm vs. 9.3cm, $p = 0.011$), even in the absence of doxycycline induction. We noted a modest increase in body mass index, but no significant change in percentage body fat or lean mass as measured by DEXA imaging. To study the role of Lin28A in the timing of sexual development, we measured vaginal opening (VO), time to first estrus, and time to first litter. We observed a 2.24 and 2.18 day delay in VO in the CD-1 and B6 strain backgrounds, respectively (both $p < 0.002$). We found that the WT and Lin28A Tg mice achieved first estrus at day 27.3 vs. day 31.8, respectively ($p = 0.011$). Furthermore, mating experiments revealed that Lin28A Tg animals had a ~3 day delay to date of first litter ($p = 0.0351$). These changes were associated with changes in gene expression in adult tissues, most notably a 7-fold Lin28A mRNA increase in the skeletal muscle, a 5-fold increase in the ovary, and a 4-fold increase in the hypothalamus. This transgenic mouse represents a fortuitous animal model to investigate the physiologic basis of the links between genetic variation in the Lin28/let-7 pathway and human phenotypes of height and sexual maturation.

Nothing to Disclose: HZ, SS, NS-C, GS, WSE, SRV, AT, CG, JNH, MRP, GQD

P1-103

Molecular Mechanism of Steroidogenic Factor 1 (SF1) Mediated Repression of CYP11B2 Expression.

NG Hattangady MS¹, P Ye PhD², E Lalli MD³ and WE Rainey PhD¹.

¹Med Coll of Georgia Augusta, GA ; ²The Univ of Queensland Brisbane, Australia and ³CNRS UMR 6097 and Univ de Nice Sophia Antipolis, France.

Introduction: Steroidogenic Factor 1 (SF1/NR5A1) is an orphan nuclear hormone receptor that is a major regulator of steroidogenesis. We recently demonstrated that while SF1 enhances the expression of most steroidogenic genes, it represses adrenal expression of the enzyme aldosterone synthase (CYP11B2). Herein we attempt to better define the mechanism for SF1 repression of CYP11B2. **Methods:** H295R-TR/SF1 (TR/SF1) human adrenocortical cells that express the SF1 transgene under the control of doxycyclin (Doxy) were used as a model. SF1 levels were regulated by incubating with 0.25 µg/mL Doxy. Expression of CYP11B2 and NURR1 was studied under basal or agonist-stimulated conditions in cells treated with or without Doxy. Total RNA was isolated and real-time quantitative RT-PCR (qPCR) was used to measure transcript levels of CYP11B2 and NURR1. RNA was also used for microarray analysis to study the effect of elevated SF1 expression on Ang II stimulated adrenal gene expression. **Results:** Doxy incubation caused a time-dependent induction of both SF1 protein and RNA. Transcript levels for SF1 plateaued after 6 h ($p < 0.001$). SF1 protein levels peaked after 6 h of Doxy treatment. Microarray analysis confirmed our previous observation that elevated SF1 expression inhibited Ang II stimulated CYP11B2 and suggested that Ang II induction of NURR1 expression was also blocked. To define SF1 effects on CYP11B2/NURR1 expression, cells with and without Doxy treatment were stimulated for 1 h (for NURR1) and 6 h (for CYP11B2) with Ang II, K⁺ and forskolin. Enhancing SF-1 expression significantly inhibited the stimulatory effects of all agonists on both NURR1 ($p < 0.001$) and CYP11B2 ($p < 0.05$), thus indicating that the effects of SF1 are broad based. Our further experiments will explore whether SF1 represses CYP11B2 transcription indirectly or by directly binding to the CYP11B2 promoter. **Conclusion:** The mechanism of SF1 mediated repression of CYP11B2 transcription is broad based. One mechanism involves blockade of agonists induction of NURR1, a key transcriptional regulator of CYP11B2. The regulation of SF1 expression or activity may play a role defining adrenal cell mineralocorticoid versus glucocorticoid production.

Nothing to Disclose: NGH, PY, EL, WER

P1-104

Regulation of SF-1-Mediated Transcription of the Human *Steroidogenic Acute Regulatory Protein* Gene by Chromatin-Loop Formation.

T Mizutani PhD¹, T Yazawa PhD¹, Y Ju¹, M Uesaka PhD¹, Y Inaoka PhD¹, Y Imamichi PhD¹, K Matsuura¹, Y Kamiki¹, A Umezawa MD, PhD² and K Miyamoto PhD¹.

¹Univ of Fukui Fukui, Japan and ²Natl Res Inst for Child Hlth and Development Tokyo, Japan.

Steroidogenic acute regulatory protein (StAR) mediates the transport of cholesterol from the outer to the inner mitochondrial membrane, the process of which is the rate limiting step for steroidogenesis. It is well known that Steroidogenic factor-1/adrenal-4-binding protein (SF-1) play an essential role for the expression of human *StAR* gene. Transcriptional regulation of the proximal promoter of human *StAR* has been well characterized, whereas analysis of its enhancer region has not. We recently reported that SF-1-induced the differentiation of bone marrow-derived mesenchymal stem cells into steroidogenic cells such as Leydig or adrenocortical cells. Here we show in the differentiated human bone marrow stem cells as well as in the granulosa tumor cell line, KGN cells that *StAR* gene expression is regulated by a novel enhancer element in an SF-1 dependent manner.

We identified novel SF-1 binding sites between 3,000 and 3,500 bp upstream (designated as the enhancer region) from the transcriptional start site of human *StAR* by gel mobility shift and ChIP assays. We demonstrated by a luciferase reporter system that the enhancer region was important for the maximal transcriptional activity of *StAR* gene. Interestingly, nucleosome eviction has been found at those SF-1 binding sites (both promoter and enhancer region) in the SF-1-induced mesenchymal stem cells by ChIP assay. Furthermore, chromosome conformation capture analysis revealed that DNA loop was formed between the promoter and the enhancer regions in both the differentiated mesenchymal stem cells and KGN cells. Finally, SF-1 knockdown study revealed the depletion of endogenously expressed SF-1 in the KGN cells prevented the loop formation between the proximal and the enhancer regions. These results indicate that the novel enhancer region participates in the human *StAR* gene activation by orchestrating chromatin loop architecture.

Nothing to Disclose: TM, TY, YJ, MU, YI, YI, KM, YK, AU, KM

P1-105

SUMOylation of Foxl2 Increases Synergistic Activation of the Mc2R Promoter by Foxl2 and SF1.

Wei-Hsiung Yang Ph.D.¹, Ninoska M. Gutierrez², Lihong Wang M.D., Ph.D.², Buffy S. Ellsworth Ph.D.³ and Chiung-Min Wang B.S.¹.

¹Mercer Univ Sch of Med Savannah, GA ; ²Univ of Michigan Ann Arbor, MI and ³Illinois Southern Univ Carbondale, IL.

Foxl2 is one of the earliest ovarian markers and plays an important role in regulation of steroid metabolism, apoptosis, and ovarian development and function. Mutations and deficiencies of Foxl2 have been shown to cause blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) as well as premature ovarian failure. Recent reports have shown that Foxl2 represses the promoter activity of the steroidogenic acute regulatory (StAR) gene by binding to StAR promoter. However, the interaction between Foxl2 and SF1 has not been studied in mammals. Herein, we demonstrate that the interplay between Foxl2 and SF1 regulates StAR and melanocortin 2 receptor (Mc2R) gene expressions in mammalian systems. First, we find that Foxl2 interacts with SF1 in mammalian two-hybrid system and immunoprecipitation assay. Both Foxl2 and SF1 are abundantly expressed in ovary and adrenal gland tissues. As expected, Foxl2 represses and SF1 enhances StAR promoter activity. Notably, Mc2R promoter activity is activated by Foxl2 in a dose-dependent manner. Surprisingly, we find that Foxl2 and SF-1 synergistically up-regulate the transcription activity of Mc2R gene. By mapping the Mc2R promoter, we provide evidence that distal SF1 response elements (-1410 and -975) are required for synergistic activation by Foxl2 and SF1. We also find that Foxl2 can be SUMOylated at Lys25 in vivo and E2 (Ubc9) and E3 (PIAS3 β , PIAS x β , and PIASy) enzymes are involved in Foxl2 SUMOylation. Furthermore, we demonstrate that deSUMOylation of Foxl2 results in attenuated transcriptional synergy between Foxl2 and SF1. Taken together, we show for the time that Foxl2 acts as a transcriptional repressor of the StAR gene but a transcriptional activator of the Mc2R gene. Our results suggest that the interplay between Foxl2 and SF1 on Mc2R promoter functions as a novel player in Mc2R-mediated cell signaling as well as steroidogenesis in adrenal glands.

Sources of Research Support: Mercer University Seed Grant to W.H.Y.

Nothing to Disclose: W-HY, NMG, LW, BSE, C-MW

P1-106

FOXL2 Interacts with Steroidogenic Factor-1 (SF-1) and Represses SF-1-Induced CYP17 Transcription in Granulosa Cells.

MR Park¹, EK Shin², MA Won¹, JH Kim¹, HY Ko², HL Kim¹, JJ Ko¹, KS Lee² and JH Bae¹.

¹CHA Univ Seongnam, Korea and ²Chung-Ang Univ Seoul, Korea.

Mutations in FOXL2 are responsible for blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) type I, in which affected women exhibit premature ovarian failure. FOXL2-null mice showed defects in granulosa cell development during folliculogenesis. We screened a rat ovarian yeast two-hybrid cDNA library to identify FOXL2-interacting proteins and found steroidogenic factor-1 (SF-1). Here, we show that human FOXL2 and SF-1 proteins interact in human granulosa cells, and that FOXL2 negatively regulates the transcriptional activation of a steroidogenic enzyme, CYP17, by SF-1. Furthermore, FOXL2 mutants found in BPES type I patients lost the ability to repress CYP17 induction mediated by SF-1. Chromatin immunoprecipitation and electrophoretic mobility shift assay results further revealed that FOXL2 inhibited the binding of SF-1 to the CYP17 promoter, while the FOXL2 mutants failed to block this interaction. Therefore, this study identifies a novel regulatory role for FOXL2 on a key steroidogenic enzyme and provides a possible mechanism by which mutations in FOXL2 disrupt normal ovarian follicle development.

Nothing to Disclose: MRP, EKS, MAW, JHK, HYK, HLK, JJK, KSL, JHB

P1-107

The Transcription Factor FoxO4 Represses Cholesterol Biosynthesis by Interacting in a Ternary Complex with SREBP2 and HIF2 α at the Promoter of Multiple Cholesterol Biosynthesis Genes.

J. Zhu M.D.¹ and F. F. Chehab Ph.D.¹.

¹Univ of California, San Francisco San Francisco, CA.

FoxO4 binds to specific DNA binding elements in the promoter of target genes and is regulated by multiple pathways such as serine/threonine phosphorylation, lysine acetylation, arginine methylation and polyubiquitination. However, FoxO4 can also regulate transcriptional responses independently of DNA binding, thus widening the range of its regulatory activities.

We recently reported that FoxO4 inhibits expression of the lanosterol 14 α demethylase gene (known as CYP51), resulting in accumulation of its substrate 24,25 dihydrostanosterol and subsequent sterol-mediated inhibition of cholesterol biosynthesis. In this communication, we address the mechanism by which FoxO4 represses CYP51 promoter activity. We found in transfection assays (n=7-10 independent experiments, each performed in triplicates) that the sterol regulatory binding protein 2 (SREBP2) and the hypoxia inducible factor 2 α (HIF2 α) stimulated CYP51 promoter linked luciferase gene activity by 11.2 and 3.5-fold, respectively, but together enhanced it to 33-fold. Although, FoxO4 alone had no appreciable effect on the CYP51 promoter, it suppressed the combined effect of SREBP2 and HIF2 α by over 50%, suggesting that a ternary complex involving SREBP2, HIF2 α and FoxO4 acts to regulate CYP51 promoter activity. Forward and reciprocal co-immunoprecipitation assays confirmed the physical interaction of SREBP2 and FoxO4, showing that FoxO4 is a cofactor to SREBP2. Furthermore, identical transfection experiments of the HMG CoA synthase or HMG CoA reductase promoters with SREBP2, HIF2 α and FoxO4 revealed the same pattern of promoter stimulation and repression, thus widening the role of the ternary complex in the regulation of cholesterol biosynthesis. HIF1 α , a homolog of HIF2 α was unable to recapitulate the HIF2 α -stimulatory effects on either promoter, demonstrating HIF2 α specificity. Since HIF2 α is stabilized in hypoxia, we determined that fibroblasts exposed to hypoxia gradually increase FoxO4 mRNA levels up to 4-fold over normoxia, substantiating an unappreciated role of FoxO4 in hypoxia. Overall, our studies show that FoxO4 regulates cholesterol biosynthesis by acting as a cofactor to SREBP2 in a ternary complex that also includes HIF2 α . Since cholesterol biosynthesis is a prominent and high oxygen-demanding pathway in liver cells, the SREBP2-HIF2 α -FoxO4 ternary complex may act to inhibit cholesterol biosynthesis in order to alleviate hepatic stress, such as in hypoxia-induced liver ischemia and steatosis.

Nothing to Disclose: JZ, FFC

P1-108

Genome-Wide Analysis Reveals PADI4 Facilitates Transcriptional Activation of a Subset of Elk-1 Target Genes in MCF-7 Cells.

XS Zhang¹, MJ Gamble¹, S Stadler², BD Cherrington¹, MS Roberson¹, WL Kraus¹ and SA Coonrod¹.

¹Cornell Univ Ithaca, NY and ²Rockefeller Univ New York City, NY.

Peptidylarginine deiminase IV (PADI4) catalyzes the conversion of positively charged arginine and methylarginine residues to neutrally charged citrulline on histone tails. This activity has been linked to the repression of gene transcription on a limited number of genes. To broaden our knowledge of the regulatory potential of PADI4, we utilized chromatin immunoprecipitation coupled with promoter tiling array (ChIP-chip) to more comprehensively investigate the range of PADI4 target genes across the genome in MCF-7 breast cancer cells. Results showed that PADI4 is enriched in gene promoter regions near transcription start sites (TSSs) and, surprisingly, this pattern of binding is primarily associated with actively transcribed genes. Computational analysis found Elk-1, a member of the ETS oncogene family, to be highly enriched around PADI4 binding sites and coimmunoprecipitation analysis then confirmed that Elk-1 physically associates with PADI4. The expression of two well characterized Elk-1 target genes, *c-fos* and *egr-1*, was then found to be inhibited following treatment of MCF-7 cells with a PADI4-specific inhibitor. The inhibitor also significantly reduced levels of acetylation at histone H4 lysine 5 at these promoters suggesting that the activating function of PADI4 at these target genes is mediated, in part, by interplay between histone citrullination and histone acetyltransferases (HAT)-mediated acetylation. These findings greatly expand our knowledge of the role of PADI4 in gene regulation by defining a new role for PADI4 and histone citrullination in mediating gene transactivation.

Nothing to Disclose: XSZ, MJG, SS, BDC, MSR, WLK, SAC

P1-109

KRAB-Zinc Finger Repressors in Liver Modulate Female Reproduction and Metabolism.

Christopher J Krebs PhD¹ and Diane M Robins PhD¹.

¹Univ of Michigan Med Sch Ann Arbor, MI.

Krüppel-associated box zinc finger proteins (KRAB-zfzs) are the largest class of transcriptional regulators encoded in vertebrate genomes, yet very few have been assigned physiological roles. The first function identified was repression of male-specific hepatic genes in mice, which we showed was due to *Regulator of sex-limitation (Rsl) 1* and *Rsl2*. Further analysis revealed that nearly 8% of the liver transcriptome is Rsl-responsive including genes with female- as well as male-specific expression patterns. Rsl targets are diverse, but a significant proportion reside in pathways of steroid and lipid metabolism.

Although *rsl* mice with mutations in both *Rsl1* and *Rsl2* appear normal, reproduction and intermediary metabolism differ detectably from wild type (wt). In female mice, the concentration in urine of Major Urinary Protein (MUP) pheromone carriers varied across the estrous cycle; *rsl* levels of MUP were higher at all phases of the cycle. In an odor preference assay, wt males were more attracted to *rsl* than wt females, suggesting the elevated MUP concentration contributed to a more robust pheromone signal. Puberty onset, as determined by vaginal opening, occurred two days earlier in *rsl* than wt females, possibly due to both hormonal and pheromonal effects. Female *rsl* mice are also leaner than wt and differentially responsive to fasting, as indicated by altered hepatic expression of key enzymes in glucose and lipid metabolism. When fed a high-fat diet (HFD) for 60 days, percent weight gain was similar; however insulin levels in *rsl* females were elevated nearly 40% compared to controls. To ascribe activity to *Rsl1* or *Rsl2*, their cDNAs were individually expressed as liver-specific transgenes. Only *Rsl2* accentuated MUP fluctuations across the estrous cycle and restored puberty onset to wt. Hepatic expression of either *Rsl1* or *Rsl2* attenuated weight gain due to HFD, showing this to be a liver intrinsic effect. In contrast, neither transgene reversed the elevated insulin levels, suggesting this might be due to Rsl action in other tissues.

In sum, in addition to previously noted effects on male-specific genes, Rsl modulates female traits by transcriptional repression of a variety of target genes in liver, interacting with hormonal and pheromonal regulation. Understanding the full extent of Rsl's impact on reproduction and metabolism will require systematic identification of targets in sites expressing Rsl, in addition to liver.

Sources of Research Support: NIH RO1DDK053998 awarded to DMR.

Nothing to Disclose: CJK, DMR

P1-110

Tissue Specific Methylation of Intronic Enhancers in the Gene Encoding Steroidogenic Factor 1.

EA Hoivik PhD¹, T Bjanesoy MSc¹, S Witso MSc¹ and M Bakke PhD¹.

¹Univ of Bergen Bergen, Norway.

Embryonic development of adrenals and gonads, and steroid hormone biosynthesis in the adult organs are dependent on the transcription factor steroidogenic factor 1 (SF-1). SF-1 is also essential for normal development of pituitary gonadotropes and of the ventromedial hypothalamic nucleus (VMH). We previously demonstrated that the DNA methylation status of the basal promoter of Sf-1 correlates completely with the SF-1 expression profile in various tissues and cell lines, clearly indicating that its activity is regulated by epigenetic mechanisms. Sf-1 contains intronic enhancers that direct SF-1 expression to the fetal adrenal gland, (fetal adrenal enhancer; FAdE), gonadotropes (pituitary gonadotrope enhancer; PGE) and VMH (VMH enhancer; VMHE). We now present data indicating that the activities of these intronic enhancers are also controlled by DNA methylation, suggesting a complex mode of transcriptional regulation of SF-1 expression.

Nothing to Disclose: EAH, TB, SW, MB

P1-111

Endothelin-Converting Enzyme-1 (ECE-1)-Mediated Differential Trafficking of Corticotropin-Releasing Factor Receptor 1 (CRF-R₁) Activated with Urocortin 1 (Ucn1) or Corticotropin-Releasing Factor (CRF).

B Hasdemir Ph. D¹, M Liao MD¹, NW Bunnett Ph.D¹ and A Bhargava Ph.D¹.

¹UCSF San Francisco, CA.

CRF and Ucn1 regulate endocrine responses to stress via activated CRF-R₁. CRF-R₁ binds to Ucn1 with a much higher affinity than to CRF, but it is unknown whether Ucn1 or CRF differentially affect trafficking or signaling of CRF-R₁. The metalloendopeptidase ECE-1 degrades internalized neuropeptides in endosomes and induces receptor dissociation from β -arrestins to promote recycling and resensitization. We determined whether Ucn1 and CRF induce endocytosis, recycling and resensitization of CRF-R₁, and if ECE-1 similarly controls post-endocytic trafficking and signaling of CRF-R₁ activated with Ucn1 or CRF. CRF-R₁ with an extracellular HA11 epitope was stably expressed in HEK-FLP cells. To examine trafficking, HEK-CRF-R₁ cells were incubated with HA11 antibody to label cell-surface receptors and were stimulated with CRF or Ucn1 (100 nM, 0-30 min). In unstimulated cells, CRF-R₁ was localized to the plasma membrane. Within 30 min of CRF or Ucn1 stimulation, CRF-R₁ was detected in endosomes, where it co-localized with early endosomal antigen 1 and ECE-1. To determine if Ucn1 and CRF serve as substrates for ECE-1, each peptide was incubated with ECE-1 at the acidity of extracellular (pH 7.4) and endosomal (pH 5.5) compartments for 0-120 min. In 2h, ECE-1 degraded Ucn1 at pH 7.4 (71%) but not at pH 5.5 (3%). Conversely, ECE-1 degraded CRF at pH 5.5 (29%) but not pH 7.4 (5%). Inhibition of ECE-1 with the selective inhibitor SM-19712 did not affect CRF or Ucn1-mediated receptor endocytosis. However, ECE-1 inhibition prevented CRF-R₁ recycling in cells stimulated with Ucn1, but not with CRF. ECE-1 inhibition prevents dissociation of GPCRs from β -arrestins in endosomes, prolonging MAPK signaling. Surprisingly, ECE-1 inhibition did not affect the magnitude, or duration of Ucn1 or CRF-induced ERK2 activation. To examine the resensitization of CRF-R₁ signaling, [Ca²⁺]_i was measured using fura-2 in response to repeated stimulation with CRF or Ucn1. Exposure of cells to CRF or Ucn1 caused a similar increase in [Ca²⁺]_i. A second challenge after 0 h of recovery resulted in a minimal [Ca²⁺]_i response, indicating desensitization, whereas, after 4 h of recovery, responses were mostly recovered, indicating resensitization. ECE-1 inhibition strongly inhibited resensitization to Ucn1 (27±9% of control), whereas CRF-mediated resensitization was unaffected (82±8% of control). Thus, ECE-1 may regulate differential trafficking and signaling of CRF-R₁ upon binding different ligands.

Nothing to Disclose: BH, ML, NWB, AB

P1-112

GnRH Regulates LHbeta Sub-Unit Expression through a FOXO3a-Mediated Mechanism.

E Stavrou¹, P Brown¹, R. P Millar¹ and A.J Pawson¹.

¹The Queen's Med Res Inst Edinburgh, UK.

Gonadotropin-releasing hormone (GnRH) is an essential regulator of the reproductive process, and stimulates production of the gonadotropins (LH and FSH) from pituitary gonadotropes, thereby regulating gametogenesis and steroidogenesis. GnRH is synthesized and secreted by hypothalamic GnRH neurons, and delivered to the anterior pituitary gland where it binds to the GnRH receptor. Diverse signaling pathways have been reported to regulate LHbeta sub-unit expression in response to GnRH, including the ERK/p38MAPK/JNK cascades and factors such as EGR1, SF1 and beta-catenin. In the present study, we asked whether FOXO3a plays a role in GnRH regulation of LHbeta expression owing to its widely reported role as a beta-catenin co-factor. FOXO3a is one of four members of the "O" sub-group of Forkhead Box (FOX) transcription factors, and plays an important role in a variety of cellular processes such as tumor suppression, cell cycle-regulation and metabolism. We have shown that FOXO3a is present in gonadotropes, and that GnRH targets its transcriptional activity. Using wild-type and mutant FOXO3a constructs we have shown that FOXO3a regulates LHbeta expression in response to GnRH. We further demonstrated that GnRH can target EGR1 expression in a FOXO3a-dependent manner, and that deletion of the EGR1 binding sites within the proximal region of the LHbeta promoter reduced FOXO3a-mediated up-regulation of LHbeta expression in response to GnRH. Chromatin immunoprecipitation assay was used to assess FOXO3a recruitment to EGR1 and LHbeta promoters. Our data suggest that FOXO3a targets EGR1 expression to, at least in part, indirectly regulate LHbeta promoter activity. We further investigated whether SF1 and beta-catenin are involved in the regulation of the LHbeta expression in co-ordination with FOXO3a in response to GnRH. Based on these data, we propose that GnRH can regulate LHbeta sub-unit expression through one or more FOXO3a-mediated mechanisms.

Nothing to Disclose: ES, PB, RPM, AJP

P1-113

Rescue of Human LH Receptor Mutant Expression and Function with an Allosteric Small Molecule Agonist.

CL Newton PhD¹, AM Whay BSc¹ and RP Millar PhD¹.

¹Med Res Council Edinburgh, UK.

Mutations in G protein-coupled receptors (GPCRs) have been identified for almost all endocrine hormone signaling deficiencies. The majority of these mutations cause protein mis-folding and consequent failure of translocation to the cell membrane, and intracellular degradation. Cell-permeant small molecule ligands (pharmacological chaperones) have been shown to “rescue” such mis-folded mutant GPCRs, presumably by stabilizing correct folding and facilitating shuttling to the cell membrane (e.g. for GnRH and vasopressin receptors). We have examined the potential of a cell-permeant, small molecule, allosteric LH agonist to “rescue” poorly-expressing human LH receptor mutants, A593P and S616Y. Myc-tagged mutant receptors stably-transfected in HEK 293 cells were retained in the cytoplasm compared with wild-type receptor which was localized at the cell membrane. Total binding of [¹²⁵I]-LH to cells expressing the two mutant receptors was 0% (A593P) and 28% (S616Y) of that observed in cells expressing the wild type receptor but there was no difference in binding affinity, indicating that the mutations only affected receptor folding and trafficking to the cell membrane. The small molecule did not displace [¹²⁵I]-LH binding, indicating that it is acting in an allosteric manner. The mutant receptors also displayed no (A593P) or markedly reduced (S616Y) LH stimulation of cAMP accumulation. Pre-incubation of cells expressing the mutant LH receptors with the small molecule increased trafficking of the myc-tagged receptors from the endoplasmic reticulum to the cell membrane and increased [¹²⁵I]-LH binding 2-3 fold. Western immunoblotting demonstrated that incubation with the small molecule increased expression of high molecular weight/mature forms of the mutant receptors by 2.5-5 fold. The small molecule was also able to stimulate a CRE-luciferase cAMP reporter gene in cells expressing the mutant receptors to the same level or greater than the wild type receptor. These studies demonstrate that a cell-permeant, small, alloagonist molecule can “rescue” cell membrane expression of human mutant LH receptors and restores cAMP signaling.

Nothing to Disclose: CLN, AMW, RPM

P1-114

Obesity and Type 2 Diabetes: A Possible Role of GPR40.

A Balhuizen MSc.¹, R Kumar MD.¹ and A Salehi PhD¹.

¹Lund Univ Malmö, Sweden.

Obesity is related to many metabolic disorders and causes metabolic derangements such as raising free fatty acids (FFA) levels as well as insulin resistance in diabetic subjects. Our recent findings show that G-coupled protein receptor 40 (GPR40) is an important player in this context. The aim of present investigation was to study the modulatory effects of hyperglycemia or hyperlipidemia on GPR40 expression and β -cells response to different nutritional stimuli (glucose or palmitate) using rodent pancreatic islets.

GPR40 expression was analysed by Confocal microscopy, Western blot and qPCR in islets from young prediabetic Zucker diabetic fatty (ZDF, fa/fa) rats or diabetic Goto-Kakizaki (GK) rats and their healthy controls. Palmitate-stimulated hormone secretion from isolated islets was RIA-determined.

Confocal microscopy of control islets showed abundant expression of GPR40 protein in insulin, glucagon and somatostatin cells. The GPR40 expression was markedly higher in islets of prediabetic fa/fa rats and had its functional counterpart in an increased response to palmitate-stimulated insulin and glucagon secretion and a pronounced inhibition of somatostatin release. Conversely, GK islets displayed an extremely faint expression of GPR40 as did high-glucose-cultured Wistar control islets. In GK islets, this was reflected in an abolished hormone response to palmitate. In accordance, both Western blot and mRNA expression of GPR40 showed a predominantly increase in fa/fa islets and a markedly decrease in GK islets as compared to control islets and high-glucose-cultured Wistar islets. GPR40 is predominantly expressed in pancreatic islets and influences palmitate-induced secretion of insulin, glucagon and somatostatin. Mild hyperlipidemia increases GPR40 expression and palmitate-induced effects on hormone secretion, whereas hyperglycemia abrogated GPR40 expression and abolished the palmitate-induced secretory effects. Present study adds novel dimensions to palmitate-stimulated hormone secretion in pancreatic islets and identifies GPR40 as a new therapeutic target of various metabolic disorders associated with obesity and type 2 diabetes.

Nothing to Disclose: AB, RK, AS

P1-115

GPR30 New Player in Type 2 Diabetes Mellitus.

R Kumar MD¹, A Balhuizen MSc¹ and A Salehi PhD¹.

¹Lund Univ Malmo, Sweden.

The lower prevalence of diabetes in females suggests that female sex steroids protect from β -cell injury. Consistent with this hypothesis, 17β -estradiol manifests antidiabetic actions in humans and rodents. Our recent findings show that the stimulatory action of 17β -estradiol on insulin secretion is mediated by the G protein coupled receptor 30 (GPR30), raising the prospect that antidiabetic action of 17β -estradiol might be due, in part, by an antiapoptotic effect on β -cells exerted through activation of GPR30. Therefore, the objective of this study was to identify expression of GPR30 in human pancreatic islets and to further clarify the role of GPR30 in pancreatic hormone secretion and beta cell survival. GPR30 expression was analyzed by confocal microscopy, Western blot and qRT-PCR in human pancreatic islets from female and male donors. Hormone secretion and cAMP content in islets were determined with RIA and apoptosis with the Annexin-V method.

Confocal microscopy revealed GPR30 expression in alpha, beta and delta cells of pancreas. GPR30 mRNA and protein expression was markedly higher in female vs male islets ($p < 0.01$). Dose-response studies of G-1 (a selective agonist of GPR30) vs 17β -estradiol

in isolated islets at 12 mM glucose showed an almost similar pattern in potentiating insulin and suppressing glucagon and somatostatin secretion.

The 17β -estradiol genomic receptor ($ER\alpha$ and $ER\beta$) antagonist ICI-182,780 (Fulvestrant) or Acolbifene (EM-652) did neither influence the amplifying effects of G-1 or 17β -estradiol on cAMP content ($p < 0.001$) nor insulin secretion from islets. Cytokine-induced ($IL1\beta + TNF\alpha + INF\gamma$) apoptosis in islets, cultured for 24 h at 5 mmol/l glucose, was almost abolished by G-1 or 17β -estradiol treatment ($p < 0.001$). These beneficial effects of G-1 or 17β -estradiol on pancreatic islets were not affected by either Fulvestrant or Acolbifene.

In view of these novel finding we suggest that drugs that could selectively modulate the activity of GPR30, represent a promising frontier in diabetes mellitus co-adjuvant therapy.

Nothing to Disclose: RK, AB, AS

P1-116

Physiological Role of Parathyroid Hormone-Related Protein (PTHrP) and Parathyroid Hormone-1 Receptor (PTH1R) Signaling in the Pancreatic β -Cell.

Xiaoying Zhang Ph.D.¹, Nagesha K Guthalu Ph.D.¹, Katoura Williams B.S.¹, Anaïs Mozar Ph.D.¹ and Vasavada Rupangi Ph.D.¹.

¹Univ of Pittsburgh Pittsburgh, PA.

PTHrP and PTH1R are expressed in the pancreas during development, in the adult β -cell of both rodent and human islets, and in insulinoma cells. Rat insulin II promoter (RIP) driven PTHrP transgenic mice, as well as rodent and human islets treated with PTHrP in vitro, show enhanced β -cell function, proliferation, and survival. Moreover, only the amino-terminus 1-36 PTHrP peptide, which binds to PTH1R, is sufficient to induce the beneficial effects of PTHrP in the β -cell, underscoring the importance of PTHrP/PTH1R interaction. Although it is evident that this pathway has obvious therapeutic potential in the β -cell, its role in normal β -cell physiology and pathophysiology has not been examined, which is the aim of the current study.

We approached this by generating β -cell specific conditional knockout (CKO) mice of the PTH1R by intercrossing RIP-Cre, PTH1R heterozygous KO, and PTH1R floxed mice. Efficient deletion of the PTH1R floxed allele in islets of CKO mice was demonstrated using genomic DNA. Importantly, using a fluorescent tagged ligand binding assay on β -cells from normal (NL) and CKO mice, we showed a functional knockdown of the PTH1R in β -cells of CKO mice.

Analysis of the phenotype of the CKO mice at 2-3 months of age shows normal body weight, fasting plasma glucose and insulin, glucose tolerance, and insulin sensitivity in both male and female mice. However, male CKO mice exhibit mild but significant hyperglycemia (182.1 ± 10.7 vs 151.7 ± 6.0 mg/dl) and hypoinsulinemia (0.57 ± 0.05 vs 0.85 ± 0.11 ng/ml) under non-fasting conditions, compared to NL littermates. Examination of β -cell homeostasis shows no changes in mass, proliferation, size, or cell death in the CKO mice relative to NL controls. As RIP-PTHrP transgenic mice are resistant to the cytotoxic effects of streptozotocin (STZ), we hypothesized that the PTH1R-CKO mice would be more sensitive. Indeed preliminary results indicate that CKO mice have increased β -cell death 16 hours after STZ administration compared to NL littermates.

Thus, PTH1R-CKO mice have normal β -cell morphology, suggesting that PTH1R signaling in the β -cell is not essential for its development or basal growth. However, whether PTH1R signaling affects β -cell function or insulin secretion is currently under investigation. Our studies suggest that the PTH1R-CKO mice are likely to be more sensitive to stress. Therefore, we will analyze the role of PTHrP/PTH1R signaling in the pathophysiological setting of Type 1 and Type 2 diabetes.

Sources of Research Support: NIH Grant DK078060&DK072264 to RCV; JDRF Research Award to RCV(1-2008-46).

Nothing to Disclose: XZ, NKG, KW, AM, VR

P1-117

The α -Subunit of the Stimulatory G Protein (G_{α}) and Its Imprinted Variants XL α s/XXL α s Are Trafficked into Distinct Subcellular Compartments upon Activation.

Z. J. Liu PhD¹ and M Bastepe MD, PhD¹.

¹Harvard Med Sch, Massachusetts Gen Hosp Boston, MA.

Certain endocrine tumors and McCune-Albright syndrome are caused by mutations of GNAS that lead to constitutive activity of G_{α} and its variant XL α s. These two proteins have identical C-terminal amino acid sequences, but differ from each other in the N-terminus encoded by different first exons. Although XL α s, as well as its N-terminally extended variant XXL α s, can mimic G_{α} regarding cAMP signaling, current data indicate that XL α s and/or XXL α s have unique cellular roles, which remain entirely unknown. Moreover, the differences between the activated forms of XL α s, XXL α s, and G_{α} have not been investigated. Using western blots, immunocytochemistry, and fluorescence microscopy, we compared the subcellular localizations of these proteins before and after activation. In transfected HEK293 cells, XL α s and XXL α s remained in the plasma membrane after stimulation by cholera toxin or the β -adrenergic agonist isoproterenol, whereas G_{α} redistributed to the cytosol. Furthermore, unlike the gsp oncogene G_{α} -R201H, the cognate XL α s and XXL α s mutants (XL α s-R543H and XXL α s-R844H) localized to the plasma membrane, which was observed in transfected HEK293 and mouse embryonic fibroblasts null for endogenous G_{α} , XL α s, and XXL α s. These distinctions in subcellular localization were also observed in the same cell by the use of a yellow fluorescent protein labeled G_{α} fusion protein and immunofluorescence directed against XL α s or XXL α s. Following cholera toxin treatment of rat PC12 cells, which express both XL α s and G_{α} endogenously, G_{α} became mostly soluble while XL α s remained in the particulate fraction. Serial truncations at the N-terminus of XL α s and XXL α s and subsequent mutational analyses indicated that two conserved cysteine residues C-terminal to the conserved proline-rich domain are critical for the membrane targeting of XL α s and XXL α s. An XL α s- G_{α} chimera, in which a 70-amino acid segment of XL α s spanning these cysteines was added onto G_{α} N-terminus, also remained in the particulate fraction upon cholera toxin treatment. These results indicate that XL α s and XXL α s demonstrate strong affinity for the plasma membrane, which may form the basis for their as-yet-undefined cellular roles. The discovery of the unique actions of the latter proteins is critical for better understanding of the pathogenesis underlying the diseases caused by GNAS mutations.

Sources of Research Support: NIH Grant R01DK073911 awarded to MB.

Nothing to Disclose: ZJL, MB

P1-118

Evidence for Allosteric Interactions between Oxytocin and β_2 -Adrenergic Receptors.

PK Wrzal MSc¹, TE Hebert PhD¹ and HH Zingg MD/PhD¹.

¹McGill Univ Montreal, Canada.

The G_q protein-coupled oxytocin receptor (OTR) mediates uterine contraction. The G_s-coupled β_2 -adrenergic receptor (β_2 AR) is co-expressed with the OTR in myometrial cells but mediates uterine relaxation. The two receptors are important pharmacological targets because OTR antagonists and β_2 AR agonists are used to control preterm uterine contractions. Our previous studies using bioluminescence resonance energy transfer (BRET) and co-immunoprecipitation suggested the formation of an OTR/ β_2 AR hetero-oligomeric complex. The goal of this study was to establish functional consequences of OTR/ β_2 AR interaction in myometrial cells.

We assessed the modulation of ERK1/2 activation, a pathway common to both receptors, using immortalized human myometrial hTERTC3 cells. We found that ERK1/2 activation induced by the β_2 AR agonist isoproterenol (ISO) was attenuated by 50% following pretreatment with either of the OTR antagonists OTA or atosiban. Similarly, ERK1/2 activation induced by the OTR agonist oxytocin (OT) was attenuated by 60% following pretreatment with either of the β_2 AR antagonists propranolol or timolol. Pretreatment with the β_2 AR agonist ISO led to a similar 60% attenuation of OTR-mediated ERK1/2 activation. By contrast, pretreatment of the cells with the β_2 AR inverse agonist atenolol resulted in a 25% increase in OTR-mediated ERK1/2 activation.

Binding studies provided further evidence for allosteric interactions between the two receptors. The effect of the β_2 AR agonist ISO on OT binding was assessed in HEK 293 cells expressing either one receptor alone or both receptors. ISO induced an 85% decrease in surface OT binding in cells expressing both receptors. No statistically significant ISO-induced decrease in OT surface binding was observed when the OTR was expressed alone. The effect of OT on β_2 AR binding is currently being evaluated.

Our analyses suggest physical and allosteric interactions between OTR and β_2 AR. Our results also emphasize the need for, and great pharmacological potential of, studying allosteric modulation in a heterodimer setting. Further investigations are required to clarify the precise mechanisms involved in the allosteric communication between OTR and β_2 AR.

Sources of Research Support: CIHR.

Nothing to Disclose: PKW, TEH, HHZ

P1-119

Cortistatin Blocks Key Steps of the Progression of Atherosclerosis, In Vitro.

Mario Duran-Prado PhD¹, Justo P Castano PhD², Michael D Culler PhD³ and Mario Delgado PhD¹.

¹CSIC, Inst of Parasitology and BioMed Lopez-Neyra Granada, Spain ; ²Univ of Cordoba Cordoba, Spain and ³Biomeasure Inc/IPSEN Milford, MA.

Increased proliferation and migration of smooth muscle cells (SMCs) as consequence of an excessive local inflammation are key steps in the progression of atherosclerosis and restenosis, leading to vessel blockade, myocardial infarction and stroke. In this context, our group has demonstrated that the neuropeptide cortistatin (CST) is highly effective against several inflammatory diseases, whereas its natural analogue somatostatin (SST) has no effect. Indeed, some functions of CST are shared with ghrelin and seem to be mediated by GHSRs. Use of molecular biology techniques enabled us to discover that CST is highly expressed in human aortic SMCs (hAoSMCs) whereas SST is expressed at a low level and ghrelin is absent. Furthermore, CST is secreted by these cells and its release is increased in two fold upon treatment with PDGF, which led us to postulate that CST could exert a protective role for these cells under pathological conditions. To test this hypothesis, we have evaluated the ability of CST to regulate PDGF-induced proliferation and migration of hAoSMCs, in vitro, focusing on the receptors and signal transduction pathways activated by the peptide, compared to SST and ghrelin. Firstly, we have found that the three peptides inhibited PDGF-stimulated proliferation of hAoSMCs showing an "u-shaped" dose-response curve, through mechanisms that involve the phosphorylation of p38 kinase and an increase in the cAMP level. This effect, in case of CST and ghrelin, also involves a decrease in PDGF-induced Akt phosphorylation. Using real time PCR, we realize that almost all SST receptors (sst5) are expressed in hAoSMCs, and both GHSRs are also detected in these cells. The use of selective antagonists for ssts and GHSRs led us to discover that SST inhibitory action in cell proliferation is mainly mediated by sst2, whereas CST action is more complex and also entails sst5 and GHSRs. CST and ghrelin inhibit the migration of hAoSMCs by blocking PDGF-induced free cytosolic calcium increase, which is mediated by GHSRs and is independent of ssts. In this line, SST failed to inhibit PDGF-induced free cytosolic calcium increase and thus migration of these cells. As a whole, and considering that CST but neither SST nor ghrelin are significantly expressed in aortic SMCs, our results suggest that CST could be a natural regulator of the functioning of SMCs under pathological conditions and let us to propose the peptide as a promising compound for the treatment of atherosclerosis.

Sources of Research Support: Programa Sara Borrell of Ministerio de Ciencia y Tecnologia (CD07/00246).

Nothing to Disclose: MD-P, JPC, MDC, MD

P1-120

Inhibition of Angiotensin II-Induced Signaling in Cardiomyocytes by Nebivolol, a beta-Adrenergic Receptor Blocker.

L Pulakat^{1,2}, VG DeMarco¹, N Rehmer^{1,2}, D Elliott^{1,2}, RI Schneider^{1,2}, J Habibi^{1,2}, A Whaley-Connell^{1,2} and JR Sowers^{1,2}.

¹Univ of Missouri - Columbia Sch of Med Columbia, MO and ²Harry S Truman VA Med Ctr Columbia, MO.

The potent vasoconstrictor hormone Angiotensin II (Ang II) activates multiple signaling pathways in heart tissue via its specific receptors, the AT1 and the AT2. Previous studies have shown that Ang II acting through the AT1, induces NADP(H) oxidase activation and subsequent generation of reactive oxygen species (ROS) in heart tissue. The Ren2 rat, an animal model with chronic elevated tissue Ang II levels due to expression of mouse Ren2 transgene, exhibits impaired glucose uptake by muscle and heart, serine phosphorylation of IRS-1 in heart tissue, and increases in NADP(H) oxidase activity and ROS. The AT1 receptor blockers (ARB) inhibit these metabolic abnormalities in Ren2 rat. Interestingly, we observed that Nebivolol, a third-generation β -adrenergic receptor blocker with vasodilator properties could also inhibit elevated NADP(H) oxidase activity and ROS generation in this animal model. Because Nebivolol is a beta-blocker and it mimicked ARB in inhibiting the NADPH oxidase activity, we hypothesized that Nebivolol-induced signaling could cross-talk with the Ang II-induced signaling in heart tissue. Therefore, we used cardiomyocyte HL-1 cell line to determine whether or not Nebivolol could directly inhibit Ang II-induced activation of the NADP(H) oxidase activity and ROS generation. HL-1 cells were cultured in medium containing 10% FBS (Sigma) supplemented with 0.1mM Norepinephrine. Cells were allowed to reach 90% confluency, washed repeatedly with PBS and were either subjected to serum starvation for 12 hours or directly used for exposure to ligands [Ang II (100nM to 1mM); Nebivolol (1mM to 10 mM) or their combinations] for different time periods (1-8 hours). NADP(H) oxidase activity was determined by using plasma membrane fractions and measuring the conversion of Radical Detector in the absence and presence of NADP(H) oxidase inhibitor apocynin (500 μ M) using spectrophotometric (450 nm) techniques. ROS levels were determined using whole cell lysates via chemiluminescence assay with 5 μ M lucigenin in dark-adapted counting vials. Samples were then normalized to total protein in the whole cell lysate, and ROS values were expressed as counts \cdot minute⁻¹ \cdot mg protein⁻¹. We observed that Nebivolol inhibited both Ang II-induced NADP(H) oxidase activity and ROS generation in HL-1 cells in all conditions tested. These observations suggest that Nebivolol could directly inhibit oxidative stress induced by other stress-inducers such as Ang II in cardiomyocytes.

Sources of Research Support: NIH and VA Merit to JRS and CDA-2 for AWC and Forest Research Institute.

Nothing to Disclose: LP, VGD, NR, DE, RIS, JH, AW-C, JRS

P1-121

A Signal Mediated through the Pituitary Cannabinoid CB₁ Receptor (CB₁R) Is Required for Normal Development of Prolactin (PRL) Cells and Angiotensin II (AII)-Induced PRL Release.

I Bodnar¹, M Olah¹, D Hecht¹, G Turu¹, J Denes², T Wenger¹, M Goth² and GM Nagy¹.

¹Semmelweis Univ Budapest, Hungary and ²State Hlth Ctr Budapest, Hungary.

Background and Aims: It has been shown that CB₁ receptor (CB₁R) and its corresponding ligands are expressed in cells of the anterior lobe of the pituitary gland and cannabinoids can modulate anterior pituitary hormone secretion. In our present studies the significance of CB₁R in dopamine- (DA) and angiotensin II (AII) induced prolactin (PRL) and adrenocorticotroph hormone (ACTH) release as well as ghrelin and growth hormone-releasing hormone (GHRH) induced growth hormone (GH) release were investigated using pituitary cell cultures derived from wild type (WT) and CB₁R knock-out (CB₁KO) mice. Dispersed anterior lobe cells were used immediately after dispersion in the PRL reverse haemolytic plaque assay (PRL-RHPA) and the number of PRL releasing cells was evaluated, or in primary cell cultures following 24 hours incubation and concentrations of PRL, ACTH and GH of the culture media were measured by specific RIA. Cells were treated with different concentrations of DA, TRH or AII, CB₁R antagonist (AM251), ghrelin and GHRH in the PRL-RHPA and in primary cultures, respectively.

Results: In male mice, DA exhibited similar inhibition on PRL release in both WT and CB₁KO animals however the proportion of PRL secreting cells were significantly higher in the CB₁KO group than in the WT controls detected in the RHPA. We also observed tyrosine hydroxylase (TH) immunoreactive glandular cells only in anterior lobes obtained from CB₁KO male mice. AII could strongly stimulate PRL as well as ACTH release in WT animals, while PRL release was diminished and ACTH release was enhanced in the CB₁KO group. Pretreatment of cultured cells obtained from WT animals with AM251, a CB₁R antagonist, prevented AII induced PRL but not ACTH response.

Summary and Conclusion: Our results clearly show that the lack of CB₁R profoundly affects PRL cell number as well as PRL and ACTH release induced by AII in male mice. All these together indicate an intra-pituitary role of the endocannabinoid system, which might manifest through a receptor cross-talk between CB₁ and AII receptors.

Sources of Research Support: OTKA-68170, 68660 and 81522, ETT-458/09.

Nothing to Disclose: IB, MO, DH, GT, JD, TW, MG, GMN

P1-122

The Presence of Somatostatin Receptor 5 Modulates β_1 -Adrenergic Receptor Mediated Signaling Pathways.

RK Somvanshi PhD¹, SA War MSc¹, N Chaudhari¹, G Kharmate¹, PS Rajput¹, D Bakshi¹, X Qiu¹ and U Kumar PhD¹.

¹Univ of British Columbia Vancouver, Canada.

The concept that G-protein coupled receptors (GPCRs) exist and function as dimers or higher order of oligomers is undisputed. Whether GPCRs from different families interact through heterodimerization is still elusive especially when receptors are regulated via different G-proteins. We here address this question by using human somatostatin receptor 5 (hSSTR5) and β_1 -adrenergic receptor (β_1 -AR) which couple to G_i and G_s respectively. Receptors were stably expressed in HEK-293 cells at physiological levels and studied for heterodimerization, receptor trafficking, coupling to adenylyl cyclase and signaling pathways using morphological, biochemical and FRET analysis in intact cells. We first determined the status of hSSTR5 and β_1 -AR in montransfected cells and then in cells cotransfected with hSSTR5 and β_1 -AR. hSSTR5/ β_1 -AR exist as heterodimers as shown by relative FRET efficiency of $12 \pm 0.7\%$ in the basal condition which was further enhanced ($17 \pm 1.2\%$) upon synergistic activation of both the receptors by using specific agonists. Interestingly, activation of individual β_1 AR or hSSTR5 displayed preferential homodimerization rather than heterodimerization. In montransfected cells expressing β_1 -AR or hSSTR5, forskolin (FSK) stimulated cAMP was increased in presence of β_1 -AR specific agonist isoproterenol and decreased upon activation of hSSTR5. In cotransfectants, β_1 AR effect was predominant however, blocking β_1 -AR with specific antagonist (CGP 20712) resulted in 60% inhibition of FSK stimulated cAMP in presence of SST or SSTR5 agonist (L-817818). In cotransfected cells, cAMP/PKA pathway was regulated in receptor specific manner. The status of phospho-ERK1/2 and PI3K/AKT was predominantly regulated by hSSTR5. In contrast, phospho-p38 expression was completely abolished in cotransfected cells. The phosphorylated levels of NFAT remained unchanged indicating blockade of calcineurin mediated dephosphorylation of NFAT and its nuclear translocation, the process predominantly regulated by activated JNK. In conclusion, data presented here has potential significance for hSSTR5 in regulation of signaling pathways such as ERK1/2, PI3K/AKT in SSTR5/ β_1 -AR heteromeric complex indicating a new role of SSTR and AR subtypes in cardio-physiology.

Sources of Research Support: CIHR, CBCF-BC&YUKON and MSFHR.

Nothing to Disclose: RKS, SAW, NC, GK, PSR, DB, XQ, UK

P1-123

Somatostatin Receptor 5 and β_2 -Adrenergic Receptor Crosstalk Modulates Intracellular Signaling.

RK Somvanshi PhD¹, SA War¹, N Chaudhari¹, G Kharmate¹, PS Rajput¹, D Bakshi¹, X Qiu¹ and U Kumar¹

¹Univ of British Columbia Vancouver, Canada.

Somatostatin receptors (SSTRs) and adrenergic receptors (ARs) belong to GPCR family and are coupled to G_i and G_s respectively. Several previous studies have shown that SSTRs and β_2 -AR display great diversity in homo-and/or heterodimerization within the family as well as with other members of GPCR family such as dopamine and opioid receptors with enhanced functional properties. In the present study, we investigated the agonist dependent heterodimerization and changes in receptor trafficking, coupling to adenylyl cyclase and signaling pathways in HEK-293 cells stably cotransfected with hSSTR5 and β_2 -AR and compared with monotransfected cells. hSSTR5/ β_2 -AR exists in a heterodimeric complex in basal state and the level of heterodimerization was further enhanced (increased FRET efficiency) upon coactivation of both the receptors in comparison to the activation of the individual receptors with their specific agonists. In cotransfected cells, inhibition of forskolin (FSK) stimulated cAMP with SST or SSTR5 specific agonist (L-817818) was blocked in the presence of β_2 -AR agonist formoterol when compared to monotransfected cells. In contrast, SST mediated inhibition of FSK stimulated cAMP was recovered by 23% in presence of β_2 -AR specific antagonist (ICI-118551). Receptor specific regulation of intracellular cAMP levels directly resulted in inhibition of G_i -cAMP/PKA pathway. In cotransfected cells, hSSTR5 mediated ERK1/2 phosphorylation was enhanced while phospho-p38 expression was completely abolished when compared with monotransfected cells. Conversely, expression levels of PI3K/AKT phosphorylation were not changed in cotransfected cells. JNK mediated phosphorylation of NFAT was observed suggesting inhibition of calcineurin induced NFAT dephosphorylation and nuclear translocation in cotransfectants. Taken together, these data describe a novel mechanism for the role of hSSTR5 and β_2 -AR in modulation of signaling pathways. In conclusion, data presented here indicate physiological significance in cardiovascular complications as normally observed in Huntington's disease and pituitary tumor (acromegaly).

Sources of Research Support: CIHR, CBCF-BC&YUKON and MSFHR.

Nothing to Disclose: RKS, SAW, NC, GK, PSR, DB, XQ, UK

P1-124

RNA Binding Protein - Mediated Regulation of Luteinizing Hormone Receptor (LHR) mRNA Expression in the Ovary: Role of PKA and ERK 1/2 Signaling.

Bindu Menon PhD¹, Megan Franzo-Romain¹ and K.M.J. Menon PhD¹.

¹Univ of Michigan Med Ctr Ann Arbor, MI.

LHR mRNA expression in the ovary undergoes downregulation during LH surge or in response to administration of a pharmacological dose of hCG. We discovered an mRNA binding protein, designated as LRBP, which binds LHR mRNA and mediates its accelerated degradation during hCG-induced LHR downregulation. The intermediary role of cAMP in this process was also established in our laboratory. LRBP has been purified and its identity has been established (Nair AK et al., 2004). Present study examined the downstream signaling events that are involved in LRBP-mediated regulation of LHR mRNA expression. Human granulosa cells were isolated from ovarian follicular aspirates after ova harvest and cultured in McCoy's medium containing 10% FBS for 48 h and in serum free medium for an additional 48 h. The cells were then treated with 10 IU hCG for 12 h, total RNA isolated and the changes in LHR mRNA levels were quantitated using real-time PCR. As expected, there was a significant downregulation in the levels of LHR mRNA in cells treated with hCG, when compared to the control (0.47 ± 0.12 - fold vs. control, $p < 0.05$, $n = 3$). Inhibition of PKA or ERK 1/2 using pharmacological inhibitors, H89 (10 μ M) or U0126 (10 μ M) respectively, significantly reduced the extent of hCG-induced LHR mRNA downregulation since LHR mRNA levels were comparable to control levels in cells that were pretreated with H89 (1.05 ± 0.084 - fold vs. control) or U0126 (1.03 ± 0.14 - fold vs. control). We then examined whether inhibition of PKA or ERK1/2 had any effect on LRBP expression and its activity by Western Blot and RNA electrophoretic mobility shift assay (REMSA), respectively. In response to hCG, there was a 2.5 fold increase in LRBP protein expression and its RNA binding activity. These increases were completely abolished by pretreatment with H89 or U0126 (fold change vs. control; hCG- 2.45 ± 0.3 ; H89+hCG- 1.17 ± 0.07 ; U0126+hCG- 1.04 ± 0.22). These data show that PKA and ERK1/2 pathways mediate the LH/hCG induced LHR mRNA downregulation, by regulating the expression and activity of LRBP.

Nair AK et al., J Biol Chem 2004; 279:14937

Sources of Research Support: NIH grant R37 HD06656 awarded to KMJM.

Nothing to Disclose: BM, MF-R, KMJM

P1-125

Expression of Somatostatin Receptors in the Diabetic Brain of *db/db* Mouse Model.

SA War M.Sc.¹, ES Foot¹, RK Somvanshi Ph.D.¹, N Chaudhari¹, G Kharmate¹, PS Rajput¹, D Bakshi¹, X Qiu¹, I Laher Ph.D.¹ and U Kumar Ph.D.¹.

¹The Univ of British Columbia Vancouver, Canada.

Diabetes is known to be a major risk factor in various neurodegenerative disorders. Somatostatin is a regulatory peptide secreted by endocrine, neuronal and immune cells which acts to regulate neurotransmission, cell proliferation and cell secretion via five G-protein coupled somatostatin receptors (SSTR1-5). SSTRs are widely distributed and highly expressed in normal as well as in several pathological conditions. In human pancreatic islet cells, SSTRs are variably expressed and colocalize with insulin, somatostatin and glucagon. Several previous studies have demonstrated changes in the expression of SSTR subtypes in Alzheimer's, Parkinson's and Huntington's disease, which are also associated with diabetes. SSTRs are expressed in the brain in a receptor and region specific manner however, their distribution and role in diabetic brain is not well understood. In the present study using immunohistochemistry and western blot analysis, we determined the expression of SSTRs in different brain regions of *db/db* mice and compared it with age-matched wild type (*wt*). In *db/db* mice, SSTR1, SSTR3 and SSTR4-like immunoreactivity was markedly reduced in the hypothalamic, cortical, striatal and hippocampal regions. In contrast, SSTR2 and SSTR5 expression was significantly elevated when compared with *wt*. Interestingly, previous studies have demonstrated inhibition of insulin secretion in pancreas upon activation of SSTR2 and SSTR5. It is not known if the increased expression of SSTR2 leads to diabetic neuropathy since SSTR2 is also associated with the induction of apoptosis in cell and tissue specific manner. Further, we also attempted to correlate these changes with glutamic acid decarboxylase (GAD) and brain nitric oxide synthase (bNOS) expression. In *db/db* mice, GAD expression was significantly decreased whereas bNOS-like immunoreactivity was highly elevated in comparison to *wt*. In conclusion, our study provides new insights for the role of SSTRs in the pathogenesis of diabetic complications in the brain.

Sources of Research Support: CIHR, CBCF-BC/Yukon, and MSFHR.

Nothing to Disclose: SAW, ESF, RKS, NC, GK, PSR, DB, XQ, IL, UK

P1-126

Effects of Moderate Global Reduction in Maternal Nutrition on β -Adrenergic Receptor (β -AR) Expression and Function in Fetal Baboon Hepatocytes.

C Li MD¹, Z-J Shu BS¹, PW Nathanielsz MD¹ and A Kamat PhD^{1,2}.

¹Univ of Texas Hlth Sci Ctr at San Antonio San Antonio, TX ; ²Univ of Texas Hlth Sci Ctr at San Antonio San Antonio, TX and ³Audie Murphy VA Hosp San Antonio, TX.

Catecholamines released by the sympathetic nervous system interact with β -ARs to regulate various physiological processes including gluconeogenesis and lipolysis. Decreased maternal and hence fetal nutrient supply leads to slowing of fetal and placental growth and compensatory changes in fetal metabolism. Numerous studies indicate that unwanted effects of altered fetal development may persist into later life and predispose to chronic diseases. Little is known about the effects of decreased fetal nutrient delivery on baboon adrenergic system development. We have previously shown increased plasma cortisol and hepatic adrenergic innervation before birth in offspring of nutrient-restricted baboon mothers (MNR) compared with offspring of *ad-libitum*-fed baboon mothers (CTR). We also demonstrated that MNR has differential gestation stage-specific effects on immunohistochemical abundance of β -AR protein in the liver lobule. To determine effects of decreased fetal nutrient delivery on baboon hepatic β -AR expression and function during development, we treated primary cultures of fetal baboon hepatocytes isolated at 0.5 gestation (G) and 0.9G with the β -AR agonist, isoproterenol (Iso) or the glucocorticoid, dexamethasone (Dex). To our knowledge, this is the first time that fetal baboon hepatocytes have been isolated and cultured. Treatment of hepatocytes isolated from 0.9G baboon fetus with 10 μ M Iso increased mRNA and protein levels of phosphoenolpyruvate carboxy kinase-1 (PCK-1), a key enzyme in gluconeogenesis. PCK-1 levels increase in mammalian cells upon β -AR activation and indicate that the isolated baboon hepatocyte cultures are a representative system to study β -AR function. A decrease in β_1 -AR and an increase in β_2 -AR mRNA levels in response to MNR were also observed in hepatocytes isolated from 0.5G fetuses. Additionally, an increase in β_1 -AR mRNA levels between 0.5 and 0.9G was demonstrated in MNR fetuses. We also treated fetal baboon hepatocytes with Dex (10 nM), and observed a decrease in fetal baboon β_1 -AR mRNA and protein levels. These results suggest that increased fetal plasma cortisol levels in MNR may suppress β_1 -AR gene transcription during development. Studies are currently being conducted using this novel nonhuman primate model system to study effects of MNR on β -AR expression, coupling and function to determine mechanisms that are involved in the programming of liver function associated with later life diabetes, obesity and metabolic syndrome.

Sources of Research Support: NIH grant HD 21350; Veterans Integrated Systems Network 17 Award (AK); American Heart Association Grant-in-Aid (AK).

Nothing to Disclose: CL, Z-JS, PWN, AK

P1-127

Molecular Characterisation Reveals Tissue-Specific, Antisense Intronic Non-Coding RNA Transcription in the *GHSR* Locus.

I Seim BSc, PhD¹, SL Carter BSc, PhD¹, AC Herington BSc, PhD¹ and LK Chopin BVSc, MSc, PhD¹.

¹Inst of Hlth and Biomed Innovation Kelvin Grove, Australia.

Here we report *GHSROS* (*GHSR* Opposite Strand), a single-exon, candidate non-coding RNA gene that overlaps the *GHSR* (growth hormone secretagogue receptor) gene on chromosome 3q26.31; a region amplified in several cancers, including lung cancer. The novel *GHSROS* gene is 1.1 kb in length and fully contained within the 2.1 intron flanking the coding exons of the *GHSR* gene. Full-length sequencing demonstrated *GHSROS* transcripts that are derived from a putative antisense promoter within an ancient MER5B DNA transposable element in the *GHSR* intron. Quantitative, real-time RT-PCR revealed that the level of *GHSROS* transcripts is very variable, with low expression in the normal tissues examined (including the normal lung) and high levels in lung tumor tissues. Although the biological role of *GHSROS* remains unclear, the data suggest that *GHSROS* is highly expressed in lung cancer and we hypothesize that the transcript could be a useful biomarker or therapeutic target for disease; or it may regulate the expression of the overlapping ghrelin receptor gene (*GHSR*).

Sources of Research Support: Grants from the Cancer Council Queensland (to LKC and ACH), the Faculty of Science and Technology, Queensland University of Technology and a QUT Early Career Researcher grant (to IS).

Nothing to Disclose: IS, SLC, ACH, LKC

P1-128

Characterization of a Putative Caveolin Interaction Motif in the Human Follicle Stimulating Hormone Receptor.

MC Dzialo BS¹, JA Dias PhD² and BD Cohen PhD¹.

¹Union Coll Schenectady, NY and ²New York State Dept of Hlth Albany, NY.

Compartmentalization of signal transducing proteins into membrane microdomains is one explanation as to why cells are capable of producing rapid and efficient signaling responses. These microdomains, or membrane rafts, provide scaffolding for cell signaling molecules to come into close proximity with other effectors. Follicle stimulating hormone receptor (FSHR) is a G protein-coupled receptor shown to localize in membrane rafts, potentially in a subtype of these rafts known as caveolae. Previous work in this lab has shown that human FSHR co-immunoprecipitates with caveolin, a scaffolding protein that serves as the molecular marker for caveolae. Presence of a putative caveolin binding motif ($\phi X \phi XXXX \phi XX \phi$) (1) in the fourth transmembrane domain of hFSHR (amino acids 479-489, FAFAAALFPIF) suggests that the receptor interacts with caveolin at this site; this interaction could prove necessary for hormone binding, signal transduction, or membrane trafficking. To test the role of caveolin interaction with the hFSHR, we created two mutant receptors with point mutations converting one of the essential phenylalanine residues in the caveolin binding domain to a leucine residue (F481L and F489L). Both mutants were successfully transfected and synthesized in human embryonic kidney cells (HEK 293). Preliminary data suggests that wild-type receptor and mutant receptor with point mutation F481L interact with caveolin while mutant receptor F489L does not. We have also found that receptor F481L is still capable of binding to hormone with a slightly higher affinity for ligand than wild-type receptor. Mutant receptor F489L is unable to bind to hormone suggesting that caveolin may be essential for hFSHR's ability to bind ligand or for trafficking to the cell membrane.

(1) Couet J et al., J Biol Chem 1997; 272:6525

Nothing to Disclose: MCD, JAD, BDC

P1-129

Follicle Stimulating Hormone Receptor Signaling Is Disrupted by Removal of Membrane Cholesterol but Not by Degradation of Membrane Sphingomyelin.

BD Cohen PhD¹ and JA Dias PhD².

¹Union Coll Schenectady, NY and ²New York State Dept of Hlth Albany, NY.

The human follicle stimulating hormone receptor (hFSHR) belongs to the G protein-coupled receptor (GPCR) family and canonically stimulates cAMP production in response to interaction with hormone. To investigate the residency of hFSHR in membrane rafts, raft disrupting agents were used to treat cells and hFSHR signaling was measured. Treatment of HEK293 cells with the cholesterol withdrawing drug methyl- β -cyclodextrin (MBCD) resulted in loss of hormone dependent cAMP production (175nM cAMP produced for control cells at 10ng hFSH vs 6nM for MBCD treated cells, $p < 0.001$). This loss of function was reversed by either washing out the MBCD or by treatment with MBCD that was preloaded with cholesterol which restored the membrane cholesterol. hFSHR lacks a putative cholesterol binding domain seen in other GPCR so the mechanism of MBCD abrogation of signaling is unclear. One possibility is that MBCD disrupted membrane rafts necessary for signal transduction. To investigate this hypothesis bacterial sphingomyelinase was used to degrade membrane sphingomyelin. This had no effect on FSH-stimulated cAMP production. The differential response to these membrane raft disrupting treatments may indicate that hFSHR resides in membrane rafts more dependent on cholesterol than sphingomyelin for structural integrity such as caveolae.

Nothing to Disclose: BDC, JAD

P1-130

Recruitment of PDK1 to the SHPS-1 Signaling Complex Enhances IGF-I Stimulated AKT Activation in Vascular Smooth Muscle Cells (VSMC).

Xinchun Shen Ph.D¹, Gang Xi Ph.D¹, Yashwanth Radhakrishnan Ph.D¹ and David R Clemmons M.D¹.

¹The Univ of North Carolina at Chapel Hill Chapel Hill, NC.

The SH2 domain-containing protein tyrosine phosphatase substrate 1 (SHPS-1) is an integral membrane protein that acts as a scaffold for multi-protein signaling complexes that are assembled in response to IGF-I in cells exposed to hyperglycemia. Our previous studies showed that insulin-like growth factor (IGF-I) stimulated SHPS-1/SHP2/Src complex formation is required for mediating IGF-I actions in VSMC and that truncation of cytoplasmic domain (CD) of SHPS-1 significantly impaired IGF-I stimulated AKT dependent functions. In the present study, we investigated the mechanism of PDK1 recruitment to the SHPS-1 signaling complex and how this regulates PDK1 activation.

PDK1 is the kinase that phosphorylates Akt Thr308. In addition, c-Src kinase and Pyk2 have been implicated in PDK1 activation. Using SHP2 silencing, Src silencing and a Pyk2 Tyr402 mutant (the Src binding site), we showed that the SHP2/Src complex mediated IGF-I stimulated Pyk2 recruitment to SHPS-1 via an interaction of Pyk2 Tyr402 and SH2 domain in Src. Following Pyk2/Src association, IGF-I stimulated Src to phosphorylate Pyk2 Tyr881 which provides a binding site for Grb2. Subsequently Grb2 recruited PDK1 to the SHPS-1 complex via the interaction of a proline rich sequence in PDK1 with a SH3 domain in Grb2. Disruption Pyk2/Grb2 via expression of either Pyk2 Y402F mutant or a Pyk2 Y881F mutant and disruption of Grb2/PDK1 association via either a Grb2/WA mutation (a mutant in which tryptophans 36 and 193 in Grb2 were substituted with alanine) or a PDK1/PA mutation (a mutant in which prolines 71 and 74 in PDK1 were substituted with alanine) resulted in decreased PDK1/Pyk2 association and PDK1 recruitment to SHPS-1. This led to impaired PDK1 activation (as evidenced by decreased Tyr373 phosphorylation), AKT Thr308 phosphorylation and cell migration in response to IGF-I. Additionally, our data indicated that assembly of this scaffold on SHPS-1 was specific for IGF-I induced activation since inhibiting PDK1 recruitment to SHPS-1 had no effect on EGF stimulated AKT Thr308 phosphorylation. Therefore, the findings in this study reveal a novel mechanism for recruitment of PDK1 to SHPS-1 that includes SHP2, Src, Pyk2, Grb2 and PDK1. Assembly of this complex in response to hyperglycemic stress is required for IGF-I stimulated AKT Thr308 phosphorylation and VSMC migration.

Nothing to Disclose: XS, GX, YR, DRC

P1-131

The Role of the Hyperglycemia Induced Increase in IAP Association with SHPS-1 in Diabetic Retinopathy.

LA Maile PhD¹, KG Gollahon PhD¹, LB Allen BSc¹, C Wai BSc¹, P Dunbar¹ and DR Clemmons MD¹.

¹UNC-Chapel Hill Chapel Hill, NC.

Cell surface expression of the transmembrane protein IAP/CD47 is increased when retinal endothelial cells are incubated in hyperglycemic compared with normoglycemic conditions. This is associated with an increase in IAP association with its binding partner SHPS-1. We hypothesized that this association may 1) contribute to increased adhesion of leukocytes to the endothelial surface of retinal capillaries characteristic of the early stages of diabetic retinopathy and 2) may enhance the biological response of endothelial cells to IGF-I.

Following induction of hyperglycemia in rats (using streptozotocin) we administered an anti-IAP antibody that we have shown disrupts the association between IAP and SHPS-1. The rats were then infused with a fluorescent dye prior to enucleation and preparation of retinal flat mounts. Hyperglycemia significantly increased, (2 fold) the number of adherent leukocytes. The anti-IAP antibody normalized the number of adherent leukocytes.

Since there is no model of diabetic proliferative retinopathy we used an in vitro model of angiogenesis. Endothelial cells in normal or hyperglycemic medium were plated on matrigel and allowed to form a capillary network. There was significantly more angiogenesis when endothelial cells were incubated in high compared with normal glucose. IGF-I enhanced this response. The anti-IAP antibody completely inhibited tube formation in high glucose medium in the presence or absence of IGF-I. VEGF has been implicated as the major growth factor driving angiogenesis. Using two inhibitors of VEGF signaling we determined IGF-I can stimulate tube formation in hyperglycemic conditions in the absence of VEGF. IGF-I did result in a significant increase in the synthesis of VEGF which was associated with activation of the VEGF receptor. However, this was completely blocked in the presence of the anti-IAP antibody. Furthermore, the ability of exogenously added VEGF to stimulate tube formation was inhibited in the presence of the the anti-IAP antibody.

Our results suggest that the hyperglycemia induced increase in IAP plays a significant role in both early and later stages of proliferative retinopathy. Furthermore our results suggest that IGF-I can stimulate endothelial cell responses in a VEGF independent manner as well as through an increase in VEGF production, however both the VEGF independent and dependent arms of the IGF-I pathway are inhibited following the disruption of IAP association with SHPS-1.

Nothing to Disclose: LAM, KGG, LBA, CW, PD, DRC

P1-132

IGF Signalling Plays an Important Role in the Prevention of *In Vitro* Vascular Calcification.

KW Siddals PhD¹, P Kalra MD¹ and JM Gibson MD PhD¹.

¹Univ of Manchester Manchester, UK.

Vascular calcification plays a significant role in the development of premature cardiovascular morbidity. Calcification is an active, cell-mediated process during which vascular smooth muscle cells (VSMCs) differentiate to an osteoblast-like phenotype. IGF-I is capable of blocking osteoblastic-conversion and preventing mineralisation. Previously we have shown that IGF signalling is particularly sensitive to altered N-linked receptor glycosylation and Ras isoprenylation. We have now investigated the role of glycosylation and isoprenylation in a cell-culture model of vascular calcification. VSMCs were obtained by explant culture from bovine aorta. Cells were induced to calcify with the addition of 5mM β-glycerophosphate and osteoblastic-conversion measured by alkaline phosphatase activity and the degree of mineralisation by the o-cresolphthalein calcium assay.

IGF-I (25ng/ml) inhibited the osteoblastic-conversion of VSMCs ($p < 0.005$) and their mineralisation ($p < 0.01$) induced by β-glycerophosphate (5mM). Mevalonate depletion [cerivastatin (100nM)] inhibited this IGF protective effect on osteoblastic-conversion ($p < 0.01$) and mineralisation ($p < 0.01$). Subsequent studies with specific glycosylation inhibitors [tunicamycin (10ng/ml, $p < 0.01$), deoxymannojirimycin (1mM, $p < 0.01$) and deoxynojirimycin (1mM, $p < 0.05$)] established this as a key mechanism in altering IGF signalling. Mevalonate depletion resulted in a combination of impaired IGF receptor processing, a decrease in mature IGF receptors and Ras at the cell surface and decreased activation of both Akt and MAPK.

These data clearly show that IGF-I protects vascular cells from calcification but that this protection is highly susceptible to disruption by changes in IGF-I signalling and receptor glycosylation. Our data suggest IGF-I is crucial for the maintenance of normal VSMC architecture and function.

Nothing to Disclose: KWS, PK, JMG

P1-133

Transforming Growth Factor- β Inhibits Muscle Differentiation by Targeting Insulin-Like Growth Factor-II.

S Gardner PhD¹, P.E. Knollman BSc², D Kuningner PhD² and P Rotwein PhD¹.

¹Oregon Hlth and Scis Univ Portland, OR and ²Life Technologies Eugene, OR.

Skeletal muscle differentiation and regeneration is regulated by interactions between signaling cascades activated by exogenous growth factors and hormones with endogenous muscle-specific transcriptional programs. The insulin-like growth factors (IGF-I and -II) can promote muscle differentiation *in vitro*, and can influence muscle repair *in vivo*. The transforming growth factor- β (TGF- β) super-family plays a prominent role in development, and can inhibit the differentiation of many cell types, including muscle. In this study we have evaluated functional interactions between IGF- and TGF- β -regulated signaling pathways during skeletal muscle differentiation. In the murine C2 muscle cell line and in human myoblasts in primary culture, addition of TGF- β 1 blocked differentiation in a dose-dependent way, inhibited production of muscle-specific proteins (myogenin, troponin-T), and impaired myotube formation. TGF- β 1 also prevented stimulation of IGF-II gene expression in myoblasts, reduced production of IGF-II precursor proteins, and thereby decreased IGF-II secretion. In addition, TGF- β 1 induced IGF binding protein-4 (IGFBP-4) mRNA, but did not up-regulate transcripts for other IGFBPs, and there was no enhanced accumulation of IGFBP-4 or other IGFBPs in culture medium. To test the hypothesis that TGF- β 1 prevents muscle differentiation primarily by blocking IGF-II biosynthesis, we added an IGF analogue to differentiation medium in the presence of inhibitory concentrations of TGF- β 1. R3IGF-I restored muscle gene expression, protein production, and promoted myotube formation, but did not reduce TGF- β 1-stimulated signaling as measured by phosphorylation of Smad 3. Taken together, our results indicate that TGF- β targets an IGF-II-induced autocrine cascade that is necessary for muscle differentiation *in vitro*. Our observations identify a potentially novel means of inhibitory growth factor crosstalk that will require further studies to discern the biochemical mechanisms by which TGF- β blocks IGF-II production in myoblasts.

Disclosures: PEK: Researcher, Life Technologies. DK: Researcher, Life Technologies.

Nothing to Disclose: SG, PR

P1-134

Free IGF-I Promotes Accelerated Mammary Gland Development.

DH Cannata MD¹, S Elis PhD¹, Y Wu PhD¹, H Sun¹, D LeRoith MD, PhD¹ and S Yakar PhD¹.

¹Mount Sinai Sch of Med New York, NY.

Insulin-like growth factor I (IGF-I) plays an important role in postnatal mammary gland growth and development. The majority of IGF-I is bound to acid labile subunit (ALS) and a family of six high affinity Insulin-like Growth Factor Binding Proteins (IGFBPs). By forming a stable complex, these binding proteins protect IGF-I from proteolytic degradation and determine its bioavailability. IGFBP-3, the most abundant IGF binding protein, accounts for 80% of all IGF binding. In the circulation, very little IGF-I is found in its unbound (free) form. In this study, we aimed to explore the effects of free IGF-I on pubertal mammary gland development in female virgin mice.

To determine the impact of free IGF-I on mammary gland development during puberty, we have employed the Cre-loxP system to create two C57/BL6 genetic mouse models with either a deletion of the first three amino acids (DES, KID mouse) or a knock-in of a single point mutation (E3R, KIR mouse) of the IGF-I ligand. In these models, mutant *igf-1s*, with substantial reductions in their affinities to IGFBPs, replace the endogenous *igf-1* gene and are expressed under the endogenous *igf-1* promoter. KID and KIR mice demonstrate increased body and organ size as compared to control mice at four, eight and sixteen weeks of age. Serum IGF-I levels are nearly undetectable in KIR and KID mice. Serum IGFBP-3 levels are decreased by 30% in KID mice and 40% in KIR mice. To characterize the effects of free IGF-I on mammary gland development, we studied the mammary gland complexity (degree of branching) of female virgin mice at four, eight and sixteen weeks of age. Mammary glands of KIR and KID female mice show a greater degree of complexity as compared to control mice at all ages analyzed. In KID mice, mammary gland complexity was increased between 20.7 and 56.3% at the different ages studied. In KIR mice, complexity ranged between 32.1 and 70.4% above the degree of complexity observed in control mice.

Taken together, our studies show that increased free IGF-I due to decreased affinity for IGFBPs leads to accelerated mammary gland development and increased ductal branching in two novel genetic mouse models. This suggests that IGF-I, in its free form, is an effective promoter of postnatal mammary gland development.

Sources of Research Support: NIH Grant R01CA128799.

Nothing to Disclose: DHC, SE, YW, HS, DL, SY

P1-135

IGF-2-Dependent Sphingosine Kinase 1 Activation Is Mediated by PLC/DAG/PKC β 2 Pathway.

Hesham M El-Shewy PhD¹, Souzan A Abdsele-Samie MD¹, Kazuyuki Kitatani PhD¹, Yusuf A Hannun MD¹, Lina M Obied MD¹ and Louis M Luttrell MD, PhD¹.

¹Med Univ of South Carolina Charleston, SC.

The insulin-like growth factor type 2/mannose-6-phosphate (IGF-2/M6P) receptor is a single transmembrane domain glycoprotein that lacks intrinsic catalytic activity. Although IGF-2/M6P receptors have been implicated in IGF signaling, little is known about the underlying mechanisms. We recently reported that IGF-2 binding to the IGF-2/M6P receptor activates the ERK1/2 cascade by triggering sphingosine kinase 1 (SK1)-dependent transactivation of G protein-coupled sphingosine-1 phosphate (S1P) receptors. Here, we investigated the mechanism of IGF-2/M6P receptor-dependent SK1 activation in HEK293 cells. Pretreating cells with the nonselective protein kinase C (PKC) inhibitor, bisindolylmaleimide-I, abolished IGF-2 stimulated translocation of green fluorescent protein (GFP)-tagged SK1 to the plasma membrane, activation of endogenous SK1, and ERK1/2 phosphorylation. Using confocal microscopy to examine membrane translocation of GFP-tagged PKC α , β 1, β 2, δ and ζ , we found that IGF-2 induced rapid, transient, and isoform-specific translocation of GFP-PKC β 2 to the plasma membrane. Similarly, IGF-2 stimulation caused persistent membrane translocation of the kinase deficient GFP-PKC β 2 (K371R) mutant, which does not dissociate from the membrane after translocation. Isoform-specific immunoblotting of PKCs phosphorylation confirmed PKC β 2 activation in response to IGF-2. To determine whether phospholipase C (PLC) is required for IGF-2-dependent PKC β 2 activation we employed the pharmacological PLC inhibitor, U73122. Pretreating cells with U73122 attenuated IGF-2-dependent PKC β 2 phosphorylation and inhibited ERK1/2 activation suggesting PLC/PKC β 2 are upstream regulators of SK1 in the pathway. Furthermore, time course stimulation by IGF-2 revealed significant increase in diacylglycerol (DAG) levels, the established activator of classical PKC. Taken together, these data provide evidence that activation of PLC and PKC β 2 by the IGF-2/M6P receptor are required for the activation of SK1.

Sources of Research Support: NIH grants DK55524 (L.M.L.), GM062887 and P20 RR17677 (L.M.O), and UDAK84213 the South Carolina COBRE in Lipidomics and Pathobiology (H.M.E.).

Nothing to Disclose: HME-S, SAA-S, KK, YAH, LMO, LML

P1-136

High Glucose Induced P66shc Inhibits IGF-I Dependent Cell Survival and Protein Synthesis Via Impairment of PI3K/AKT Activation in SMC.

Gang Xi PhD¹, Xinchun Shen PhD¹ and David R Clemmons MD¹.

¹Univ of North Carolina at Chapel Hill Chapel Hill, NC.

High glucose has been shown to increase p66shc in several cell types. In this study, we showed that high glucose (25 mM) increased p66shc in pSMC. Although p66shc has been shown to mediate apoptosis via enhanced reactive oxygen species generation, we have shown that p66shc impairs IGF-I stimulated SMC growth by inhibiting Src kinase activation. Therefore, we hypothesized that p66shc may enhance the apoptotic response to high glucose via impairment of IGF-I survival signaling. To test this hypothesis, we quantified IGF-I stimulated AKT activation in SMC expressing different levels of p66shc, showing that IGF-I stimulated PI3K/AKT activation was enhanced by knockdown of p66shc and impaired by its overexpression. When the p66shc level was increased by changing the glucose from 5 mM to 25 mM, the increase of IGF-I stimulated AKT activation was greater in p66shc knockdown cells than in the control cells. Consistently, high glucose induced cell death, cleaved caspase-3 protein level and caspase-3 enzymatic activity were positively correlated with p66shc protein level. For example, exposure to high glucose in serum free media for 72 h resulted in $20.1 \pm 0.8\%$, $10.8 \pm 1.1\%$ and $6.6 \pm 0.3\%$ cell death in p66shc overexpressing, control and p66shc knockdown cells, respectively. Importantly, the ability of IGF-I to prevent high glucose induced cell death was significantly impaired when p66shc was overexpressed. In addition, IGF-I stimulated AKT dependent phosphorylation of TSC2 Ser939 and mTOR Ser2448, and protein synthesis were enhanced by knockdown of p66shc and inhibited by its overexpression. Since we have shown that p66shc directly binds to Src via a PXXP-SH3 domain interaction, initiating the impairment of IGF-I stimulated Src activation, we disrupted the Src/p66shc interaction by either a blocking peptide or expression of a p66shc mutant which substituted 3 prolines with alanines. The disruption rescued IGF-I stimulated PI3K/AKT activation and its dependent biological actions in the presence of high level of p66shc. Taken together, our data suggest that hyperglycemia induced p66shc inhibits IGF-I stimulated Src activation, leading to impairment of PI3K/AKT activation, cell survival and protein synthesis in response to IGF-I. Of note, the inhibitory effect of p66shc is highly correlated its protein level, the increase of p66shc induced by high glucose maybe insufficient to overcome the glucose induced enhancement of mitogenic response to growth factors in SMC.

Nothing to Disclose: GX, XS, DRC

P1-137

IGF-I Suppresses AMPK Activity through Stimulation of Akt Mediated Phosphorylation of AMPK at S485.

Junyu Ning MD PhD¹ and David R. Clemmons MD¹.

¹Univ of North Carolina at Chapel Hill Chapel Hill, NC.

As a metabolic sensor and effector, the serine/threonine protein kinase AMP-activated protein kinase (AMPK) is believed to promote the adaptation of cells to signals arising from nutrients and growth factors. Activation of AMPK has been shown to inhibit protein synthesis via activation of the TSC1/2 complex which suppresses mTOR phosphorylation thus leading to inhibition of P70 S6 kinase. IGF-I which stimulates protein synthesis has been reported to suppress AMPK activity. However the mechanism by which IGF-I inhibits AMPK has not been described. Here we report that IGF-I dose-dependently suppresses phosphorylation of AMPK α subunit at Thr172 in porcine smooth muscle cells (pSMCs) in culture. This response correlated with a reduction in AMPK activity. IGF-I induced the phosphorylation of Akt Ser473 and P70 S6 kinase (P70S6K) Thr389; simultaneously the level of AMPK S485 phosphorylation also increased. Administration of IGF-I to mice showed that it induced same responses in Akt, P70S6K and AMPK in the aorta. The addition of either the PI3K inhibitor, LY294002, or a specific Akt inhibitor inhibited the IGF-I induced decrease in AMPK/Thr172 phosphorylation and the increase in Ser485 phosphorylation. Transduction of the mutant AMPK α in which the Ser485 was substituted with alanine (AMPK S485A) resulted in inhibition of IGF-I stimulated phosphorylation of P70S6K. In contrast expression of an AMPK S485D mutant (resulting in constitutive suppression of AMPK activity) was associated with increases in IGF-I stimulated P70S6K phosphorylation. Moreover the decrease in AMPK/Thr172 phosphorylation in response to IGF-I was inhibited by expression of AMPK S485A and accentuated by expression of AMPK S485D. The protein synthesis response to IGF-I was inhibited in pSMCs expressing AMPK S485A but enhanced in cells expressing AMPK S485D compared to control cultures. We conclude that AMPK Ser485 phosphorylation negatively regulates AMPK activity by modulating the Thr172 phosphorylation response to Akt and that this change accounts for suppression of AMPK activity following treatment with IGF-I.

Nothing to Disclose: JN, DRC

P1-138

Significant Alterations in the Expression of Extracellular Matrix Proteins, Ion Channels and Novel Signaling Proteins Caused by IGF-I Overexpression in the Pancreatic Islets.

Subrata Chowdbury MSc¹, Xiao Wang MD, PhD^{1,2}, Bing Li MD, PhD¹, Guang Ning MD, PhD² and Jun-Li Liu PhD^{1,3}.

¹McGill Univ Hlth Ctr Montreal, Canada ; ²Shanghai Jiao Tong Univeristy Sch of Med Shanghai, China and ³Montreal Diabetes Res Ctr Montreal, Canada.

We have reported MT-IGF mice which overexpress IGF-I gene under the metallothionein I promoter(1). The transgene expression, although widespread, was *highly concentrated to the β -cells* of the pancreas. Yet, the β -cell mass and pancreatic morphology were unaffected, supporting the notion that IGF-I alone does not stimulate islet cell growth under normoglycemia. IGF-I overexpression led to significant hypoglycemia, hypoinsulinemia and improved glucose tolerance. Moreover, MT-IGF mice were significantly resistant to streptozotocin-induced diabetes, exhibiting diminished hyperglycemia and abolished weight loss and mortality. In order to explore novel targets of IGF-I action, we have performed a whole-genome DNA microarray analysis on total RNA prepared from the pancreatic islets and found ~100 genes specifically up- or down-regulated in MT-IGF vs. wild-type mice. According to potential functions, they were classified into major groups, e.g. the consistently increased expression of extracellular matrix proteins (including fibulin-2 and various collagens) might enhance islet integrity; quite a few genes either up- or down-regulated seemed to regulate cell growth, survival or signaling (such as PI3K-C2 γ and WISP2); changes in ion channels (KCNF1 K⁺ channel and CATSPER2) might affect insulin secretion. There were also novel membrane and intracellular proteins whose functional involvement remains to be determined. We have reconfirmed part of the microarray results using real-time PCR, Western blots and/or immunohistochemistry. Although our microarray and real-time PCR analysis showed a significant increase in the mRNA level of 11 β -hydroxysteroid dehydrogenase 1 (11 β -HSD1) in the islets of MT-IGF mice, a decreased protein level was revealed by Western blot and immunohistochemistry. Its staining in wild-type mice was evenly distributed in the cytosol of most islet but not acinar cells; the level diminished per islet cell, some totally devoid of 11 β -HSD1 in MT-IGF mice. Regardless, the change in 11 β -HSD1 level in the islets caused by IGF-I and its β -cell-enriched expression were novel. Our result suggests that IGF-I exerts multiple, potent effects to promote pancreatic islet integrity and function, e.g. IGF-I might affect insulin secretion and β -cell survival by regulating the intracrine level of glucocorticoids. Revealing these molecular targets would validate novel IGF-I effects on islet cells and develop methods to elucidate the mechanism of IGF-I actions.

(1) Robertson K et al., Am J Physiol Endocrinol Metab 2008; 294:E928

Sources of Research Support: NSERC grant 341205-07; CIHR grants MOP-84389 & CCI-85675; Chercheurs boursiers Fondamental, Senior, Fonds de la recherch  en sant  Quebec.

Nothing to Disclose: SC, XW, BL, GN, J-LL

P1-139

IGF-I Induces Cyclin D1 Expression Via CREB Ser 133 Phosphorylation and Activation of the Cyclin D1 Promoter in Oligodendroglial Cells.

Yun Yan MD.¹, Qichen Hu MD. PhD.¹, Hedi Liu MD.¹, Ping Ye MD. PhD.¹ and A. Joseph D'Ercole MD.¹.

¹Univ of North Carolina Chapel Hill, NC.

Introduction: Insulin-like growth factor-I (IGF-I) is widely expressed in the developing central nervous system, and is known to stimulate cell cycle progression in a number of cell types, including neuronal and glial progenitors. The precise mechanisms of this regulation, however, have not been elucidated. **Objective:** In this study, we investigated the effect of IGF-I on cyclin D1 expression, a critical regulator of the cell cycle and proliferation, in a rat oligodendroglial cell line (OL-1). **Methods:** OL-1 cells were grown in serum-free medium supplemented with 30% conditioned medium derived from B104 neuroblastoma cells. OL-1 cells were treated with IGF-I at a concentration of 100ng/ml. Cyclin D1 mRNA levels were estimated by quantitative real-time PCR, and Western blots were used to assess cyclin D1 protein expression, cAMP responsive element binding protein (CREB) and ERK1/2 phosphorylation status. To assess cyclin D1 promoter activity, transient transfections were performed with two constructs: a 350 bp length cyclin D1 promoter containing a CREB binding site (-174 to +130 relative to the transcription start site) and a similar fragment containing a CREB binding site mutant. Each was inserted into a pGL3-luciferase reporter vector. Lipofectamine™ 2000 reagent was used for transfection of these constructs. HiPerFect reagent was used for CREB and negative siRNA transfections. **Results:** We found that IGF-I significantly increased cyclin D1 mRNA and protein levels ($p < 0.05$). As judged by luciferase reporter assay, IGF-I strongly stimulated the activity of the transfected cyclin D1 promoter ($p < 0.01$), but not that of the promoter containing the mutant CREB binding site. The CREB binding site, thus, appeared responsible for IGF-I induction of cyclin D1 expression. After introducing CREB siRNA into OL-1 cell, the levels of cyclin D1 mRNA significantly decreased ($p < 0.05$). Furthermore, we found that IGF-I induced phosphorylation of CREB at serine133 and ERK1/2, an upstream kinase responsible for CREB phosphorylation. Phosphorylation of ERK1/2 and CREB were suppressed by MAPK kinase inhibitor PD98059, but were not suppressed by wortmannin, an inhibitor of the Akt activating enzyme PI3 kinase. **Conclusions:** In OL-1 cells IGF-I can induce cyclin D1 expression, at least in part, by stimulating CREB phosphorylation and activation, via the MAPK pathway. These findings point to a potential mechanism by which IGF-I stimulates glial cells proliferation.

Sources of Research Support: NIH T32 Institutional fellow training grant; Children's Promise Grant, University of North Carolina.

Nothing to Disclose: YY, QH, HL, PY, AJD

P1-140

Lipophilic Pyruvate Derivatives Maintain IGF-I Sensitivity and mTOR Activity in Myotubes Exposed to Inflammatory Cytokines.

RA Frost Ph.D.¹ and CH Lang Ph.D.¹.

¹Penn State Univ Coll of Med Hershey, PA.

Bacterial infection decreases skeletal muscle protein synthesis via inhibition of the mammalian target of rapamycin (mTOR), a key regulator of translation initiation. To better define the mechanism by which muscle mTOR activity is decreased we used an in vitro model of C2C12 myotubes treated with endotoxin (LPS) and interferon (IFN) γ to determine whether stable lipophilic pyruvate derivatives restore mTOR signaling. Myotubes treated with a combination of LPS and IFN γ dramatically down regulated the phosphorylation of mTOR and its substrates S6K1 and 4EBP-1. The phosphorylation of ribosomal protein S6 was decreased whereas phosphorylation of elongation factor-2 (eEF-2) was enhanced, results consistent with defects in both translation initiation and elongation. LPS/IFN γ decreased protein synthesis 60 % in myotubes. Co-treatment with LPS/IFN γ and either methyl or ethyl pyruvate partially protected against the fall in mTOR signaling compared to LPS/IFN γ -treated myotubes. The protective effect of ethyl and methyl pyruvate could not be replicated by an equimolar amount sodium pyruvate. Although LPS/IFN γ treated myotubes were initially IGF-I responsive, prolonged exposure resulted in IGF-I resistance at the level of mTOR activity despite normal IGF-I receptor phosphorylation. Ethyl pyruvate co-treatment restored IGF-I sensitivity by shifting the dose response curve for IGF-I stimulation (1000-fold) and maintaining IGF-I responsiveness for a longer period of time. Ethyl pyruvate also restored IGF-I-stimulated protein synthesis. The data suggest that LPS and IFN γ in combination inhibits mTOR activity and that prolonged exposure induces IGF-I resistance in myotubes. Lipophilic pyruvate derivatives show promise at rescuing mTOR activity and muscle protein synthesis by maintaining IGF-I sensitivity in this model.

Sources of Research Support: NIH Grant GM38032 (CHL).

Nothing to Disclose: RAF, CHL

P1-141

Harmful Microalgae, *Amphidinium cartarae*, Regulates the IGF-IR Signaling Pathway in Human Colon Cancer Cells.

JA Ryu MA¹, SJ Park MA¹, IH Kim PhD¹, HJ Hwang PhD¹, CH Kim PhD¹ and TJ Nam PhD¹.

¹Pukyong Natl Univ Busan, Korea.

Marine microorganisms such as bacteria, cyanobacteria, dinoflagellates, and others have attracted many natural product chemists as the real producers of marine toxins such as fish and algal poisons as well as bioactive substances isolated from marine invertebrates such as sponges and tunicates. Among marine microorganisms, dinoflagellates have proved to be important sources of marine toxins and have been investigated worldwide by natural product chemists. This study examined how *A. cartarae*, of a genus of symbiotic marine dinoflagellates, regulates HT-29 cell proliferation and the influence of *A. cartarae* on the IGF-IR signaling pathway. We prepared the *A. cartarae* powder by freezing dry. *A. cartarae* stimulated HT-29 cell growth in a dose-dependent manner. The immunoprecipitation and Western blotting studies revealed that *A. cartarae* increased the phosphorylation of IGF, Akt and ERK 1/2. In conclusion, we demonstrated that *A. cartarae* induces the cell proliferation in HT-29 cells, and that this may be mediated by its ability to activate the IGF-IR signaling, especially, PI3K and ERK 1/2.

Nothing to Disclose: JAR, SJP, IHK, HJH, CHK, TJN

P1-142

The Unique Efficacy Profile of a β -Arrestin Pathway-Selective Parathyroid Hormone Receptor Agonist Revealed by Metabolic Pathways Analysis.

S Maudsley PhD¹, S Liao MD PhD², L Yuan BS², RJ Lefkowitz MD², LM Luttrell MD PhD³ and D Gesty-Palmer MD PhD².

¹Natl Inst of Hlth Baltimore, MD ; ²Duke Univ Med Ctr Durham, NC and ³Med Univ of South Carolina Charleston, SC.

Biased GPCR agonists are orthosteric ligands that possess pathway-selective efficacy, activating or inhibiting only a subset of the signaling repertoire of their cognate receptors. We have recently shown in vitro, that dTrp12,Tyr34-bPTH(7-34) (PTH- β arr), a biased agonist for the type 1 parathyroid hormone receptor (PTH1R), antagonizes receptor-Gs coupling but activates β -arrestin-dependent signaling. When administered to WT, but not β -arrestin2 null mice, PTH- β arr, like the full agonist, PTH(1-34), stimulates bone formation. Here, we employed Affymetrix gene arrays to compare transcriptional activity in calvarial bone from mice treated for 8 weeks with vehicle, PTH- β arr or PTH(1-34). Treatment of WT mice with PTH- β arr affected gene clusters associated with a limited number of metabolic pathways, notably cell cycle regulation, Akt/PI3K, p53 and ATM signaling. These responses were absent in β -arrestin2 null mice, identifying them as downstream targets of β -arrestin2-mediated signaling. In contrast, PTH(1-34) treatment of WT mice affected pathways more classically associated with mature osteoblast function, including collagen synthesis and matrix mineralization. PTH(1-34) actions were less dependent on β -arrestin2, as might be expected of a ligand capable of G protein activation. Surprisingly, there was minimal overlap in the genomic signatures elicited by PTH(1-34) and PTH- β arr, suggesting either that PTH- β arr stabilizes PTH1R conformations that represent, at most, a minor proportion of those produced by the full agonist, or that activation of β -arrestin-mediated signaling without concomitant G protein activation preferentially activates pathways that are repressed by G protein activity. These results illustrate the uniqueness of biased agonism and demonstrate that functional selectivity can be exploited to change the quality of GPCR efficacy.

Nothing to Disclose: SM, SL, LY, RJL, LML, DG-P

P1-143

Antagonist Minigenes Identify Genes Regulated by Parathyroid Hormone through G Protein-Selective and G Protein Co-Regulated Mechanisms in UMR-106 Osteoblastic Cells.

J Wang Ph D¹, A Gilchrist Ph D^{1,2} and P H Stern Ph D¹.

¹Northwestern Univ Feinberg Sch of Med Chicago, IL and ²Chicago Coll of Pharmacy, Midwestern Univ Downers Grove, IL.

Parathyroid hormone (PTH), an endocrine factor secreted by the parathyroid glands, is the major hormone regulating bone remodeling. PTH has both anabolic and catabolic effects on bone *in vivo* mediated through its activation of the PTH1 receptor (PTH1R) expressed on osteoblastic cells. PTH1R mediates intracellular responses largely through heterotrimeric guanine nucleotide binding proteins (G proteins) and thus is a member of the superfamily of G protein coupled receptors (GPCRs). Binding of PTH to the PTH1 receptor can potentially trigger multiple signal transduction pathways mediated through several different G proteins, including the Gas-cAMP-PKA pathway, the Gq-PLC-PKC pathway and the G12-RhoA-PLD pathway. In order to dissect out which of these G proteins were responsible for effects of PTH(1-34), we transfected osteoblastic cells with G protein antagonist minigenes to selectively inhibit Gas, Gq or G12. After the cells were treated with PTH(1-34) for 24 hours, gene expression profiles were studied using qPCR-based targeted arrays for signaling and osteogenesis, consisting of 159 genes. Among the 32 genes significantly regulated by PTH treatment, 9 genes (Myc, Egf, Pdgfa, Igfbp3, Cyp19a1, Rbp1, Fasn, Flt1 and Fgf1) were exclusively regulated through Gs, while 6 genes (Tgfb1, Egr1, Igf1, Tnf, Bmp3 and Bmp7) were solely mediated through Gq, and 3 genes (Mmp 8, Foxa2 and Gadd45a) were only controlled through G12. Such findings support the concept that there is some absolute specificity in downstream responses initiated at the G protein level following binding of PTH to the PTH1R. On the other hand, we found that 6 PTH-regulated genes (Hsf1, Fgf4, Cdh1, Mmp7, Col3a1 and Fn1) were regulated by both Gs and Gq, 3 genes (Cebpb, Itgav and Col14a) were regulated by both Gs and G12, and an additional 3 genes (Fos, Birc3 and Vegfa) were controlled by Gs, Gq and G12. These findings indicate potential overlapping or sequential interactions among different G protein-mediated pathways. In addition, two PTH-regulated genes (Pmepa1 and Hk2) were not regulated through any of the G proteins examined, suggesting that additional signaling mechanisms may be involved. In conclusion, we have dissected out effects of PTH on gene regulation and have identified differential effects mediated exclusively or conjointly through three different G protein pathways. This study provides a more complete understanding of PTH signaling in osteoblastic cells.

Sources of Research Support: NIH Grant AR11262 awarded to PHS.

Nothing to Disclose: JW, AG, PHS

P1-144

Impaired Bone Anabolic Response to PTH Therapy in the Th3/+ Mouse Model of Thalassemia.

J Tsay MD¹, RW Grady PhD¹, PJ Giardina MD¹, AL Boskey PhD² and MG Vogiatzi MD¹.

¹Weill Cornell Med Coll New York, NY and ²Hosp for Special Surg New York, NY.

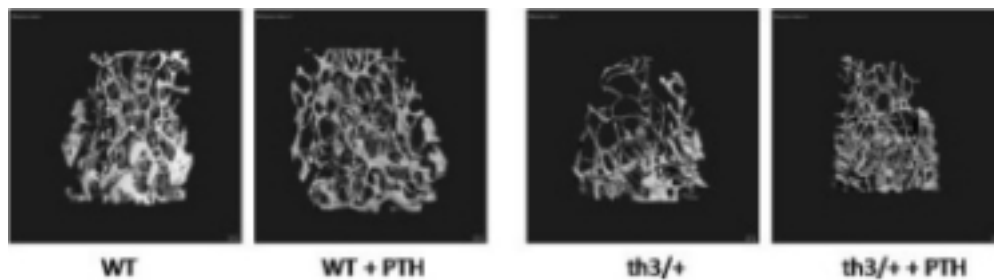
Background: Beta thalassemia is a congenital hemolytic anemia characterized by high rates of osteoporosis and fractures. We described previously that the th3/+ thalassemia mouse has bone loss that resembles that of humans with the disease, associated with decreased bone formation. To determine new therapies for the treatment of osteoporosis in thalassemia, we treated the th3/+ mouse with parathyroid hormone (PTH), a bone anabolic agent.

Methods: 2 groups of C57/BL6 and 2 groups of th3/+ mice (n=6/group, 3M-3F/group) were treated with either placebo or PTH (40 µg/kg/day, sc) from age 2 to 4 months. Femurs were analyzed for bone mass and morphology by Micro-CT.

Results: Compared to untreated WT animals, the WT animals treated with PTH showed significant increases in cortical bone thickness, bone area, and bone mineral density (p < 0.05 for all three parameters). These improvements were not seen in the trabecular bone. Surprisingly, thalassemic mice did not appear to benefit from PTH therapy in either cortical or trabecular parameters (see table and figure).

Conclusions: As expected, PTH therapy appears to have an anabolic effect on the cortical bone in WT mice. However, the thalassemia mouse model does not respond as robustly to PTH as do WT mice. The poor anabolic response of thalassemic mice to PTH compared to WT mice may be partially due to the impaired bone formation that has been described in the th3/+ mouse. Further studies will be needed in this area to further elucidate the most efficacious target for therapeutic agents in thalassemia bone disease.

| Group | Cortical Bone | | | | Trabecular Bone | | |
|-----------|---------------------|------------------------------|-------------|-----------|------------------------------------|---------------------------|-------------|
| | Bone Thickness (mm) | Bone Area (mm ²) | BMD (mg/cc) | BV/TV (%) | Trabecular No. (mm ⁻¹) | Trabecular Thickness (µm) | BMD (mg/cc) |
| WT | 0.179 | 0.754 | 1104.5 | 0.127 | 4.07 | 0.042 | 836.7 |
| WT + PTH | 0.193 | 0.889 | 1130.2 | 0.108 | 3.54 | 0.046 | 809.4 |
| th3 | 0.161 | 0.724 | 1122.7 | 0.088 | 3.47 | 0.042 | 846.2 |
| th3 + PTH | 0.170 | 0.777 | 1096.0 | 0.061 | 3.06 | 0.042 | 838.9 |



Nothing to Disclose: JT, RWG, PJG, ALB, MGV

P1-145

Differential Effects of Continuous and Intermittent PTH on Bone.

M Babey MD¹, Y Wang MD PhD¹, H Elalieh¹ and D Bikle MD PhD¹.

¹Univ of California, San Francisco San Francisco, CA.

Intermittent administration of parathyroid hormone (PTH) stimulates bone formation, whereas continuous exposure of PTH induces bone loss. After demonstrating that IGF-I signaling is a crucial mediator of the anabolic actions of intermittent PTH, we are currently investigating the role of IGF-I signaling in mediating the catabolic actions of continuous PTH. In this context, we first examined the degree to which continuous PTH affects murine bone structure and bone marrow stromal cell (BMSC) differentiation in comparison to intermittent PTH. Three-month-old male FVB/N mice were either administered rat PTH 1-34 (80 microg/kg body weight/d) continuously via mini osmotic pumps or intermittently for two weeks in comparison to continuous and intermittent vehicle (n=3 in each group). Structural measurements of femurs and tibiae were made by microCT. Long bone mRNA levels were extracted to determine osteoblast differentiation markers. BMSCs were cultured to determine osteoblast progenitor proliferation and mineralization. By microCT analysis, the trabecular bone parameters were only minimally affected in both the continuous and intermittent PTH groups compared to vehicle. However, cortical thickness tended to be reduced (p=0.06) in the continuous PTH group and was increased in the intermittent PTH group in comparison to the control groups. Long bone mRNA levels of *IGF-I*, *AP* and *OCN* were respectively 1.7, 5.4 and 4.8 fold increased over controls in the intermittent PTH group, but in the continuous PTH group mRNA expression levels of *IGF-I* and *AP* were not altered and *OCN* mRNA levels were increased only 2.3 fold. In BMSC cultures after continuous PTH, colony numbers and mineralized nodules were markedly reduced by 63% and 52%, respectively, relative to those from vehicle bones, but were increased by 31% and 50% relative to BMSC cultures after intermittent PTH. Two-week administration of continuous and intermittent rat PTH (1-34) showed opposite effects on murine bone and BMSC differentiation. The anabolic actions of PTH due to stimulation of osteoblast function were lost when PTH of the same amount was given continuously. The basis of the biphasic action of PTH remains under active investigation in our laboratory, but appears to involve a differential effect on IGF-I signaling.

Sources of Research Support: Janggen-Poehn-Foundation; NIH Grant R01DK054793 awarded to DB.

Nothing to Disclose: MB, YW, HE, DB

P1-146

Intermittent In Vivo Parathyroid Hormone (PTH) Decreases Apoptotic Rates of Hematopoietic Stem Cells (HSCs) Prior To Expanding Their Numbers.

I Shafiq MD¹, R M Porter PhD¹, J M Weber¹, B J Frisch PhD¹, J N Smith PhD¹, O Bromberg¹, M J Basil¹, R S Dhillon MD¹, E M Schwarz PhD¹ and L M Calvi MD¹.

¹Univ of Rochester Rochester, NY.

HSCs are rare primitive cells capable of reconstituting all blood cell lineages throughout the life of an individual. The microenvironment in which stem cells reside regulates their survival, self-renewal, and differentiation. Our group and others have established that PTH-dependent activation of osteoblastic cells increases HSCs. Apoptosis is an important HSC fate choice, thus decreased HSC apoptosis could cause the PTH-dependent HSC expansion. To determine if *in vivo* PTH could decrease HSC apoptosis rate, C57B/6 mice were treated intermittently with saline or PTH (40ug/Kg/day) for 28 days. Flow cytometric analysis can enumerate the HSC-enriched lineage⁻ Sca-1⁺ c-kit⁺ (LSK) cell pool. However, the LSK compartment is heterogeneous and contains at least three subpopulations of cells: long term HSCs (LT-HSCs), short-term HSCs (ST-HSCs), and multipotent progenitors (MPPs), all with multi-lineage potential, but with progressively more limited self-renewal. The HSC subsets can be quantified by flow cytometric analysis. When bone marrow mononuclear cells from vehicle and PTH-treated mice were analyzed by flow cytometric analysis, there was a PTH-dependent significant increase in LSK and all 3 subsets as early as the 10th day of treatment. The PTH increase in MPPs, ST-HSCs and LT-HSCs was confirmed both by competitive repopulation assays and by secondary and tertiary transplantation. The increase in LSK and subsets persisted through day 28 of PTH treatment. Therefore this is an excellent time-dependent model to study the early effects of PTH on HSC apoptosis. PTH effects on HSC apoptotic rates were quantified by flow cytometry using Annexin V cell membrane expression. As early on 7th day of PTH treatment, LT-HSCs apoptotic rates were decreased in the PTH treated group compared to vehicle (VEH/ PTH 10.482 ± 2.25 vs. 6.27 ± 1.93, p=0.01) suggesting that changes in LT-HSC apoptosis predate the HSC increase. LT-HSCs apoptosis rates were decreased also 10 and 14 days after PTH, and later also in ST-HSCs and MPPs. These results suggest for the first time that PTH may exert its beneficial effect on bone marrow reconstitution at least in part by decreasing HSC apoptotic rates. Since stressful manipulation of HSC *ex vivo* is essential for their use in transplantation, defining factors regulating and decreasing their apoptosis may dramatically improve their engraftment efficiency, potentially expanding their clinical use even when their numbers are limited.

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3)J.M. Weber, Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. *Bone* 39 (2006) 485-93.

4)NIH/NIDDK 1R01DK076876-01A2 Laura Calvi (PI) "Hematopoietic-Osteoblastic Interactions"

5)NYSTEM Investigator Initiated Project # N08G-322 Laura M. Calvi " Therapeutic Stimulation of the Hematopoietic Stem Cell Niche"

Nothing to Disclose: IS, RMP, JMW, BJF, JNS, OB, MJB, RSD, EMS, LMC

P1-147

A New GCM2 Mutation as a Cause of Congenital Hypoparathyroidism in Two Siblings.

DA Doyle MD¹ and SM Kirwin BS².

¹A I duPont Hosp for Children/Thomas Jefferson Univ Wilmington, DE and ²A I duPont Hosp for Children Wilmington, DE.

Background: GCM2 or Glial cell missing 2 mutations have been previously described in 4 kindreds with familial isolated hypoparathyroidism. We present two siblings born in the US to parents who recently emigrated from Guatemala. The first child presented at 7 days of age with a hypocalcemic seizure and her asymptomatic younger sister was subsequently screened and diagnosed with hypocalcemia on day of life 3. Both had low iPTH, hyperphosphatemia and negative FISH probe for DiGeorge syndrome. They required IV calcium, followed by PO calcium, calcitriol and a low phosphorous formula for maintenance of eucalcemia. Neither child has yet shown evidence of hypercalciuria or nephrocalcinosis and both are growing well with normal development at ages 4 and 3 years respectively.

Objective: To identify the presence of a homozygous GCM2 mutation in each affected child. Also to prove heterozygosity or no mutation in their unaffected parents and living sibling.

Methods: PCR of DNA obtained from peripheral lymphocytes was performed using primers for each exon to amplify the 5-exon GCM2 gene in both parents, the two affected sisters and their unaffected brother.

Results: A previously undescribed homozygous Y136X mutation (Tyrosine to Stop codon) at position 136 in exon 3 was discovered in each of the affected siblings. Their unaffected parents were both heterozygous for this change. Their unaffected living brother inherited the normal allele from each parent.

Conclusion: A previously undescribed mutation resulting in a stop codon in exon 3 of the GCM2 gene was identified as the cause of familial congenital isolated hypoparathyroidism in these two siblings. An autosomal recessive inheritance pattern is likely in this family. There are 2 affected children with hypoparathyroidism and a history of another male sibling that expired at 7 months of age due to intractable seizures while the family was still living in Guatemala. This deceased sibling may have carried the Y136X mutation. Issues going forward include the known complications of treating hypoparathyroidism such as developmental delay, basal ganglia calcifications, short stature and nephrocalcinosis. There has also been the challenge of maintaining a low phosphorous intake as the children grow into a more diversified diet. If recalcitrant hypocalcemia, hyperphosphatemia or any of the known complications of conventional therapy should develop, recombinant PTH may be a useful alternative treatment.

Nothing to Disclose: DAD, SMK

P1-148

Activating Mutations of the Calcium-Sensing Receptor: The Calcilytic NPS-2143 Mitigates Excessive Signaling of Novel Mutants Causing Autosomal Dominant Hypocalcemia.

S Letz¹, C Haag², R Rus¹, E Schulze², K Frank-Raue², HG Dorr¹, D Schnabel³, B Mayr¹, F Raue² and C Schofl¹.

¹Friedrich-Alexander-Univ Erlangen-Nuremberg Erlangen, Germany ; ²Endocrine Practice Heidelberg, Germany and ³Charité-Univ Med Berlin Berlin, Germany.

Introduction: The calcium sensing receptor (CaSR) is a G-protein-coupled receptor located on the surface of parathyroid and kidney cells and plays an important role in the calcium homeostasis of mammals. Activating mutations of this receptor cause autosomal dominant hypocalcemia (ADH) characterized by low serum calcium, relative hypercalciuria and inappropriately low PTH. Until today there is no definitive medical treatment for ADH patients. In this study we characterised four mutations of the CaSR gene (P221L, E767Q, G830S and A844T) found in ADH patients, compared in vitro function with clinical parameters, and measured the effect of the calcilytic NPS-2143 on receptor signalling of the mutant receptors.

Methods: Wild type and mutant CaSR proteins were expressed in human embryonic kidney 293T cells and receptor signalling was studied by measurement of intracellular free calcium ([Ca²⁺]_i) in response to different concentrations of extracellular calcium ([Ca²⁺]_o). To investigate the effect of the calcilytic NPS-2143, cells were stimulated with 3 mM [Ca²⁺]_o either in the presence or absence of NPS-2143 (300 nM or 1 μM).

Results: All four mutations demonstrated elevated maximal responses to [Ca²⁺]_o, elevated absolute responses at [Ca²⁺]_o concentrations in the physiologic range (2-3 mM) and a left shifted dose response curve (EC₅₀: wt 4.3 mM; P221L 1.6 mM; E767Q 2.4 mM; G830S 2.0 mM; A844T 3.2 mM) confirming the clinical diagnosis of ADH. The patient with the lowest urinary calcium excretion had the mutation P221L with the lowest EC₅₀, while the mutation A844T with the highest EC₅₀ was found in the patient with the highest serum calcium and highest urinary calcium excretion. The responsiveness to 3 mM [Ca²⁺]_o of 3 novel mutations in the transmembrane region (E767Q, G830S, A844T) could be lowered by the calcilytic NPS-2143. When coexpressed with the wildtype receptor the activity of the mutant P221L located in the extracellular domain was also decreased by NPS-2143.

Conclusion: In vitro receptor testing confirmed the diagnosis of ADH and despite the low number of patients there appeared to be a correlation between receptor sensitivity to extracellular calcium in vitro and patients' calcium metabolism in vivo. Allosteric calcilytics like NPS-2143 could control the enhanced responsiveness to [Ca²⁺]_o of activating mutants of the CaSR. This might offer medical treatment for ADH patients harbouring activating CaSR mutants.

Nothing to Disclose: SL, CH, RR, ES, KF-R, HGD, DS, BM, FR, CS

P1-149

PTHrP-Induced Wnt Signaling Plays a Role in Specification of the Mammary Mesenchyme.

M Hiremath PhD¹, P Dann BS¹ and JJ Wysolmerski MD¹.

¹Yale Univ New Haven, CT.

PTHrP regulates mammary cell fate by specifying the mammary mesenchyme during embryonic mammary development. Loss of PTHrP or its receptor allows mammary bud cells to adopt an epidermal fate, whereas, overexpression of PTHrP in the epidermis induces inappropriate differentiation of the ventral epidermis into nipple-like skin. Previous studies have suggested that the Wnt pathway is one of the downstream pathways activated by PTHrP in specifying the mammary mesenchyme. We confirmed that PTHrP modulates Wnt signaling in the mammary mesenchyme using a reporter of Wnt signaling, TOPGAL-C. Reporter expression is completely abolished by loss of PTHrP signaling and ectopic mesenchymal Wnt signaling is induced by overexpression of PTHrP. To further investigate the requirement for Wnt signaling, we examined mutant mice overexpressing PTHrP but lacking Lef1, an essential component and target of the Wnt pathway. Our observations suggest that loss of Lef1 partially reverses the nipple-like differentiation of the ventral skin. Therefore, we concluded that PTHrP-driven mammary mesenchyme specification is partially mediated by Wnt signaling. Surprisingly, ectopic TOPGAL-C expression is maintained in K14-PTHrP;Lef1-null mice. These data suggest that PTHrP induces a Wnt signal that activates Lef1 expression in the mesenchyme. To determine if Wnt signaling regulates mesenchyme specification and Lef1 expression, we deleted beta-catenin, the primary mediator of the canonical Wnt pathway, in the mammary mesenchyme. Reduced mesenchymal Wnt signaling correlates with abnormal bud morphology and reduced expression of mammary mesenchyme markers. To identify other Wnt pathway components that are regulated by PTHrP during mammary mesenchyme specification, we performed microarray analyses of RNA from ventral skins of WT and K14-PTHrP mice and from RNA extracted from mammary buds of PTHrP-null and WT mice. We identified genes that are upregulated in PTHrP-overexpressing ventral skin, compared to WT, and genes that are downregulated in PTHrP-null mammary buds, compared to WT, as candidate genes that are regulated by PTHrP. Of these, we are currently investigating RSPO1 and WNT11 as Wnt pathway regulators that potentially act downstream of PTHrP during mesenchyme specification.

Sources of Research Support: NIH Grant DK05501 to JW; DOD Postdoc Fellowship W81XWH0910580 to MH.

Nothing to Disclose: MH, PD, JJW

P1-150

Stanniocalcin Is an Ancient Hormone as Revealed by Genomics.

GJ Roch BSc¹ and NM Shewood PhD¹.

¹Univ of Victoria Victoria, Canada.

Stanniocalcin (STC) is a protein hormone involved in calcium homeostasis and identified by structure in many vertebrates from humans to bony fish. In humans, the first stanniocalcin characterized, STC1, has been implicated in a variety of roles including phosphate and calcium regulation (1). A second homolog of human stanniocalcin, STC2, was more recently identified and characterized, although its functions are not as well understood. In fish, STC1 is an important hypocalcemic regulator, and STC2 has also been isolated. In this study, we used genomic resources for the two major invertebrate chordate models, the tunicate *Ciona intestinalis* (sea squirt), and the amphioxus *Branchiostoma floridae* (lancelet), to search for stanniocalcin homologs. In *C. intestinalis*, we found and isolated a single pro-STC homolog of 237 amino acids, and in *B. floridae*, we isolated two pro-STC homologs of 207 and 210 amino acids; all three are shorter than the human stanniocalcins. These hormones are not highly homologous with their human counterparts (14-23% identity), however, they share important structural conservations including the invariant 10 cysteine residues found in vertebrate stanniocalcins, critical to internal disulfide bridging (2). The 11th conserved cysteine, critical for dimerization, is only found in one of the amphioxus homologs. Both amphioxus STCs possess an N-glycosylation motif like the vertebrate hormones, although not at the canonical site. Phylogenetic analysis indicates that the invertebrate homologs form a distinct group, separate from vertebrate STC1 and STC2. Expression analysis of tunicate *stc* mRNA using qPCR was conducted on *C. intestinalis* tissues. Predominant expression was detected in the tunicate heart, with lesser expression present in the branchial basket, neural complex and endostyle. STC1 was previously detected in developing mouse hearts (3) and chick hearts (4). In humans, patients with heart failure have upregulated STC1 expression, and the hormone inhibits calcium L-channels in cardiomyocytes, slowing their contractions (5). Although the physiology of the invertebrate stanniocalcins has not been explored, it appears both tunicate and amphioxus possess homologs that may have functions similar to the vertebrate stanniocalcins. The conservation from invertebrates to vertebrates suggests an important role for stanniocalcin.

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(3) Jiang WQ et al., J Endocrinol 2000; 165:457-466

(4) Mittapalli VR et al., Anat Embryol (Berl) 2006; 211:519-523

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Sources of Research Support: Natural Sciences and Engineering Research Council of Canada.

Nothing to Disclose: GJR, NMS

P1-151

Identification of Novel Vitamin D Receptor Target Genes Based on Promoter Interaction with the Vitamin D Response Element Binding Protein.

TS Lisse PhD¹, JS Adams MD¹ and M Hewison PhD¹.

¹Orthopaedic Hosp Res Ctr Los Angeles, CA.

The vitamin D-activated transcriptional complex comprises 1,25-dihydroxyvitamin D₃ (1,25D₃)-liganded vitamin D receptor (VDR) and its retinoid X receptor partner. However, accessory proteins are required for VDR-directed chromatin remodeling and transcriptional functionality. In previous studies of a patient with hereditary vitamin D resistant rickets (HVDRR), we identified a novel accessory protein which inhibited VDR signaling via blockade of the vitamin D response element (VDRE). The VDRE-binding protein (VDRE-BP) corresponds to heterogeneous nuclear ribonucleoprotein (hnRNP) C1/C2. Although over-expressed in the HVDRR patient, the VDRE-BP is also involved in normal VDR-mediated signaling. To identify VDR target genes specifically influenced by VDRE-BP, DNA array analyses were carried out using B-cells from the HVDRR patient and a matched control with or without 1,25D₃ (10 nM, 6hrs.). Heat map analyses defined a sub-cluster of VDRE-BP target genes induced by 1,25D₃ in control B-cells but not in HVDRR B-cells. To explore the generalisability of these differentially regulated genes, we next determined whether they were regulated by 1,25D₃ in other cells. Using the MG-63 osteoblastic cell line and primary cultures of normal human osteoblasts, expression of the DNA-damage-inducible transcript 4 (*DDIT4/REDD1*) gene, an inhibitor of mTOR signaling, was induced in a dose-dependent manner by 1,25D₃ (1-100 nM). Chromatin immunoprecipitation (ChIP) assays were then carried out to assess binding of the VDR and/or VDRE-BP to the proximal promoter region of the *DDIT4* gene. Treatment of MG-63 cells with 1,25D₃ (10 nM, 15 mins) induced a normalized 50-fold positive change in VDR binding to DDIT4-1kB dsDNA and a 37-fold increase in VDRE-BP binding to the same DDIT4-1kB dsDNA, suggesting that both VDR and VDRE-BP are necessary for 1,25D₃-induced expression of this gene. Transient over-expression of the VDRE-BP within MG-63 cells suppressed 1,25D₃-induction of DDIT4 mRNA, as well as induction of other known VDR-target genes such as *CYP24A1* and osteocalcin. These data show for the first time that the VDRE-BP (hnRNP C1/C2) is an important component of the transcriptional apparatus required for normal 1,25D₃-VDR-mediated transcription. Furthermore the suppressive actions of over-abundant VDRE-BP on VDR-regulation of osteogenic genes, provides new evidence supporting the role of this protein in vitamin D resistance.

Sources of Research Support: NIH grant R01 AR50626-01A1 awarded to JS Adams.

Nothing to Disclose: TSL, JSA, MH

P1-152

Long-Term Low Dose Calcitriol Treatment Reduces Blood Pressure and Decreases Diet-Induced Atherosclerosis in Tsukuba Hypertensive Mice (THM).

Karen M. Tordjman MD¹, Maya Ish Shalom MD¹, Jessica Sack MD¹, Michal Vechoropoulos PhD¹, Alex Litvak DVM¹ and Naftali Stern MD¹.

¹Tel Aviv Sourasky Med Ctr Tel Aviv, Israel.

Epidemiologic studies have suggested vitamin D might have favorable cardiovascular effects. Suppression of the renin-angiotensin system (RAS), through downregulation of renin, its rate-limiting step, has been proposed as one of several possible mechanisms. We reported that calcitriol administered for 3 weeks to young Tsukuba Hypertensive Mice (THM) prevented hypertension in this model of hypertension and atherosclerosis secondary to the transgenic expression of the human renin (hRen) and angiotensinogen (hAGT) genes. We questioned whether long-term calcitriol treatment would have a sustained effect on blood pressure (BP), and whether it would affect the atherosclerosis that develops in these mice.

Methods: Starting at 7-9 weeks, THM animals were fed an atherogenic Western diet. 17 animals received calcitriol as an intraperitoneal injection of 0.25 ng.g body weight⁻¹ every other day for 12 weeks (1/2 the dose used in our previous study). 19 control mice received the vehicle only. BP was measured noninvasively. The extent of atherosclerosis at the aortic sinus was assessed by quantification of Oil-Red-O-stained lesions. Expression of the RAS in the aorta was studied by real-time PCR.

Results: BP was unchanged in control animals but was significantly lower in calcitriol-treated mice: systolic 142.7±1.8 vs 111.9±3.2 mm Hg; diastolic 88.6±1.4 vs 69.6±2.5, $P<0.0001$ for both. Calcitriol treatment significantly increased cholesterol concentrations 186.4±8.7 vs. 144.8±8.3 mg/dl, $P=0.0028$, and serum calcium 10.3±0.2 vs 8.9±0.3 mg/dl, $P<0.001$. In addition, calcitriol-treated animals weighed more. Nonetheless, the extent of atherosclerosis was reduced 42.9% by calcitriol, $P=0.015$. This was accompanied by a significant 77% suppression of the hRen gene at the aorta, $P=0.005$. hAGT, ACE and the angiotensin II type 1 receptor were not affected.

Conclusions: Long-term calcitriol treatment provided sustained BP reduction as well as a significant attenuation of atherosclerosis in THM animals. The concomitant suppression of hRen is in agreement with the notion that the beneficial effect is mediated by downregulation of the RAS. However, even at this lower dose which had no untoward effect in the short-term, prolonged treatment resulted in hypercalcemia. Moreover, the unfavorable metabolic profile seen with treatment is a major concern. Animal studies with analogs devoid of these effects will have to confirm the above findings before clinical trials can be contemplated.

Nothing to Disclose: KMT, MIS, JS, MV, AL, NS

P1-153

The Metabolic Phenotype and Islet Cell Function of the Vitamin D Receptor Knockout Mouse.

SL Lau MBBS¹, R Stokes BSc.¹, B Ng¹, SM Lau PhD¹, R Clifton-Bligh PhD² and JE Gunton PhD¹.

¹Garvan Inst Darlinghurst, Australia and ²Kolling Inst St Leonards, Australia.

Previous studies implicate vitamin D signaling in glucose metabolism. Mice expressing a functionally inactive mutant VDR have impaired insulin secretory capacity and glucose tolerance (1). In this model, however, the VDR may still bind partners such as RXR, without activating transcription, thus may act as a dominant negative mutation. We examined mice with targeted ablation in the DNA-binding domain of VDR. In this line, the mutant VDR is not expressed. Decreased fat mass and greater energy expenditure has been reported in these mice (2). Their islet function has not been reported.

42 homozygous, 37 heterozygous and 25 wild-type offspring of heterozygous pairs were studied. All mice were fed rescue diet from weaning. At 7 and 24 weeks of age, mice underwent glucose tolerance testing. Glucose stimulated insulin secretion (GSIS) was measured at 25 weeks and insulin tolerance testing at 26 weeks. A subset underwent DEXA scanning at 27 weeks. At sacrifice at 27-28 weeks, organ weights were collected and 4-6 mice per group had islets isolated for ex-vivo GSIS. In a separate study, islets from 9 week old females were transplanted under the kidney capsule of age-matched C57Bl6 recipients with streptozotocin-induced diabetes. Glucose levels were assessed over 1 month.

Comparing VDR KO and WT mice, there was no significant difference in weight, glucose tolerance, GSIS, or insulin-response. Male hets had worse glucose tolerance at 7 wks, and were significantly heavier than KO or WT littermates at all timepoints. This corresponded to greater fat mass in hets, with no difference between KO and WT. KOs had shorter snout to tailbase length than WTs ($p=0.02$), lower gastrocnemius weights ($p<0.01$) and lower bone density (KO vs WT: females 0.053 vs 0.061g/cm^2 , $p<0.01$; males 0.052 vs 0.057g/cm^2 , $p=0.01$). After isolation, KO islets had reduced GSIS compared to WT. The ratio of insulin secretion at high vs low glucose was diminished (KO vs WT: female 1.06 vs 1.72 , $p=0.03$; male 1.60 vs 2.14 , $p=0.15$). Mice which received transplants from KO donors had significantly higher random glucose levels versus WT recipients.

In the whole animal model, VDRKO mice do not have glucose intolerance. Differences in body composition may confound comparison of beta-cell function. When islets were assessed by ex-vivo GSIS or transplantation, VDRKO islets potentially have diminished function.

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Sources of Research Support: Royal Australian College of Physicians McCaughey Scholarship; NHMRC postgraduate scholarship; Diabetes Australian Research Trust grant; L'Oreal Women in Science Award for JEG.

Nothing to Disclose: SLL, RS, BN, SML, RC-B, JEG

P1-154

Intestinal Cell Phosphate Uptake and the Targeted Knockout of the 1,25D₃-MARRS Receptor/PDIA3/Erp57.

I Nemere Ph.D.¹, N Garbi Ph.D.² and GJ Hammerling Ph.D.².

¹Utah State Univ Logan, UT and ²German Cancer Res Ctr DKFZ Heidelberg, Germany.

We have crossed Erp57^{flx/flx} mice with commercially available mice expressing villin-driven cre-recombinase. Enterocytes isolated from 3-4 wk old littermate (LM) male mice responded to 1,25(OH)₂D₃ with enhanced phosphate uptake, relative to corresponding controls within 1 min of addition, whereas in cells from targeted knockout (KO) mice to response was severely blunted. Unlike chick enterocytes, mouse enterocytes did not respond to phorbol ester with enhanced phosphate uptake. However, forskolin, which does not stimulate phosphate uptake in chick intestinal cells did so in enterocytes isolated from either young male LM or KO mice. Intestinal cells isolated from young female LM mice also responded to 1,25(OH)₂D₃ with enhanced phosphate uptake within 5 min of hormone addition, whereas cells from KO mice did not. Forskolin also stimulated phosphate uptake in enterocytes from either young female KO or LM mice. As with intestinal cells from adult male chickens or rats, cells from adult (8 wk) male LM mice lost the ability to respond to 1,25(OH)₂D₃ with enhanced phosphate uptake. In contrast, intestinal cells from adult female LM mice did respond with enhanced phosphate uptake within 1 min of steroid hormone addition, relative to corresponding controls, and the magnitude of the effect was greater than that observed in enterocytes of young females. Cells isolated from young or adult male or female LM mice failed to respond to 1,25(OH)₂D₃ with enhanced PKC activity. Finally, we have previously reported that mouse enterocytes have cell surface VDR; however preincubation of such cells with anti-VDR antibodies demonstrated that the classical receptor is not involved in the rapid 1,25(OH)₂D₃-stimulated uptake of phosphate.

Sources of Research Support: A seed grant from the Center for Integrated BioSystems and the Utah Agricultural Experiment Station, Utah State University, Logan, UT.

Nothing to Disclose: IN, NG, GJH

P1-155

Vitamin D Status and Muscle Mass, Muscle Strength and Physical Function in a Diverse Sample of Men.

AB Araujo PhD¹, GR Chiu MS¹ and SS Harris DSc².

¹New England Res Inst Watertown, MA and ²Jean Mayer USDA HNRCA at Tufts Univ Boston, MA.

Introduction: Evidence is accumulating that reduced muscle strength and performance in older adults is associated with lower levels of serum 25-hydroxyvitamin D (25(OH)D). Data examining this relationship in diverse populations of men are limited. In this study we examined the association between 25(OH)D and muscle mass, muscle strength, and physical function in a diverse male population.

Methods: The Boston Area Community Health/Bone (BACH/Bone) Survey is a population-based cross-sectional survey of skeletal health in a random sample of 1,219 Boston men aged 30-79 y. Age and race/ethnicity were obtained via self-report. Serum 25(OH)D was assessed using a competitive binding protein (CPB) assay without prior chromatography. Lean mass was measured by DXA. Grip strength was measured with a hand dynamometer. A composite physical function score was derived from walk and chair stand tests. Least-square means were used to quantify the relationship between 25(OH)D and outcomes, adjusted for confounding influences.

Results: Mean age of the 1115 men with complete data (Black n=334, Hispanic n=362, White n=419) was 48 y. Associations between 25(OH)D and outcomes were consistent across race/ethnicity and age group. In most cases, 25(OH)D was not significantly related to outcomes, whether treated continuously or categorically (Table). In an age-adjusted model, 25(OH)D was significantly related to the physical function, but this association was no longer present once adjusted for confounding variables.

Conclusion: In this multi-racial study of men, 25(OH)D was not associated with lean mass, upper body strength, or lower body physical function.

Age and multivariate-adjusted least-square means of lean mass, grip strength, and physical function by quartiles of 25(OH)D

| Outcomes | Lean mass (kg) | | Grip strength (kg) | | Physical function (0-8) | |
|------------------|----------------|------------------------|--------------------|------------------------|-------------------------|------------------------|
| | N=852 | | N=753 | | N=898 | |
| 25(OH)D Quartile | Age-adjusted | Multivariate-adjusted* | Age-adjusted | Multivariate-adjusted* | Age-adjusted | Multivariate-adjusted* |
| ≤20.8 ng/ml | 55.1 | 54.4 | 39.1 | 38.6 | 3.51 | 3.81 |
| 20.9-31.3 ng/ml | 54.7 | 55.1 | 38.9 | 38.9 | 4.11 | 4.00 |
| 31.4-42.7 ng/ml | 55.9 | 55.6 | 42.3 | 41.8 | 4.16 | 3.96 |
| ≥42.8 ng/ml | 55.2 | 54.8 | 39.3 | 39.1 | 4.11 | 4.02 |
| p-value | 0.56 | 0.29 | 0.28 | 0.29 | 0.03 | 0.78 |

*Adjusted for age, race/ethnicity, calcium intake, physical activity, BMI, education, income, self-reported health, arthritis, and alcohol use.

Sources of Research Support: NIH Grant R01AG020727.

Nothing to Disclose: ABA, GRC, SSH

P1-156

Novel Mutations in 25-Hydroxyvitamin D3 1alpha Hydroxylase Gene in Patients with Pseudovitamin D Deficiency Rickets.

N Coudray¹, G Abeguile¹, N Richard PhD¹, A Linglart MD;PhD² and M-L Kottler MD;PhD¹.

¹Univ Hosp Caen, France and ²INSERM U561 Paris, France.

Pseudovitamin D deficiency rickets also called vitamin D-deficiency rickets type 1 (VDDR 1) is an autosomal recessive disorder in which *25-hydroxyvitamin D3 1 alpha-hydroxylase* gene (*CYP27B1*) is deficient. VDDR1 is characterized by hypocalcemia, severe hypophosphatemia, elevated serum PTH levels, normal 25(OH)D level and low or undetectable serum concentrations of 1,25(OH)₂D₃.

We screened for mutations *CYP27B1* in ten individuals from seven unrelated families with VDDR-1. In three families parents were consanguineous. The first symptoms appeared within the first year of life. In F5 et F7 the diagnosis was made in two boys later at 8yr and 5 yr respectively. In these patients laboratory abnormalities were mild with normal serum 1 α ,25(OH)D₃ but elevated serum alkaline phosphatase activity and PTH. Deformations of the legs and radiographic signs of vitD deficiency were evidenced when calcium needs were increased in the early growth. All patients responded well to treatment with 1 α -OHD.

The nine exons of the gene were amplified by PCR and sequenced. We identified eight unclassified variants including six new one which were not found in a panel of normal control. In five families the mutation was found at homozygous state. The mutations were severe, affecting the heme and / or substrate binding site or leading to truncated protein if translated. Two false-sense mutations P105F and G398S were not evaluated.

Table 1 : characterization of the mutations

| F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|--|---------|----------------------|---------------------------|---------|-----------------------------|----------------------|
| IVS1 and exon 8 | Exon 2 | Exons 2 and 8 | Exon 2 | Exon 7 | Exon 8 | Exon 8 |
| c.196-2A>G; c.1319_1325*dup pCCCACCC | p.P105F | p.E106X ; p.R459C | p.A129T | p.G398S | c.1319_1325*dup pCCCACCC | p.R453H |
| | F | | F | C | C/F | C/F |
| Splice site mutation | | Heme binding-site | Substrate binding-site | | Truncated protein | Heme binding-site |

C=consanguineous; F: familial; * Wang et al. 1998

The classical laboratory criteria for the diagnosis of VDDR1 may fail to identify patients with partial but significant defect in this enzyme and hence, this syndrome may be more common than previously appreciated.

Wang JT et al., Am J Hum Genet. 1998; 63:1694.

Nothing to Disclose: NC, GA, NR, AL, M-LK

P1-157

A Sensitive Method for Simultaneous Quantitation of Serum 25-Hydroxy Vitamin D₃ and D₂ by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).

Anqi Zhang Ph D¹, Shalender Bhasin MD¹ and Michael Holick MD Ph D¹.

¹Boston Univ Sch of Med Boston, MA.

Background: Hypovitamin D, in addition to its well known links to bone disorders such as rickets and osteoporosis, has been associated with a wide range of conditions, including cancer, hypertension, diabetes, multiple sclerosis, depression and falls. The measurement of circulating 25-hydroxy vitamin D, the main form of Vitamin D in blood, is recognized as an important indicator of vitamin D status in vivo.

Objective: To develop an accurate, sensitive, fast method for the simultaneous measurement of circulating 25(OH)D₃ and 25(OH)D₂ in blood.

Methods: The isotope-dilution liquid chromatography- tandem mass spectrometry and the online extraction technology by turbo column were used. Internal standard, deuterated 25(OH)D₃ was added to 75µL of serum, and serum was precipitated by adding acetonitrile. The supernatant from acetonitrile precipitation was extracted online by turbo column and the extract was automatically transferred to C18 column for separation. Ion transition of 383.5>257.2, 395.5>211.0 and 389.5>263.1 were detected by mass spectrometry for 25(OH)D₃, 25(OH)D₂ and 25(OH)D₃-d6 respectively.

Results: The limit of quantitation for both 25(OH)D₃ and 25(OH)D₂ was 1 ng/mL. The accuracy was determined by using National Institute of Standard and Technology certified standard for human serum vitamin D (NIST972).

Result of Measurement NIST 972 Standard

| NIST Standard | 25(OH)D ₃ (ng/mL) | | 25(OH)D ₂ (ng/mL) | |
|---------------|------------------------------|-------------|------------------------------|-------------|
| | NIST Value | This method | NIST Value | This method |
| Level 1 | 23.9 | 22.7 | | |
| Level 2 | 12.3 | 10.3 | 1.7 | 1.8 |
| Level 3 | 18.5 | 19.5 | 26.4 | 23.6 |
| Level 4 | 70.7 | 68.3 | 2.4 | 2.4 |

The inter-assay variations for 25(OH)D₃ were 4.8% and 2.6% at concentration of 26.2 and 46.0 ng/mL respectively. The inter-assay variations for 25(OH)D₂ were 5.1% and 5.4% at concentration of 21.6 and 41.9 ng/mL respectively. The intra-assay variations were 5.9% at concentration of 38.7 ng/mL for 25(OH)D₃, and 8.2% at concentration of 13.7 ng/mL for 25(OH)D₂ respectively. The recovery of 25 ng/mL were 112.5% and 98.0 % for 25(OH)D₃ and 25(OH)D₂ respectively. Biological validation was established by demonstrating increases in serum 25(OH)D levels after UV treatment of patients with vitamin D deficiency.

Conclusion: This LC-MS/MS method is sensitive, accurate, fast, and reliable, and therefore suitable for the evaluation of vitamin D in vivo status.

Nothing to Disclose: AZ, SB, MH

P1-158

High Prevalence of Hypovitaminosis D in a Young Adult South Florida Population: The Effect of Vitamin D Supplementation.

AC Apaza MD¹, AM Sosa Melo MD¹, H Florez MD, MPH, PhD^{1,2}, V Obeso MD¹, L Guzman MD¹, R Campo MD¹ and S Levis MD^{1,2}.

¹Univ of Miami, Miller Sch of Med Miami, FL and ²Miami Veterans Affairs Healthcare Syst Miami, FL.

Vitamin D blood levels are the result of sun exposure, food intake and supplement use. Beneficial health outcomes have been associated with serum 25OH-vitamin D [25(OH)D] concentrations of at least 32 ng/mL. In a cross sectional study, we evaluated the prevalence of hypovitaminosis D (HVD: 25(OH)D<32 ng/mL) in a group of young and apparently healthy university students and employees in South Florida, a region of year-round sunny weather. Additionally, we tested whether daily supplementation with less than 800 IU (International Units) of vitamin D is enough to keep adequate 25(OH)D levels. A total of 132 subjects (83 women and 49 men) age 41.3±14.6 years underwent a Preventive Medicine assessment at the University of Miami and had their 25(OH)D levels measured during the month of November. The overall prevalence of HVD was 68.2% and no significant difference in 25(OH)D level was found between men and women (28.5±13 ng/ml vs. 29±13.1 ng/ml, p=0.8, respectively). Thirty two participants reported current vitamin D supplementation and were compared to 32 age- and gender- matched participants not taking supplements. The prevalence of HVD was much higher in those not receiving supplements compared to those that reported vitamin D supplementation (96.9% vs. 56.3%, p<0.001, respectively). Those taking less than 800 IU daily or no vitamin D supplement, had more than eight times higher risk of HVD compared to those on 800 IU daily or more [OR: 8.5 (2.3-31.8)]. In the group taking vitamin D, only those taking 2000 IU daily or more achieved 100% adequacy (25(OH)D≥32 ng/ml), while this was much lower (26.3%) in those taking <800 IU per day. These results highlight the elevated prevalence of HVD in apparently healthy young individuals living in a subtropical latitude with year-round sun exposure. Oral supplementation with 2000 IU daily reliably reached adequate circulating 25(OH)D levels, suggesting that the current recommended daily intake of vitamin D (400 IU-600 IU) is insufficient to achieve an adequate vitamin D status. Health promotion on the use of vitamin D supplements in this population may be warranted. Ana C. Apaza and Andrea Sosa Melo contributed equally to this work.

Nothing to Disclose: ACA, AMSM, HF, VO, LG, RC, SL

P1-159

Age and Sex-Specific Changes of Vitamin D and Parathyroid Hormone in an Elderly Population in Rural Areas of Korea.

MI Kang M.D.¹, GS Kim M.D.¹, EH Jang M.D.¹, MH Kim M.D.¹, MK Kim M.D.¹, DJ Lim M.D.¹, KH Baek M.D.¹, KW Oh M.D.², KH Yoon M.D.¹, KW Lee M.D.¹ and HY Son M.D.¹.

¹Seoul St Mary's Hosp, The Catholic Univ of Korea Seoul, Republic of Korea and ²Kangbuk Samsung Hosp, Sungkyunkwan Univ Sch of Med Seoul, Republic of Korea.

Purpose: The association of 25-hydroxyvitamin D[25(OH)D] and intact parathyroid hormone [iPTH] with age, sex and anthropometric variables in old Asians is poorly understood. Our objective was to determine age and sex-specific changes of 25(OH)D and iPTH, and the association of 25(OH)D and iPTH with bone mineral density [BMD] in elderly Koreans.

Methods: Anthropometric parameters, serum 25(OH)D and iPTH, BMD of the lumbar spine and femur by dual-energy X-ray absorptiometry [DXA] were measured in 467 men and 602 postmenopausal women.

Results: The threshold of vitamin D inadequacy was 20 ng/mL. Mean 25(OH)D was lower in women than in men. Age negatively correlated with 25(OH)D and positively correlated with iPTH in the whole population. When men and women were separately analyzed, only iPTH in women, and only vitamin D in men significantly correlated with age. When vitamin D was inadequate, individuals older than 75 years had higher iPTH than those aged ≤ 65 years. When study participants were categorized by age and sex, 25(OH)D was lower in women than men in most age groups in both the winter and summer seasons. Age-associated increase of iPTH was steeper in the winter than in the summer in women. 25(OH)D positively correlated with femoral BMD but not with lumbar BMD, and iPTH negatively correlated with femoral BMD in both sex.

Conclusion: The prevalence of vitamin D inadequacy in elderly Asians increases with age, is higher in women and in the winter. Elderly women with vitamin D inadequacy in the winter are most vulnerable to age-associated hyperparathyroidism.

Nothing to Disclose: MIK, GSK, EHJ, MHK, MKK, DJL, KHB, KWO, KHY, KWL, HYS

P1-160

Prevalence of Vitamin D Deficiency in Rural School Children in North India and Effect of Calcium Replenishment.

V Bhatia MBBS, MD¹ and JB Kanwar MBBS, MD¹.

¹Sanjay Gandhi Postgraduate Inst of Med Scis Lucknow, India.

Background: Vitamin D deficiency is common in urban Indians. Data in rural children, who have abundant sun exposure, are scarce. Dietary calcium deficiency may deplete vitamin D stores. Since milk is expensive in India, this may be a mechanism for secondary vitamin D depletion in poor rural children. **Objective:** To measure serum 25 hydroxyvitamin D (25OHD) in rural children at baseline and after 3 months of calcium carbonate replenishment.

Design: 101 school children (55 female, age 12.7 ± 3.4 years) from rural low economic background were evaluated. Serum calcium, alkaline phosphatase (ALP), albumin and 25OHD were measured. Dietary calcium was recorded by food frequency questionnaire and sun exposure from log of daily activity and clothing pattern. Children were randomised to receive 500 mg elemental calcium twice daily (as calcium carbonate) or placebo. Parent, teacher and school education about the importance of sunlight and calcium, as well as tablet counting, were methods employed to improve compliance. Spot urinary calcium / creatinine ratio was assessed at baseline, and after 1 month and 2 months of therapy.

Result: Serum 25OHD at baseline was 16.1 ± 6.7 ng/ml. Sixty seven percent subjects had 25OHD <20 ng/ml. Boys had higher 25OHD than girls (19.2 ± 5.3 vs 13.8 ± 6.4 ng/ml, $p < 0.001$). Serum 25OHD in girls and boys ≤ 11 years was not significantly different (18.6 ± 5.7 vs 20.4 ± 5.4 ng/ml). However boys > 11 years had significantly higher 25 OH D than girls (19.6 ± 5 vs 11.5 ± 4.9 ng/ml, $p < 0.0001$). Serum ALP was raised in 15% children. Calcium intake was 337 ± 200 mg/d, similar in boys and girls. Sun exposure during winter and summer was higher in boys (85.2 ± 17.1 and 68.2 ± 13.1 min/d) than in girls (77.2 ± 19.2 and 60.7 ± 17.6 min/d, $p < 0.05$). Serum 25 OHD was negatively correlated with age, and positively with sunlight exposure. Serum 25OHD at 3 months was 16.6 ± 6.2 and 17.5 ± 6.7 ng/ml in calcium versus placebo groups. The change was not different in the 2 groups. Urinary calcium / creatinine was significantly higher on therapy than at baseline for the treated group, but there was no difference between the 2 groups over time. **Conclusion:** Vitamin D deficiency is common in rural Indian children, especially adolescent girls. Inadequate sun exposure is a modifiable risk factor. In children receiving 300 mg per day dietary calcium, addition of 1 gm calcium daily for 3 months does not improve serum 25 OHD.

Sources of Research Support: Intramural grant, SGPGIMS.

Nothing to Disclose: VB, JBK

P1-161

Vitamin D Status and Supplementation in Bariatric Surgery Patients in an Inner City Hospital.

JT Tan MD¹, S Gorantla MD¹, S Mohan MD¹, D Jardine MD¹, L Ahmed MD¹ and CM Park MD,PhD^{1,2}.

¹Harlem Hosp Ctr, Columbia Univ Coll of Physicians and Surgs New York, NY ; ²Harlem Hosp Ctr, Columbia Univ Coll of Physicians and Surgs New York, NY and ³Columbia Univ Coll of Physicians and Surgs New York, NY.

Chart review of 193 patients undergoing bariatric surgery at Harlem Hospital from January 2006 to July 2008 showed 77.3% with 25-hydroxyvitamin D total (25OHD) < 30ng/ml and 41.5% < 20ng/ml. The cohort was largely female (91.3%), and Hispanic (82%, with 15% AA and 2% Caucasian), with mean age 43.2 years and mean BMI 48kg/m². All patients were prescribed cholecalciferol (D3) 800-1200IU/day (800IU in calcium citrate tablets plus 400IU if their multivitamin had D3); and patients with 25OHD < 30ng/ml were also prescribed ergocalciferol (D2), 50,000-100,000 IU/week. Adherence is not known, but we found a significant increase in 25OHD (23.8±11.6 to 36.5±14.4ng/ml, $p<0.001$) on retesting at least 8 weeks later. Total 25OHD was inversely related to PTH before ($r=-0.29, p<0.001$) and after ($r=-0.18, p=0.03$) treatment. A subset of 73 patients with increases in blood levels of D2 (suggesting a 50% adherence rate with taking some D2) had a similar increase in 25OHD to the overall cohort (22.5±10.5 to 36.4±14.1 ng/mL, $p<0.001$), with increased 25-hydroxyvitamin D₂ (25OHD₂) (2.7±6.5 vs 24.5±14.7 ng/ml, $p<0.001$) and decreased 25-hydroxyvitamin D₃ (25OHD₃) (19.8± 9.2 vs 12.4±7.9ng/ml, $p<0.001$). The drop in 25OHD₃ occurred in 77.8% of patients with blood level evidence of D2 ingestion, despite their also having been prescribed D3 (adherence unknown). Increases in 25OHD₂ correlated with increases in 25OHD ($r=0.76, p<0.001$) and decreases in 25OHD₃ ($r=0.49, p<0.001$). Patients with elevated PTH (22.1% of the 193 patients) had a significant decrease in PTH (95.5±28.5 to 75.3±30.2 pg/ml, $p=0.015$) after treatment. Those without evidence of D2 ingestion had a greater decrease (93.3±27.6 to 67.1±27.2pg/ml, $p=0.01$) than those with evidence of D2 ingestion (97.1±29.9 to 81.3±31.7pg/ml, $p=0.20$) despite a similar rise in 25OHD (18.1±10.1 to 28.9±14.0ng/ml, $p=0.015$ vs 20.6±7.8 to 33.3±17.0ng/ml, $p=0.003$). CONCLUSION: In this chart review study, prescribing vitamin D (either D2 or D3 and high dose D2) pre-operatively to inner city bariatric surgery patients was associated with increased 25OHD. Elevated PTH levels fell. In patients with evidence of D₂ ingestion 78% showed a fall in 25OHD₃ accompanying increases in 25OHD₂ and 25OHD. We are including adherence assessments in ongoing studies of whether high dose D2 provides benefit in raising 25OHD and lowering PTH when added to our standard pre-operative prescription of the daily D3 routinely used post-operatively.

Nothing to Disclose: JTT, SG, SM, DJ, LA, CMP

P1-162

Vitamin D Deficiency in a Male Veteran Population: Prevalence and Correlation with C-Reactive Protein.

C Kovcsdy MD¹, B Dunn PA¹ and A Iranmanesh MD¹.

¹VA Med Ctr Salem, VA.

The purpose of the present study was to assess the prevalence of vitamin D deficiency in outpatient male veterans, and to explore the notion of potentially more representative lower threshold values by correlating serum 25 OH vitamin D (25OHD) with its metabolic surrogates (calcium, PTH, bone alkaline phosphatase), and a marker of systemic inflammation (CRP). Study population consisted of 1230 men with (mean ± SD) age of 64 ± 12 yrs (range: 20-90) and BMI of 29 ± 6 kg/m². The results indicated prevalence of 6.7%, 36.5%, 71.5%, and 76.7% for 25OHD (ng/mL) levels of <10, <20, <30, and <32, respectively. Fractional polynomial regression and restricted cubic splines analysis of non-linear association of 25OHD with PTH and CRP revealed stronger negative correlation of PTH and CRP with 25OHD at levels of less than 19 ng/mL. The association of 25OHD with calcium and bone alk-p was linear, but with a more significant correlation at levels 19-28 ng/mL.

Table 1: Changes (95%CI) in PTH, Ca, Bone alk-P, and CRP in response to 10 ng/mL decrements in 25OHD in entire group and sub-groups with various 25 OHD levels.

| 25OHD range(ng/mL) | All | <19 | 19-28 | >28 |
|--------------------|---------------------|--------------------|---------------------|--------------------|
| PTH(pg/mL) | 6.2(4.9, 7.4) | 16.1(8.8, 23.4) | 11.7(2.4, 21.0) | 2.7(0.7, 4.7) |
| Calcium(mg/dL) | -0.02(-0.04, -0.01) | -0.02(-0.12, 0.08) | -0.19(-0.35, -0.03) | -0.01(-0.05, 0.03) |
| Bone Alk-P(U/l) | 0.8(0.4, 1.3) | 2.2(-0.3, 4.7) | 2.8(-2.1, 7.8) | 0.3(-0.3, 0.9) |
| CRP(mg/dL) | 1.02(0.35, 1.69) | 5.79(1.56, 10.01) | 0.30(-3.98, 4.59) | -0.16(-1.17, 0.84) |

Comparison of the areas under receiver operator curves (AUROC) identified 25OHD levels of < 20ng/mL as a stronger predictor of PTH, CRP, and calcium at respective values of >65 pg/mL, >10 mg/dL, and <9.4 mg/dL. Regression analysis did not disclose significant correlation between BMI and CRP. In conclusion, by virtue of more robust association with biochemical markers of bone mineral homeostasis, 25OHD levels of less than 19 ng/mL appear to be a definite representation of hypovitaminosis D in this population. While continuing at a lesser intensity for 25OHD levels between 19 and 28 ng/mL, such associations are not marked for vitamin D concentrations more than 28 ng/mL. Consideration of lower threshold for diagnosis of vitamin D deficiency, and the role vitamin D in systemic inflammation warrant future studies.

Sources of Research Support: Salem V.A. Medical Center Research Institute.

Nothing to Disclose: CK, BD, AI

P1-163

Prevalence of Decreased Vitamin D Is High among Veterans with Diabetes and/or CKD.

S Yaturu MD¹ and J Davis².

¹Stratton VA Med Ctr Albany, NY and ²Overton Brooks VAMC Shreveport, LA.

Vitamin D deficiency is associated with a variety of skeletal and extraskeletal problems, including cardiovascular disease, infection, malignancy, and death. This study evaluated the prevalence of vitamin D deficiency among veterans in sunny Louisiana, a region of year-round sunny weather. Using the VA computerized patient record system, we collected all 25- (OH) Vitamin D and 1, 25 (OH) vitamin D levels that were measured between 2007 and 2009. The information collected include age, body mass index, creatinine, history of diabetes, hypertension along with vitamin D levels and PTH. We determined the number of individuals who were vitamin D insufficient and deficient. The mean concentrations of 25(OH) D were 22.5 ± 0.2 ng/ml and that of 1, 25 (OH) vitamin D were 29.2 ± 0.4 ng/ml, among 2990 studies evaluated. Among them only 695 subjects (23%) had normal values, where as 889 (30%) had insufficiency and 1405 (47%) had deficiency. Subjects with diabetes (1041) had significantly ($p < 0.0001$) lower levels (21 and 25ng/ml) of both 25 (OH) and 1, 25(OH) vitamin D levels compared to subjects without diabetes (23 and 32 ng/ml). Similarly subjects with chronic kidney disease (1128) had much lower vitamin D levels than subjects without CKD. Among subjects with diabetes, those with chronic kidney disease (512) had much lower levels of both 25 (OH) and 1, 25(OH) vitamin D levels than with those with normal creatinine levels. We conclude that vitamin D insufficiency and deficiency is highly prevalent in veterans, more so among subjects with diabetes and / or CKD.

Nothing to Disclose: SY, JD

P1-164

Prevalence of Vitamin D Deficiency and Association with Glycemic Control in Patients with Type 2 Diabetes Mellitus: A Retrospective Analysis.

R Kant MD¹, R Chandra MD¹, H Arzumanyan MD¹ and El Krug MD¹.

¹Sinai Hosp of Baltimore Baltimore, MD.

Introduction: Hypovitaminosis D has long been suspected to be a risk factor for glucose intolerance. Several reports suggested an active role to vitamin D in functional regulation of the pancreatic beta cells (1,8). Hypovitaminosis D may be an independent risk factor for type 2 diabetes (T2DM) and metabolic syndrome (2-7). **Objective:** To estimate the prevalence of 25(OH)Vit D [vit D] deficiency in T2DM and association of Vit D level with HbA1c. **Methods:** We performed retrospective continuous chart review of 124 T2DM patients seen at Endocrine outpatient clinic from 2003 to 2008. The data included the age, race, HbA1c, Vit D, PTH level, family history of T2DM, calcium intake. Vit D levels were divided in 4 quartiles: Normal (Vit D > 32 ng/dL), mild deficiency (25 < Vit D < 33 ng/dL), moderate deficiency (14 < Vit D < 26 ng/dL), severe deficiency (Vit D < 15 ng/dL). SPSS software was used to apply T-test, ANOVA and Chi-square tests for analysis of data. **Results:** A total of 113 T2DM patients (91.1%) were found to be Vit D deficient (35.5% - severely, 38.7% - moderately, 16.9% - mildly). Serum Vit D level was inversely related to HbA1c (Pearson correlation -0.208, P=0.029). Mean HbA1c was higher in patients with severe Vit D deficiency when compared with patients with normal Vit D (7.1 Vs 8.18%, P=0.065). 90 out of 124 patients had their race documented in charts (54 whites, 33 black and 3 Asian). Mean HbA1c was higher in Blacks than in Caucasians (8.59 Vs 7.0%; P < 0.05), but mean Vit D level was lower (15.3 Vs 23.4 ng/dL; P < 0.05). At the time of presentation 8 out of 124 patients (6.4%) were on Vit D supplementation (2 with normal Vit D, 4 with moderate Vit D deficiency, 2 with severe Vit D deficiency). **Conclusions:** Our results showed high prevalence (91.1%) of Vit D deficiency in T2DM. Only 6.4% of patients were taking vit D when first seen at endocrine clinic despite regular primary care visits. Inverse relationship between serum Vit D level and glycemic control in our sample supports active role of Vit D in pathogenesis of type 2 DM. Finding of lower Vit D and higher HbA1c level in Black patients underscores the importance of aggressive screening and supplementation in this population. Since majority of type 2 diabetic patients are diagnosed and treated by primary care providers, screening and Vit D supplementation as part of routine primary care may improve health outcomes of this highly prevalent condition.

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Nothing to Disclose: RK, RC, HA, EIK

P1-165

Plasma 25-Hydroxyvitamin D Is Associated with High Levels of HDL-Cholesterol in Patients with Type 2 Diabetes.

U Kampmann MD¹, D Grove MD¹, L Rejnmark MD¹, L Mosekilde Professor, MD¹, N Moller Professor, MD¹ and L Orskov MD².

¹Aarhus Univ Hosp Aarhus, Denmark and ²Silkeborg Regional Hosp Silkeborg, Denmark.

Background: Vitamin D deficiency is a worldwide problem and causes rickets, osteomalacia and osteoporosis. The discovery that most organs and immune cells in the body have vitamin D receptors and that some also have the capacity to metabolize 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D has provided new insights into the effects of vitamin D. There is increasing evidence that altered vitamin D metabolism affects the risk of diabetes and cardiovascular disease. However, most observational studies concern vitamin D status among healthy subjects and the risk of developing diabetes. Fewer studies deal with the effects of vitamin D in patients with type 2 diabetes.

Aim: In this study we wish to describe vitamin D status among patients with type 2 diabetes in a Danish outpatient clinic and to explore the relations between vitamin D concentrations and metabolic control in diabetic patients.

Design and Methods: The participants in this cross-sectional study were patients with type 2 diabetes from the outpatient clinic at Silkeborg Regional Hospital. The recruitment period was from June 2008 to March 2009. 152 patients were included. Exclusion criteria were malabsorption and renal failure. 131 of the patients (86%) participated in a questionnaire survey concerning social class, medical conditions, vitamin D and calcium intake, sun exposure, smoking habits and physical activity. Blood samples were collected once in the period from November 2008 to March 2009.

Results: Median serum 25OH vitamin D in the 152 patients with type 2 diabetes was 54.5 nmol/l (35.0; 80.0). The prevalence of vitamin D insufficiency, defined as 25OH vitamin D < 50 nmol/l, was 47 %. In a linear regression model 25 OH vitamin D was positively associated with HDL-cholesterol (R=0.22; P=0.013) and after adjusting for age, BMI and smoking in a multivariable regression model the association remained significant (R=0.32; P=0.041). HbA1c was negatively but not significantly associated with 25 OH vitamin D (R=0.11; P=0.172).

Conclusion: Among individuals with type 2 diabetes we showed that higher vitamin D status is positively associated with HDL-cholesterol. Previous studies have concluded that 25OH vitamin D might be protective against coronary heart disease but the underlying mechanisms are not clear. Our findings suggest that HDL-cholesterol may be linked to some of these mechanisms.

Nothing to Disclose: UK, DG, LR, LM, NM, LO

P1-166

Vitamin D Deficiency and Inflammatory Markers in African American Type 2 Diabetics.

CV Villabona MD¹, IM Espinosa DO², R Kazlauskaitė MD³, S Barcham MD², MB Sevilleja-Smith BS², J Miernik MD², W Niklinski MD², JA Borgia PhD³ and L Fogelfeld MD².

¹Cleveland Clin, Florida Weston, FL ; ²JH Stroger Hosp of Cook County Chicago, IL and ³Rush Univ Med Ctr Chicago, IL.

Background

Recent studies showed that low Vitamin D [25(OH)D] levels are associated with increased prevalence of cardiovascular disease (CVD) in Type 2 diabetic (T2DM) patients, probably mediated by elevations in inflammatory markers. Clinical trials in non diabetics showed that 25(OH)D supplementation markedly reduced these markers. In the present study we examined the relationship between vitamin D deficiency and these markers in T2DM African American (AA) patients. We also examined if vitamin D replacement would improve these markers of CVD.

Methods

T2DM AA patients were evaluated for 25(OH)D, PTH, and inflammatory markers: high sensitivity C-reactive protein (hsCRP), fibrinogen, matrix metalloproteinase-9 (MMP-9), soluble intercellular adhesion molecule (sICAM-1), soluble vascular cell adhesion molecule (sVCAM1), and tissue-type plasminogen activator inhibitor-1 (tPAI-1). Patients with low 25(OH)D (< 20ng/dl) were randomized into placebo or intervention. The intervention consisted of administration of ergocalciferol 50,000 IU/orally weekly for 12 weeks while the control subjects received placebo oral tablets weekly for 12 weeks after which labs were repeated.

Results

At baseline, among 117 patients, 81 (69%) had low 25(OH)D and 36 (31%) had non-low 25(OH)D. Of these 117 patients, 83% and 43% had hsCRP >1 and >3 mg/L which can denote moderate and high cardiovascular risk. Similar percentages of hsCRP were found in low and non-low 25(OH)D patients (85%, 49% vs. 79%, 47%). Similarly, there were no differences in the levels of the other inflammatory markers between the low and the non-low 25(OH)D groups, nor were there correlations between these markers and 25(OH)D. No correlations were found even when a cutoff of 25(OH)D <10ng/dl was used. Among the low 25(OH)D patients, elevated PTH was found only in 28.4%. There was however a significant inverse relationship between them.

After the intervention, 25(OH)D rose significantly (not in the control) but there was no difference in the levels of the inflammatory marker between the intervention (n=22) and control (n=24).

Conclusion

In T2DM AA patients, high levels of CRP and other markers were not associated with vitamin D deficiency at baseline and after the intervention. This may be due to the fact that much stronger factors influence these markers or that AA patients with low 25(OH)D are relatively protected from its metabolic and inflammatory consequences. Larger studies are needed to confirm these findings.

Nothing to Disclose: CVV, IME, RK, SB, MBS-S, JM, WN, JAB, LF

P1-167

Hypovitaminosis D and Macrovascular Complications in Diabetic Patients.

E Garcia MD¹, G Martinez MD¹, M Calatayud MD¹, R Sanchez MD¹, M Partida MD¹, C Vargas MD¹ and F Hawkins MDPH¹.

¹Hosp Univ 12 de Octubre Madrid, Spain.

Background: Recently, vitamin D deficiency has been identified as a potential risk factor for the development of CVD, possibly through its association with other risk factors, such as diabetes and hypertension.

Methods: We conducted this cross-sectional study in 92 in-hospital patients with different types of diabetes, analyzing the relationship between serum 25-hydroxyvitamin D (25-OHD) concentration and macrovascular diabetic complications. **Results:** 51,1% of patients were women and 48,9% were men. The average age was 51 ± 21 years (mean \pm SD). 36,2% had Type 1 diabetes, 57,4% had Type 2 diabetes, 2,3% had pancreatoprive diabetes and 4,3% had latent autoimmune diabetes in adults (LADA). Average duration of diabetes was $8,8 \pm 11$ years. Mean glycated hemoglobin (HbA_{1c}) was $11,5 \pm 2,7\%$. Mean serum concentration of 25-OHD was $19,6 \pm 11,9$ ng/ml. Prevalence of hypovitaminosis D (<20ng/ml) was 56,5%. Its concentration decreased according to the number of macrovascular complications; 7,6% had coronary heart disease, 6,5% had cerebrovascular disease, and 7,7% had peripheral vascular disease. In patients with hypovitaminosis D, prevalence of coronary heart disease was 11,5%, while 9,6% had cerebrovascular disease, and 11,5% had peripheral vascular disease, versus 2,5%, 2,5% and 2,5% in diabetic patients with normal 25-OHD levels. There was no significant association between 25-OHD and age, gender, BMI, type and duration of diabetes, type of treatment, number of macrovascular complications or HbA_{1c}. Only serum albumin were significantly associated with decreased 25-OHD ($p=0,025$).

Conclusions: We found an elevated prevalence of hypovitaminosis D in our patients. Macrovascular complications of diabetes were found to be increased in the subgroup of patients with hypovitaminosis D without statistical significance.

Nothing to Disclose: EG, GM, MC, RS, MP, CV, FH

P1-168

Serum 25-Hydroxyvitamin D and the Metabolic Syndrome: A Population-Based Study.

MM Oosterwerff MD¹, EMW Eekhoff MD, PhD¹, P Lips MD, PhD¹ and NM Van Schoor Msc, PhD¹.

¹VU Univ Med Ctr Amsterdam, Netherlands.

Recent evidence suggests that low serum levels of 25-hydroxyvitamin D (25(OH)D) may be associated with an increased risk of the metabolic syndrome, although the results are inconsistent. Metabolic syndrome and vitamin D insufficiency are both common among older individuals. The objective of this study was to examine a possible association between serum 25-hydroxyvitamin D and the metabolic syndrome in a population-based sample of older persons.

The study was performed as a substudy of the Longitudinal Aging Study Amsterdam (LASA), an ongoing multidisciplinary cohort study in a representative sample of the older population in the Netherlands.⁽¹⁾The current study was performed in persons aged 65 years and older, who participated in the medical interview in 1995/96 (n=1509). Data on 25(OH)D and metabolic syndrome were available for 1289 persons. Metabolic syndrome was determined in 1995/1996 and defined as the presence of three or more of the following criteria: triglycerides ≥ 1.7 mmol/l; HDL < 1.0 mmol/l for men and < 1.3 mmol/l for women; blood pressure $\geq 160/90$ mmHg or antihypertensive medication; waist circumference > 102 cm for men and > 88 cm for women; and fructosamine ≥ 0.247 mmol/l or antidiabetes medication. (Adult Treatment Panel III)

The data were analyzed with 25(OH)D levels below 50 nmol/l versus above 50 nmol/l with logistic regression analysis. Potential confounders were age, sex, season, years of education, alcohol use, total activity, chronic diseases and smoking.

In total, 630 men and 659 women were included in the analysis with a mean age of 75.4 years. Of the total sample 36.9% had metabolic syndrome. The mean vitamin D level in the metabolic syndrome group was 50.78 nmol/l and in the non-metabolic syndrome group 55.09 nmol/l. No interaction was found with sex $p=0.49$.

Persons having serum 25(OH)D levels below 50 nmol/l had a higher risk on metabolic syndrome, odds ratio 1.32 (95% CI 1.02-1.71) after adjustment for confounders. Investigating the individual components of metabolic syndrome in relation to 25(OH)D revealed that 25(OH)D was most associated with low HDL levels 1.58 (1.26-1.99), after adjustment for confounders 1.37 (1.06-1.77) and waist circumference 1.55 (1.24-1.94), after adjustment for confounders 1.27 (0.99-1.63).

In conclusion, a serum 25(OH)D level below 50 nmol/l appears to be associated with a higher risk of the metabolic syndrome in older men and women. The association was mainly determined by low HDL and waist circumference.

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Nothing to Disclose: MMO, EMWE, PL, NMVS

P1-169

Serum 25-Hydroxyvitamin D Is an Independent Determinant of Whole-Body Insulin Sensitivity.

JA Alvarez MS, RD¹, AP Ashraf MD¹, GR Hunter PhD¹ and BA Gower PhD¹.

¹Univ of Alabama at Birmingham Birmingham, AL.

Background: Circulating 25-hydroxyvitamin D (25(OH)D) has been shown to be associated with insulin sensitivity, however adiposity may be a confounder in this relationship. Furthermore, most studies have used proxy measures of insulin sensitivity, such as HOMA-IR, which primarily reflect hepatic insulin resistance. The objective of this study was to examine the relationship of 25(OH)D with whole-body insulin sensitivity after adjusting for adiposity and fat distribution using robust techniques. A secondary aim was to investigate the relationship between 25(OH)D and other cardiometabolic risk factors.

Methods: Subjects were 62 women (age range: 18-67 yrs; % fat range: 22.1-53.7%). Whole-body insulin sensitivity index (S_i) was determined with an intravenous glucose tolerance test and minimal modeling, % fat with dual energy X-ray absorptiometry, intra-abdominal adipose tissue (IAAT) with computed tomography, and 25(OH)D with liquid chromatography-tandem mass spectrometry. HOMA-IR was used as an index of hepatic insulin resistance. Other cardiometabolic risk factors tested were systolic and diastolic blood pressure, total cholesterol, triglycerides, HDL, and LDL.

Results: Mean 25(OH)D was 22.2 ± 13.6 ng/ml. Multiple linear regression analysis indicated that 25(OH)D was a significant determinant of S_i (standardized $\beta = 0.24$, $P = 0.04$), independent of age, race, IAAT, and parathyroid hormone. 25(OH)D was not significantly associated with HOMA-IR. 25(OH)D was positively associated with HDL ($r = 0.29$, $P = 0.05$), independent of % fat, IAAT, and ethnicity, but was not associated with other cardiometabolic risk factors.

Discussion: 25(OH)D was independently associated whole-body insulin sensitivity in a cohort of healthy women. The lack of a significant association with HOMA-IR suggests that circulating 25(OH)D is more closely related to peripheral than hepatic insulin sensitivity, although glucose tracer studies are required to confirm this.

Sources of Research Support: NIH grants R01DK49779, R01DK51684, R01DK58278, R01DK067426, M01-RR-00032 (GCRC), P30-DK56336 (CNRC), and P60DK079626 (DRTC); JAA was supported in part by the American Heart Association (Greater Southeast Affiliate), the NIH National Center for Research Resources (5TL1 RR025775-02), and the Alabama Louis Stokes Alliance for Minority Participation. APA is supported in part by the Child Health Research Center K12 HD043397 (T0909180013).

Nothing to Disclose: JAA, APA, GRH, BAG

P1-170

How Do We Determine How Much Vitamin D To Recommend to Our Pediatric (Diabetic) Patients?.

AS Dothard¹ and CD Tapidaor M.D.¹.

¹East Tennessee Children's Hosp Knoxville, TN.

Background: In late 2008, the American Academy of Pediatrics (AAP) revised its daily vitamin D recommendation from 200 IU to 400 IU. There has been very little research to confirm whether this revision is adequate for diabetic patients.

Objective: To review 25-(OH)D levels and recommended doses of vitamin D among pediatric diabetic patients from January 2009 to December 2009 accounting for age, race, changes in season, and initial vitamin D levels.

Methods: Patients were divided by gender into three age groups: 0-5 yrs old, 6-10 yrs old, and 11 and older. The study was compartmentalized into four seasons labeled as follows: Winter (January 1-March 20), Spring (March 21-June 20), Summer (June 21-September 21) and Fall (September 22-December 22). At their initial evaluation, each patient was measured for baseline 25-(OH)D levels and then classified as deficient (<20 ng/ml), insufficient (20-29 ng/ml), or sufficient (>30 ng/ml). An additional African-American/Biracial group was created in order to evaluate the effects of darker skin pigmentation. After their initial evaluation, the deficient, insufficient, and sufficient groups were prescribed 2000 IU, 1000 IU, and 400 IU of vitamin D respectively.

Results: 125 patient charts were reviewed with 73 males and 52 females (ages 3-18 years). We found 23 (18%) to be sufficient, 74 (59%) insufficient, and 28 (23%) deficient.

Average 25-(OH)D Levels per Season (ng/ml)

| | Winter | Spring | Summer | Fall |
|---------------|--------|--------|--------|------|
| Sufficient | 32.3 | 32.9 | 40.6 | 32.3 |
| Insufficient | 24.4 | 26.7 | 31.6 | 29.4 |
| Deficient | 16.2 | 24.4 | 30.3 | 29.9 |
| AA & Biracial | 17.8 | 23.4 | 22.8 | 25.2 |

Treatment of 400 IU for those in the sufficient group prevented a drastic decline of final 25-(OH)D levels. However, it was not effective in substantially increasing the vitamin D levels of these patients.

For those in the insufficient and deficient categories, treatments of 1000 IU and 2000 IU did improve vitamin D levels. Recommendations above 400 IU appear necessary for these groups.

Of the African American/biracial patients, 91% were insufficient or deficient and their increase in 25-(OH)D level was only 54% of those of their Caucasian counterparts.

Conclusions: 400 IU of vitamin D daily may not be adequate for all pediatric diabetic patients, especially those known to be vitamin D insufficient or deficient. Instead, physicians should consider increasing dosage recommendations for individual patients based on age, race, season, and baseline vitamin D level.

Nothing to Disclose: ASD, CDT

P1-171

The Effect of Weight on Vitamin D Dose Response.

EE Fuller MD¹, AT Drincic MD¹, LAG Armas MD¹, RP Heaney MD¹ and MS Dowell RN, PhD¹.

¹Creighton Univ Med Ctr Omaha, NE.

Background: Obesity is a very common problem in the United States. It has been well documented that obesity is associated with low 25(OH) vitamin D levels.^{1, 2, 3, 4, 5, 6, 7, 8, 9} Although the causes of vitamin D deficiency in this population have not been well elucidated, the prevalence of and need for treatment of vitamin D deficiency is well accepted. There have been vitamin D dose response studies conducted in populations that are normal to overweight (BMI 20-30 kg/m²)^{2, 10, 11, 12} but there are no vitamin D dose response studies in a frankly obese population. Clinicians wishing to treat obese patients for vitamin D deficiency have no guidelines to follow. This project will give clinically relevant information to clinicians who are treating obese patient with vitamin D deficiency.

Trial Design: This is a randomized, single-blind, controlled study. Subjects were enrolled during winter months to minimize sun exposure. Subjects were randomly assigned to take either 1,000 IU/d, 5,000 IU/d, or 10,000 IU/d of vitamin D3 for a period of four months. At baseline, we measured 25(OH)D, vitamin D3, PTH, calcium, and creatinine in fasting serum. Subjects are scheduled for visits on or near target dates that mark 1, 3, 6, 10, and 21 treatment weeks. At visits 2, 3, 4, and 5, we will measure 25(OH)D and calcium. At visit 6, we will measure 25(OH)D, PTH, calcium and vitamin D3. At visits 1 and 6 a 2 hr urine collection for calcium and creatinine clearance is done. In addition, we will measure body composition on the Hologic 4500 DXA and skin tone using the IMS Smart Probe.

Inclusion/Exclusion Criteria: Healthy women without an outdoor job during the previous summer and who do not plan to travel during the study, between ages 19 and 70 years, BMI ≥ 30.0 kg/m², $\leq 1,000$ IU/d usual intake of vitamin D, no history of hepatic or renal disease, not be taking medications known to affect vitamin D metabolism, no diagnoses of diseases causing malabsorption of vitamin D.

Primary Clinical Endpoint: To characterize the dose response of 25(OH)D to 1,000 IU/day, 5,000 IU/D, and 10,000 IU/D in a group of obese women.

Preliminary Data: 41 Caucasian and Hispanic women were enrolled between November 2009 and January 2010.

Average age 44.9 years (range 28.8-68.2 years), average BMI 36.8 kg/m² (range 29.1-58.5 kg/m²). All subjects have completed the baseline visit and serum samples are currently being analyzed.

- (1) Rosenstreich SJ et al., J Clin Invest 1971;50:679-87.
- (2) Arunabh S et al., J Clin Endocrinol Metab 2003;88:157-61.
- (3) Hypponen E et al., Diabetes Care 2006;29:2244-6.
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- (5) Looker AC. J Clin Endocrinol Metab 2005;90:645-40.
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- (11) Harris SS et al., J Am Coll Nutr 1999 Oct;18(5):470-4.
- (12) Heikkinen A et al., Calcif Tissue Int 1998 Jan;62(1):26-30.

Sources of Research Support: Health Futures Foundation, Creighton University, Omaha, NE.

Nothing to Disclose: EEF, ATD, LAGA, RPH, MSD

P1-172

Relationship between Vitamin D Status and ICU Outcomes in Veterans.

JD MCKinney DO¹, BA Bailey PhD², LH Garrett PhD², P Peiris MD³, T Manning BA¹ and AN Peiris MD¹.

¹Mountain Home VAMC Johnson City, TN ; ²East Tennessee State Univ Johnson City, TN and ³Mayo Clin Jacksonville, FL.

Vitamin D deficiency remains a poorly recognized pandemic and is closely linked to increased health care costs in Veterans. Projected health care needs in Veterans are expected to increase over the next decade. Intensive care unit (ICU) costs contribute significantly to hospital costs and stem from intervention services and management of sepsis including nosocomial infections. Vitamin D has immunomodulating and antimicrobial properties through antimicrobial peptides such as cathelicidin. A retrospective study was undertaken to evaluate if Vitamin D insufficiency was associated with less than optimal ICU outcomes in Veterans. The study included 136 Veterans with 25(OH)D within a month of admission to ICU. The average 25(OH)D level was 24.6 ng/ml [normal range 30-100] with 38 % of patients falling in the Vitamin D deficient category (<20 ng/ml) ICU survivors had a significantly lower percent of Vitamin D deficiency compared to non-survivors (28.4 versus 52.7%). Twenty nine percent of patients with a Vitamin D replete state were in ICU 3 days or longer whereas, 56 % of patients with Vitamin D deficiency stayed in ICU 3 days or longer.

Table 1

| Length of ICU stay | Vit D level(sd) | %Deficient |
|--------------------|-----------------|------------|
| < 3 days* | 26.7(11.0)*a | 26.8%*b |
| 3+ days | 21.1(11.7) | 55.6% |

*A natural break at 3 days both in the distribution for all patients, and in differences between the Vit D status group. *a p=.006, *b p=.001

These differences were highly significant translating to two-fold increased risk (2.0 RR) for 3 day or longer stay in ICU in patients with Vitamin D deficiency.

Table 2

| Survival Status | Vit D levels(sd) | %Deficient |
|-------------------------|------------------|------------|
| Died (in ICU or later*) | 21.6(11.8)*a | 52.7%*b |
| Survived | 26.4(11.1) | 28.4% |

*No significant difference on Vit D status between those who died in the ICU and those who died later. *a p=.018, *b p=.001

Moreover, the relative risk of death was significantly higher in ICU patients related to Vitamin D deficiency (RR 1.81). We propose that a Vitamin D state of replete may confer survival advantages in critical illness and recommend that 25(OH)D levels be checked on admission to ICU.

Nothing to Disclose: JDM, BAB, LHG, PP, TM, ANP

P1-173

Effect of Vitamin D on Falls and Fallers: a Meta-Analysis of Randomized Control Trials.

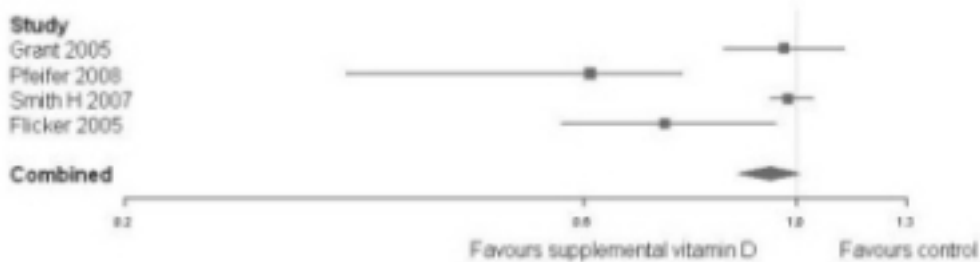
AJ Sai M.D.¹, JC Gallagher M.D.¹ and X Fang PhD¹.

¹Creighton Univ Med Ctr Omaha, NE.

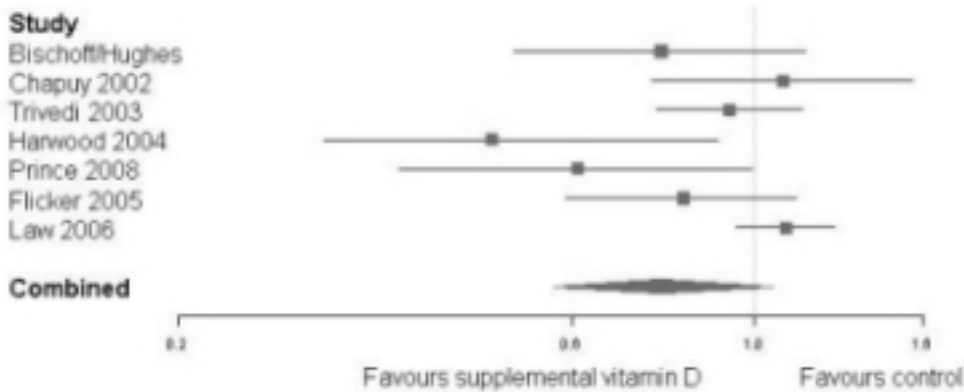
Background: In recent year there has been an interest in the effect of Vitamin D on falls. In a meta-analysis published recently, it was concluded that high dose supplemental vitamin D (700-1000 IU/d) significantly reduced fall risk by 19%.

Methods: We did an extensive search of literature- Pubmed, Cochrane, abstracts, references for randomized trials of Vitamin D and falls. 16 studies were identified that used Vitamin D3/D2. The inclusion criteria were: a randomized placebo control study design, using Vitamin D2/D3, ascertaining falls/fallers and describing them as outcome and duration ≥ 10 months. We did not include short-term studies (< 10 months) and those involving few people (< 100) because of concerns about the statistical power to predict an effect.

Results: Based on above criteria only 4 studies were included for meta-analysis of falls involving 15,599 people with mean age 79 yrs and 7 studies for fallers involving 8,508 people with mean age of 79.6 yrs. The pooled risk ratio for falls was 0.94 (95 % Confidence Interval (CI) = 0.87-1.01).



We also examined the effect of short term studies of vitamin D on falls with similar results (not shown). The pooled risk ratio for fallers was 0.77 (CI = 0.57-1.05).



Thus, there was no significant effect of Vitamin D intervention compared to placebo on either falls or fallers.

Conclusion: We found no significant effect of vitamin D intervention in contrast to a recent meta-analysis that included studies as short as 3 months with group sizes as low as 23 subjects in each intervention arm. The difference in our meta-analysis from positive published meta-analysis probably reflects selection bias. **Summary:** Few studies are adequately powered to predict an effect of vitamin D intervention on falls and fallers, and larger studies over longer time need to be performed.

Sources of Research Support: NIH Grant AG28168.

Nothing to Disclose: AJS, JCG, XF

P1-174

Impact of Vitamin D on Bone Mineral Density among Different Ethnic Groups in Premenopausal Women.

Rudrudee Karnchanasorn MD^{1,2} and Ken C Chiu MD^{1,2}.

¹City of Hope Natl Med Ctr Duarte, CA and ²Harbor-UCLA Med Ctr Torrance, CA.

A positive association between serum 25-hydroxyvitaminD (25OHD) and bone mineral density (BMD) has been reported, but little is known about this association among different ethnicities in premenopausal women.

We analyzed the data from premenopausal female participants age ≤ 20 years old (y/o) in the National Health and Nutrition Examination Survey (NHANES) 2005-2006, a cross-sectional survey of the U.S. population to study the health and nutritional status of noninstitutionalized resident in the United States. This sample consists of 355 non-Hispanic whites [NHW, age 35 ± 10 y/o, body mass index (BMI) 26.43 ± 6.23 kg/m², mean \pm STD], 160 Non-Hispanic blacks [NHB, age 35 ± 9 y/o, BMI 28.45 ± 6.21 kg/m²], and 178 Mexican Americans [MA, age 35 ± 10 y/o, BMI 28.35 ± 5.72 kg/m²]. We divided 25OHD into 4 groups by an increment of 10 mg/dl within each ethnic group. The relationship of serum 25OHD and femoral neck BMD was examined with the consideration of age, BMI, family history of osteoporosis in parents, and prior history of osteoporotic fracture as potential confounders.

Hypovitaminosis D (≤ 30 ng/mL) was extremely prevalent in this premenopausal female population: 80% in NHW, 99% in MA, 98% in NHB. Serum 25OHD concentrations were 25 ± 9 ng/mL in NHW, 17 ± 6 ng/mL in MA, and 14 ± 7 ng/mL in NHB. We observed a positive association between 25OHD and BMD in NHW ($P_{\text{trend}}=0.05$) and MA ($P_{\text{trend}}=0.05$), while a negative association was observed in NHB ($P_{\text{trend}}=0.01$). The positive associations remained significant after adjustment for age and BMI ($P_{\text{trend}}=0.009$ for NHW and $P_{\text{trend}}=0.01$ for MA) and after adjustment for age, BMI, family history of osteoporosis in parents, and prior history of osteoporotic fracture ($P_{\text{trend}}=0.01$ for NHW and $P_{\text{trend}}=0.009$ for MA). However, the inverse association in NHB was no longer observed after adjustment for the covariates.

Our observations show a positive association between 25OHD and femoral neck BMD in NHW and MA premenopausal women. The inverse association in NHB premenopausal women, to our knowledge, has not been reported, albeit the association becomes only marginally significant after adjustment for covariates. This may be in line with the observation that NHB have fewer osteoporotic fractures than other ethnic groups despite lower 25 OHD. This paradox may reflect decreased sensitivity to vitamin D, parathyroid hormone resistance, and increased renal calcium conservation in NHB. Further studies are needed in this field.

Nothing to Disclose: RK, KCC

P1-175

Vitamin D Status in Multiethnic Malaysian Pregnant Women.

F Harun MBBS,FRCP¹, I Taufik BSc¹ and SZ Omar MBBS,MRCOG¹.

¹Univ of Malaya Kuala Lumpur, Malaysia.

Introduction : Exclusively breast fed babies are at risk of hypocalcaemic fits or vitamin D deficiency rickets especially if their mothers are vitamin D deficient during pregnancy as the maternal transfer of vitamin D is adequate only for the 1st two months after birth. As breast milk has little supply of vitamin D, exclusively breast fed babies thus need vit D supplementation. This policy is not practiced universally in tropical countries like Malaysia as health professional assumed that the adult population would have acquired adequate supply of vitamin D from sun exposure. We determine the vitamin D status of pregnant women during the last trimester of pregnancy as maternal vitamin D deficiency may affect the fetus and baby.

Method : Blood was taken for Vitamin D (25 - hydroxy vitamin D) and PTH from 197 pregnant women of 3 main ethnic groups, i.e. Malay (n=108) Chinese (n=34) and Indian (n=55) during their last trimester of pregnancy in a single tertiary hospital. Plasma 25-hydroxyvitamin D and PTH were analyzed using electrochemiluminescence immunoassay analyser. Demographic data on the mothers, their working status, type of work, and type of clothing were obtained using preset questionnaires. A Vitamin D level of <20ng/ml was considered deficient, that between 20-30ng/ml as insufficient and >30ng/ml as adequate (Horlick 2007).

Results: There were 31.9% (63/197) of this cohort who were deficient in vitamin D, 50.3% insufficient and only 17.8% have adequate vitamin D. The majority of whom who were deficient were Malays (44%) followed by Indians (22%). These two ethnic groups have darker skin and were culturally fully clothed in comparison to the Chinese. Plasma PTH was negatively correlated with plasma 25-OH vitamin D ($r = -0.326$; $p < 0.01$).

Conclusion : This initial study showed that a third of pregnant mothers in this cohort despite residing in the tropics were deficient in vitamin D, a result which concurs with other studies which showed that 30 to 50% of their adult population have 25-hydroxyvitamin D levels < 20ng/ml (Horlick 2007). Inadequate vit D has implications in their babies as well as their own later health. Our next study would be to determine the baby's vitamin D status in those who are exclusively breast fed.

Holick MF, N Eng J Med 2007;357: 266-281

Nothing to Disclose: FH, IT, SZO

P1-176

Vitamin D Supplementation during Pregnancy.

A Sachan MD, DM¹, CV Harinarayan MD, DM¹, D Gunasekhar M.Sc.¹, P Sreevani M.Sc.¹, B Bhargavi MBBS², GS Subramanyam MD², V Bhavani MD², G Rajagopal MD¹, PA Reddy MD¹, P Satish MD¹ and V Suresh MD, DM¹.

¹SV Inst of Med Scis Tirupati, India and ²Government Maternity Hosp Tirupati, India.

Introduction: Vitamin D deficiency is prevalent in India. Impact of hypovitaminosis D during pregnancy on neonates and effect of replacement strategies is not known.

Methods: From the obstetric unit, 107 pregnant women were recruited in beginning of 3rd trimester, who received 3 doses of vitamin D, 60,000 I.U. each, once a month in the last trimester along with 1000 mg of calcium/day (supplemented group). Another group of 110 women, recruited at term, received routine calcium and vitamin D supplements (nonsupplemented group). Maternal and neonatal anthropometry was recorded.

Samples were collected at baseline (3rd trimester) for 25 hydroxyvitamin D {25(OH)D} and intact parathormone (PTH)(in supplementation group only) and at term for serum calcium (Ca) , phosphorus, albumin, heat labile alkaline phosphatase (ALP), 25(OH)D and PTH (for both supplemented and nonsupplemented groups). Cord blood serum 25(OH)D, ALP, albumin and 3rd postpartum day neonatal Ca and PTH were collected from neonates born to the recruited subjects.

Results: Among the vitamin D supplemented women, no improvement was seen in serum 25(OH)D at term but serum PTH was lower at term (19.5 ± 12.2 vs 24.2 ± 13.0 pg/ml, $p < 0.005$) as compared to baseline. Supplemented women had lower PTH than nonsupplemented women (19.5 ± 12.2 vs 24.2 ± 12.6 pg/ml, $p < 0.01$) despite having similar 25(OH)D (19.6 ± 10.3 vs 17.1 ± 12.6 ng/ml, $p = \text{NS}$).

Neonates born to supplemented women had higher birth weight (3.0 ± 0.3 vs 2.8 ± 0.2 Kg, $p < 0.001$), head circumference (32.8 ± 0.7 vs 32.4 ± 0.9 cm, $p < 0.005$), mid upper arm circumference (9.1 ± 0.2 vs 9.0 ± 0.2 cm, $p < 0.001$) and serum albumin (3.8 ± 0.4 vs 3.6 ± 0.3 g/dl, $p < 0.01$) as compared to those born to nonsupplemented women.

None of the newborns had hypocalcemia. Serum PTH was lower among the neonates born to the supplemented women (32.0 ± 23.1 vs 42.8 ± 34.2 pg/ml, $p < 0.01$) as compared to nonsupplemented women despite having similar cord blood 25(OH)D (13.6 ± 9.7 vs 13.9 ± 6.3 ng/ml, $p = \text{NS}$). A lesser number of neonates born to supplemented women, had secondary hyperparathyroidism (PTH > 55 pg/ml) as compared to nonsupplemented women (10/107 vs 25/110, $p < 0.01$).

Conclusion: Three doses of 60,000 I.U. of vitamin D in the last trimester of pregnancy were not adequate to treat the preexisting vitamin D deficiency in pregnant women. However, this schedule improved anthropometry of the neonates and ameliorated secondary hyperparathyroidism to some extent, both in women and in their newborns.

Sources of Research Support: Grant No. BT/PR 8093/SPD/11/926/2006, Department of Biotechnology, Ministry of Science and Technology, Government of India.

Nothing to Disclose: AS, CVH, DG, PS, BB, GSS, VB, GR, PAR, PS, VS

P1-177

A Randomized Controlled Trial of Prenatal Vitamin D Supplementation To Prevent Vitamin D Deficiency in Mothers and Their Infants: Interim Results.

HF Saadi MD¹, A Dawodu MD², G Bekdache MD³, M Altaye PhD², JY Pathan BSc¹ and BW Hollis PhD⁴.

¹United Arab Emirates Univ Al Ain, United Arab Emirates ; ²Cincinnati Children's Hosp Med Ctr Cincinnati, OH ; ³Tawam Hosp Al Ain, United Arab Emirates and ⁴Med Univ of South Carolina Charleston, SC.

Background: Vitamin D (vD) deficiency is common in Arab women of childbearing age and is a risk for vD deficiency in infants and young children.

Objectives: To determine the efficacy and safety of high dose (2000 or 4000 IU/day) prenatal vD to prevent vD deficiency in Arab pregnant women and their infants.

Methods: Arab expectant mothers were randomized at 12 weeks gestation to one of three vD3 treatment groups (400, 2000 or 4000 IU/day) to be continued throughout pregnancy (goal 180 subjects). Maternal serum calcium (Ca), phosphorus, albumin, parathyroid hormone (PTH) and 25-hydroxyvitamin D [25(OH)D] concentrations and urinary calcium/creatinine (UCa/Cr) ratio were monitored. Serum 25(OH)D was also measured in cord blood. We defined hypervitaminosis D limits as 25(OH)D >250 nmol, or serum Ca >2.75 mmol/L with UCa/Cr ratio >1.0 mmol/mmol.

Results: Baseline 25(OH)D in 143 subjects ranged from undetectable (<12.5 nmol/L) in 33(23.1%) subjects to 86.7 nmol/L (mean ± SD 18.3 ± 11.7 nmol/L). 25(OH)D correlated negatively with PTH at baseline and at follow up (r -0.22 to -0.37, P <0.05). In the whole group, mean 25(OH)D at follow up was significantly higher than the baseline values (P <0.001) (Table). Ninety-two subjects have delivered thus far. 25(OH)D ranged from undetectable (<12.5 nmol/L) in 2(1%) subjects to 167 nmol/L at delivery. Cord blood 25(OH)D ranged from undetectable (<12.5 nmol/L) in 3(1.6%) subjects to 126.4 nmol/L. Corrected serum Ca concentration and UCa/Cr ratio increased significantly from baseline (P <0.001) but did not reach hypervitaminosis D limits in any subject.

Conclusions: The results confirm a high prevalence of severe vD deficiency in expectant Arab mothers. The ongoing study indicates that prenatal vD3 supplementation at all dose levels was associated with improved vD status without hypervitaminosis D.

Mean (SD) of biochemical parameters at baseline & follow up

| Parameter/Time | 12 wk gestation | 16 wk gestation | 28 wk gestation | Delivery | Cord blood |
|-----------------------|-----------------|-----------------|-----------------|-----------|------------|
| 25(OH)D (nmol/L) | 18.3±11.7 | 51.6±23.8 | 72.8±36.1 | 69.8±33.1 | 51.5±25.2 |
| PTH (pmol/L) | 2.4±1.2 | 1.6±1.0 | 1.6±0.8 | 3.0±2.5 | 1.2±1.9 |
| Corrected Ca (mmol/L) | 2.31±0.09 | 2.35±0.09 | 2.38±0.09 | 2.38±0.09 | 2.49±0.22 |
| Phosphorus (mmol/L) | 1.15±0.16 | 1.21±0.15 | 1.13±0.17 | 1.14±0.31 | 1.77±0.31 |
| UCa/Cr (mmol/mmol) | 0.29±0.21 | 0.38±0.26 | 0.43±0.27 | NA | NA |

NA: Not Applicable

Sources of Research Support: Thrasher Research Fund.

Nothing to Disclose: HFS, AD, GB, MA, JYP, BWH

P1-178

Associations between Vitamin D Receptor Genotypes and Mortality.

RT de Jongh¹, P Lips¹, KJ Rijs¹, NM van Schoor¹, MH Kramer¹, JP Vandenbroucke² and OM Dekkers².

¹VU Univ Med Ctr Amsterdam, Netherlands ; ²VU Univ Med Ctr Amsterdam, Netherlands ; ³Leiden Univ Med Ctr Leiden, Netherlands ; ⁴Leiden Univ Med Ctr Leiden, Netherlands and ⁵VU Univ Med Ctr Amsterdam, Netherlands.

Context: Vitamin D receptor (VDR) polymorphisms are associated with a variety of diseases which may translate into an effect on mortality.

Objective: To investigate associations between VDR gene variants and mortality in older individuals.

Design and subjects: The analyses were conducted in a population-based, prospective cohort of the Longitudinal Aging Study Amsterdam. We included 935 individuals (≥ 65 yr) with adequate DNA analysis. We aimed to assess associations between mortality and three VDR polymorphisms (Cdx-2, GATA and FokI) and three haplotypes of the BsmI, ApaI and TaqI polymorphisms (baT, Bat and bAT).

Results: During a median follow-up of 10.7 years 480 participants deceased (51%). The GATA GG genotype was associated with a 30% higher mortality risk compared to the AA genotype (HR 1.30, 95%-CI 1.01-1.68). Homozygosity for the baT haplotype was associated with 22% reduction in mortality risk compared to the absence of copies (HR 0.78, 95%-CI 0.68-1.01). Adjustment for cardiovascular risk factors and 25-hydroxyvitamin D did not affect these hazard ratios. The GATA AG genotype tended to be associated with a twofold increased risk of osteoporotic fractures (HR AG genotype 2.02, 95%-CI 0.99-4.08). After adjustment for fractures, the GATA GG genotype was no longer associated with higher mortality risk (HR 1.10, 95% CI 0.84-1.45).

Conclusion: The GATA G allele was related to increased mortality risk, which may be partly explained by fractures. The baT haplotype tended to be associated with decreased mortality risk. As the biological mechanism is uncertain, our results should be interpreted as hypothesis generating.

Nothing to Disclose: RTdj, PL, KJR, NMvS, MHK, JPV, OMD

P1-179

Vitamin D Receptor Single Nucleotide Polymorphisms in Somali Immigrants.

AA Moheet MBBS¹, AA Khowaja MBBS², SA Jones MD², S Nazarian MD¹, T Keyler², JV St Peter PharmD³, A Madar MD² and C O'Riordan.

¹Univ of Minnesota Minneapolis, MN ; ²Hennepin County Med Ctr Minneapolis, MN and ³Coll of Pharmacy, Univ of Minnesota Minneapolis, MN.

Vitamin D receptor (VDR) single nucleotide polymorphisms (SNPs) have been associated with metabolic, immunologic, and infectious disease. (1-5) However, these associations are not consistently found in all populations. Estimates of VDR SNP prevalence in different ethnic groups have been previously published though there are no published data characterizing SNPs in Somali people. Because of the lack of available data in this population we assessed the prevalence of common SNPs related to infectious and metabolic disease in a group of newly arriving Somali immigrants. SNPs studied included VDR SNPs (Bsm1, Taq1, Apa1, C621T, CTAG27307, and Fok1). SNPs of the mannose binding lectin gene and others were also studied.

VDR SNP in Somali Population

| SNP | Genotype | N (%) |
|---------------|----------|------------|
| VDR_Apa1 | CC | 40 (19) |
| | AC | 101 (48) |
| | AA | 70 (33) |
| VDR_Bsm1 | TT | 78 (40.2) |
| | TC | 97 (50) |
| | CC | 19 (9.8) |
| VDR_Taq1 | GG | 13 (12.8) |
| | AG | 55 (54.4) |
| | AA | 33 (32.6) |
| VDR_Fok1 | GG | 152 (75.2) |
| | AG | 46 (22.8) |
| | AA | 4 (2) |
| VDR_C621T | TT | 17 (8.7) |
| | CT | 81 (41.3) |
| | CC | 98 (50) |
| VDR_CTAG27307 | CC | 0 (0) |
| | CT | 2 (2) |
| | CC | 99 (98) |
| MBL_C169T | TT | 160 (82) |
| | CT | 35 (18) |
| | CC | 0 (0) |

Further studies are underway to determine relationships between these SNPs and metabolic and infectious diseases in Somali people. Somali immigration to the United States has increased dramatically over the past two decades and, thus, it is important to determine these relationships. A significant percentage of these new arrivals are immigrating to the Minneapolis-St. Paul area.

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- (2) Chiu KC et al., BMC Med Genet 2001;2:2.
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- (6) Oh JY et al., Metabolism 2002;51:356-9
- (7) Van Schooten FJ et al.,FASEB J 1998;12:1409-17
- (8) Štefaniæ M et al., Croat Med J 2005;46(4):639-646

Nothing to Disclose: AAM, AAK, SAJ, SN, TK, JVSP, AM, CO

P1-180

High Throughput and Highly Sensitive LC/MS/MS Assay for Quantification of 25-Hydroxy Vitamin D2 and 25-Hydroxy Vitamin D3 in Dried Blood Spots.

Suma Ramagiri PhD¹, Adam Latawiec MSc¹ and Robert Ellis PhD¹.

¹ABSCIEX Toronto, Canada.

Vitamin D is known for its role in prevention of wide range of diseases. Its deficiency can lead to childhood rickets, cardiovascular diseases, various forms of cancers, type I and II diabetes and even schizophrenia. Universally accepted means to monitor vitamin D deficiency is by quantitative measurement of 25-hydroxy vitamin D2 (25-OH Vit D2) and 25-hydroxy vitamin D3 (25-OH Vit D3) levels in serum, plasma or whole blood.

An LC/MS/MS assay specially focusing on pediatric and diabetic patients is desirable in dried blood spots, which is a more convenient sampling technique. Here, we have developed a simple, accurate and high throughput LC/MS/MS based assay to monitor 25-OH Vit D2 and 25-OH Vit D3 in dried blood spots with 3.0 mm and 6.0 mm punch sizes. The LC/MS/MS assay was developed on AB SCIEX QTRAP® 5500. Calibration curve ranging from 0.5 ng/mL-40 ng/mL was prepared in dried blood spots and evaluated for its linearity, accuracy and precision. 25-OH Vit D2-d6 was used as an internal standard. Two sample extraction methods were evaluated, one with simple protein precipitation using acetonitrile and the other with protein precipitation followed by liquid-liquid extraction using hexane. The current LC/MS/MS assay showed linearity ($R > 0.99$) with accuracy ranging from 80-120% and % CV below 15. Both extraction methods had good sample recovery (75-125%) but later showed ion suppression and matrix effects. Details of these experiments and results will be further discussed.

The above LC/MS/MS based assay quantifying simultaneously 25-OH Vit D2, 25-OH Vit D3 in dried blood spot looks promising and could open new opportunities in Vitamin D deficiency testing in neonatal screening and also in diabetic patients.

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2. Ravinder J. Singh, Robert L. Taylor, G. Satyanarayana Reddy, and Stefan K. G. Grebe; Clin Endocrinol Metab 91: 3055-3061, 2006

3. Rinaldo P, Tortorelli S, MaternD. Curr Opin Pediatr, 16: 427-33, 2004.

Nothing to Disclose: SR, AL, RE

P1-181

Quantification of Serum 25-Hydroxyvitamin D₂ and 25-Hydroxyvitamin D₃ Using High-Performance Liquid Chromatography, Tandem Mass Spectrometry (LC-MS/MS): Correlation with PTH and Other Assay Methods.

WA Salameh MD¹, BX Holmquist PhD¹, G Lee Ph.D¹, A Caston-Balderama Ph.D¹, X Huang¹, NJ Clarke Ph.D¹, K Zhang MD, PhD¹, E Reitz MD¹ and MF Holick MD, PhD².

¹Quest Diagnostics Nichols Inst San Juan Capistrano, CA and ²Boston Med Ctr Boston, MA.

Background: Much discussion abounds relating to measurement of 25-hydroxyvitamin D [25(OH)D]: 1) what concentration reflects 25(OH)D sufficiency; 2) does 25(OH)D₂ influence the level of parathyroid hormone (PTH) differently than 25(OH)D₃; 3) are there advantages to measuring 25(OH)D₂ and 25(OH)D₃ by LC-MS/MS; and 4) are there differences between LC-MS/MS and Diasorin RIA and Liaison[®] platform assays?

Methods: We developed an automated, liquid chromatography, tandem mass spectrometry (LC-MS/MS) method, which quantifies separately 25(OH)D₂ and 25(OH)D₃ in human sera. To explore impact of type of 25 (OH) vitamin D, age and sex on iPTH levels, we analyzed data from 169, 685 subjects in the Quest data repository. Accuracy of the LC/MSMS assay was determined by using NIST standards in 486 measurements across multiple Quest laboratories performing the assay. Results derived from this assay were also compared to those from another LC-MS/MS method, the Diasorin RIA, and the Liaison[®] platform assay.

Results: Data from the Quest repository showed 51,411 subjects had detectable 25(OH)D₂ and 25(OH)D₃ levels, while only 25(OH)D₂ was detected in 3,999 individuals. The relative proportions of 25(OH)D₂ and 25(OH)D₃ in the sample significantly impacted the PTH concentration at which maximum PTH suppression occurred. In addition there were statistically significant differences in iPTH plateau levels that were both gender and age dependent. Accuracy by NIST standard analysis showed 25(OH)D₃ level 1, 24.7 ± 2.7 (target: 23.9 ± 0.8); 25(OH)D₃ level 2, 12.8 ± 1.7 (target: 12.3 ± 0.6); 25(OH)D₃ level 3, 19.0 ± 2.1 (target, 18.5 ± 1.1); and 25(OH)D₂ level 3, 26.3 ± 2.2 (target 26.4 ± 2.0). The correlation between the two LC-MS/MS methods by Deming regression showed good agreement. Performance of the comparison of LC-MS/MS with the Diasorin RIA and Liaison methods showed a proportional bias indicating under-recovery of 25(OH)D₂ in both immunoassay methods. Similarly, Bland Altman analysis showed a negative bias in excess of 35% between our LC-MS/MS method and the Liaison method, again indicating under-recovery by the immunoassay.

Conclusions: Our data shed new insights on the relationship between type of 25(OH)D and PTH and indicate that age and gender may be important considerations when defining biochemical sufficiency. These data also highlight the need for harmonization between 25(OH)D assays to improve the accuracy of measurements in patients receiving vitamin D pharmacotherapy.

Disclosures: WAS: Researcher, Quest Diagnostics. GL: Researcher, Quest Diagnostics. AC-B: Researcher, Quest Diagnostics. XH: Researcher, Quest Diagnostics. NJC: Employee, Quest Diagnostics. KZ: Researcher, Quest Diagnostics. ER: Employee, Quest Diagnostics.

Nothing to Disclose: BXH, MFH

P1-182

A Simplified Workflow for the Clinical Analysis of 25-Hydroxyvitamin D₃ and 25-Hydroxyvitamin D₂ Using a Commercially Available Plasma Calibrator and Control Kit and LC/MS/MS.

AP Latawiec¹, R Ellis¹ and L Sapp¹.

¹AB Sciex Concord, Canada.

Liquid chromatography-tandem mass spectrometry (LC/MS/MS) is a powerful technique for the clinical determination of 25-hydroxyvitamin-D₃ and 25-hydroxyvitamin-D₂ in serum or plasma samples. LC/MS/MS also has the benefits of lower detection limits, greater specificity, and a broader dynamic range when compared to many commercial immunoassays. This paper evaluates the use of commercially available plasma calibrators and controls for the purpose of LC/MS/MS analysis. The off-line sample preparation is based on a simple pre-treatment step using a methanolic precipitation reagent. This is followed by a quick and simple on-line sample cleanup and high performance liquid chromatography tandem mass spectrometry to detect and quantify 25-hydroxyvitamin-D.

The LC/MS/MS assay showed excellent linearity over the concentration range with calibration regression coefficients ranging from 0.9994 to 0.9996. The intra-assay accuracy and precision (n=5) was less than 10%. Interassay accuracy ranged from 92-105% for the low level quality control samples, and %CVs ranging from 4-10%.

Nothing to Disclose: APL, RE, LS

P1-183

A 38 Year-Old Male with Recurrent Nephrolithiasis, Osteoporosis, and Hypervitaminosis D Associated with a Novel Mutation of *CYP24A1*.

MF Bouchonville MD¹ and PL Kapsner MD¹.

¹Univ of New Mexico Albuquerque, NM.

Background: Impaired catabolism of 1,25-dihydroxyvitamin D and defective bone mineralization have been demonstrated in animal models with targeted inactivation of 24-hydroxylase. However, this defect has not been previously described in humans.

Clinical Case: A 38 year-old male with a history of recurrent nephrolithiasis, hypercalciuria, hypercalcemia, and elevated 1,25-dihydroxyvitamin D levels without a history of calcitriol ingestion was referred to the Endocrinology and Metabolism department. Medical history was otherwise unremarkable. Biochemical evaluation revealed a calcium of 10.7 (8.4-10.4 mg/dL), normal phosphorus, normal creatinine, undetectable PTH level, 25-hydroxyvitamin D of 54 (10-55 ng/mL), 1,25-dihydroxyvitamin D of 160 (21-65 pg/mL), and 24-hr urine calcium of 405 (50-300 mg/24hr). Serum and urine protein electrophoresis, fibroblast growth factor-23, ACE, and PTH-related peptide levels were normal. Imaging studies including chest radiograph, CT abdomen, and a whole body gallium scan revealed no evidence of granulomatous disease. DEXA bone mineral density demonstrated a Z-score of -2.8 in the spine, -1.4 in the hip, and -2.8 in the distal radius. Consideration was given to a defect in the enzymes involved in vitamin D metabolism and the patient was referred to the NIH Section of Undiagnosed Diseases for further evaluation. The inactive catabolite 24,25-dihydroxyvitamin D was found to be low at 0.39 (1-2 pg/mL) and subsequent sequencing of *CYP24A1* (24-hydroxylase) revealed a 3-base pair deletion in the coding region of exon 2. This mutation has not been previously described but may be resulting in a loss of function defect of 24-hydroxylase.

Conclusion: Emerging evidence suggests that regulation of 1,25-dihydroxyvitamin D levels occurs locally in bone. Maintenance of appropriate concentrations of 1,25-dihydroxyvitamin D at the local level may play an important role in normal bone function. We present a novel mutation in *CYP24A1* (24-hydroxylase) and resulting clinical sequelae which emphasize the relevance of local regulation of 1,25-dihydroxyvitamin D levels.

Nothing to Disclose: MFB, PLK

P1-184

Congenital Rickets Due to Mutation of the CYP2R1 Gene Causing Selective Vitamin D 25-Hydroxylase Deficiency in a Saudi Family.

Angham N MD AIMutair MD¹, Daphne D.phD Head PhD² and David WphD Russell PhD².

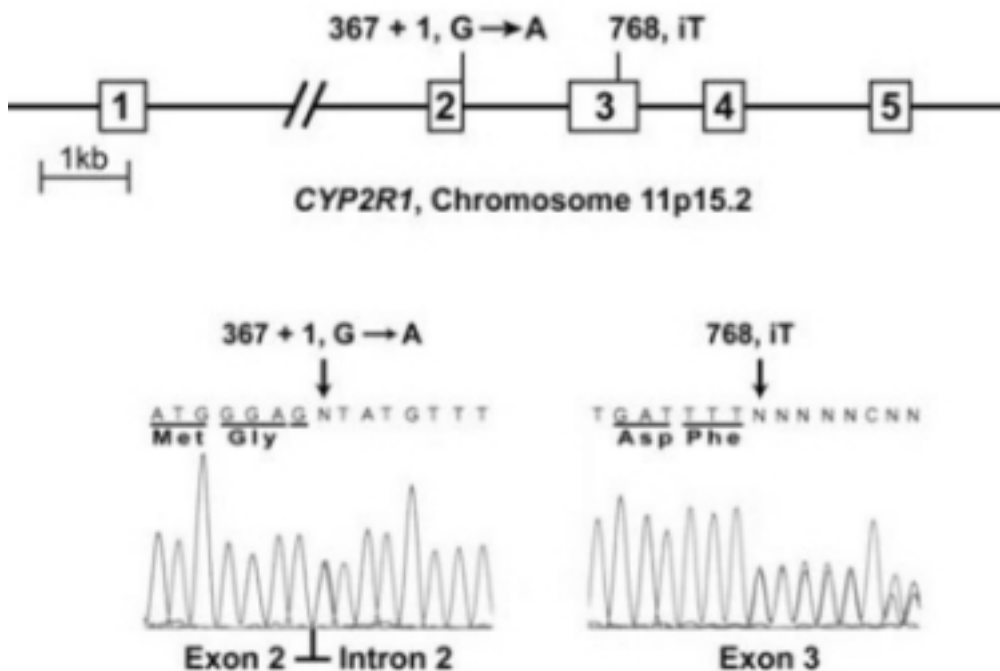
¹King Abdulaziz Med City Riyadh,, Saudi Arabia and ²UT Southwestern Med Ctr Dallas, TX.

BACKGROUND: Vitamin D (Vit D) deficiency rickets remains prevalent in some countries like Saudi Arabia(1). Inherited mutations causing this disease have been identified in several genes, including those encoding the Vit D receptor and two cytochrome P450s that hydroxylate Vit D. The identity of the Vit D 25-hydroxylase has been controversial; however, recently a homozygous mutation in exon 2 of the CYP2R1 gene on chromosome 11p15.2, which specifies a hepatic Vit D 25-hydroxylase, was identified in members of two Nigerian families and shown to cause selective 25-hydroxyVit D₃ deficiency (2,3).

AIMS : We present two patients from a Saudi family with a clinical picture of severe rickets that started in childhood and improved on Vit D therapy but relapsed when Vit D therapy was stopped. DNA sequencing revealed that both patients were compound heterozygotes for two previously undescribed mutations in the CYP2R1 gene.

METHODS: Here the clinical scenario and lab will be presented

RESULTS : DNA sequencing of the five exons in the CYP2R1 gene revealed two mutations in the affected siblings. One mutation was a G to A transition in the splice donor sequence of intron 2; this change is predicted to disrupt splicing of the mRNA transcribed from the mutant allele. The second mutation was a T insertion in the coding sequence of exon 3; this change disrupts the translational reading frame and is predicted to produce a truncated CYP2R1 protein. Both patients are compound heterozygotes for these mutations. Although we have not reproduced these mutations in an expressible gene, both lesions are predicted to inactivate CYP2R1 causing Vit D 25-hydroxylase deficiency.



CONCLUSIONS: Although we report only the third family in which mutation of the CYP2R1 gene is associated with selective Vit D 25-hydroxylase deficiency, it seems likely that this disorder is under-diagnosed. A similar molecular basis should be considered in patients with a clinical picture of chronic 25-hydroxy Vit D deficiency rickets with a childhood presentation who respond to Vit D treatment but relapse once off treatment. This diagnosis should be given further consideration in Vit D deficiency endemic area.

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- (2) Cheng J.B et al., Proc. Natl. Acad. Sci.; 2004; 101:7711-7715
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Nothing to Disclose: ANA, DDH, DWR

P1-185

Familial Tumoral Calcinosis: Challenges in the Diagnosis and Management of Two Brothers.

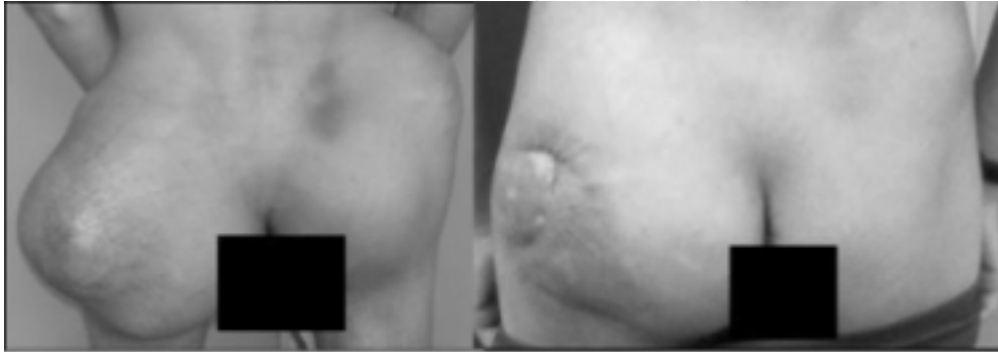
D Espindola-Antunes PhD¹, CL Nascimento MD², LB Araujo MD², RBN Anjos MD², PN Rabelo medical student¹, M Rassi-Cruz medical student¹, SA Conceicao medical student¹ and FS Meirelles MD³.

¹Fed Univ of Goias Goiania, Brazil ; ²Gen Hosp of Goiania Goiania, Brazil and ³Emergency Hosp of Goiania Goiania, Brazil.

Background: Familial tumoral calcinosis (FTC) is a rare condition characterized by massive soft tissue deposit of calcium phosphate predominantly periarticular. Mutations in the FGF23 fosfatonin and in the GALNT3 gene lead to hyperphosphatemia owing to enhanced renal phosphate retention, and inappropriately normal or high calcitriol.

Case 1: A 22-year men presented with soft consistency tumors in hips, sacrum and elbows. He underwent several subsequent surgeries for recurrence of the lesions, pathologic reported as "calcinosis with xantogranulomas". One of his seven brothers had similar clinical picture. Physical exam: BMI= 18,46 kg/m², extensive lesion in the left iliac crest (15 cm in longest axis) and smaller tumors in the sacrum and elbows. Initial laboratory showed phosphorus (P)= 6 mg/dl (2.5-4.5 mg/dL), calcium (Ca)= 9.5 mg/dL (8,8-11 mg/dL), PTH= 13.6 pg/mL (10-65 pg/ml) and calcitriol= 59.9 pcg/ml (16-60 pcg/mL), consistent with FTC. Patient was prescribed with hypophosphatemic diet and aluminum hydroxide, later associated with acetazolamide. Despite significant clinical improvement and calcitriol decrement in 2 years of pharmacological treatment, the phosphatemia remained high.

Case 2: Brother, 24 years old. First lesions occurred at age of 14 in the right elbow, which were surgically resected, but recurred within 1 year. New lesions developed in the hip at age of 22, limiting movements. Physical examination: BMI= 17,71Kg/m², pale (2+/4+), HR= 124 bpm, tumors in the left elbow, first and fifth right fingers and large bilateral lesions in hips (10 cm in longest axis). Initial laboratory: P= 5.7 mg/dL, Ca= 10.2 mg/dL, PTH= 5.0 pg/mL and calcitriol= 57.3 pg/mL. Patient was hospitalized for surgical treatment of the hip lesions; however it was not performed due to significant and fast volume reduction with pharmacological treatment. After 1 year using aluminum hydroxide and acetazolamide, lesions disappeared, general state improved, but the phosphatemia also remained high.



Patient 2 before and after pharmacological treatment.

Conclusion: FTC represents a diagnosis challenge, despite the exuberant clinic. There is no specific treatment available and long term lesions recurrence is frequent.

Nothing to Disclose: DE-A, CLN, LBA, RBNA, PNR, MR-C, SAC, FSM

P1-186

A Novel *GNAS* Mutation in an Infant Boy with Pseudohypoparathyroidism Type Ia and Normal Calcium and Phosphate Levels.

MTA Reis¹, A Cattani², C Castillo², BB Mendonca MD, PhD¹, PHS Correa MD, PhD¹ and RM Martin MD, PhD¹.

¹HC-FMUSP Sao Paulo, Brazil and ²Pontificia Univ Catolica de Chile Santiago, Chile.

Background: Pseudohypoparathyroidism type Ia (PHP Ia) is a rare genetic disorder characterized by the association of multi-hormonal resistance and clinically abnormal features, called Albright's hereditary osteodystrophy (AHO). This condition is an inherited disease and results from heterozygous loss of function mutation within the *Gsα* gene (*GNAS*).

Clinical case: An 8-month-old boy was admitted to the Endocrinology Unit of Universidad Catolica in Chile due to obesity and low growth velocity. His laboratory profile showed normal calcium (9.8 mg/dL, NV = 8.8 - 10.8) and phosphate (6.6 mg/dL, NV = 5.0 - 10.8), high PTH levels (132.1 pg/mL, NV = 11 - 62) and elevated TSH (18.8 uU/mL, NV = 0.8 - 6.3). Noteworthy, his mother exhibited typical AHO phenotype such as short stature, round face, short neck and brachydactyly. In addition, she had paresthesia, hypocalcemia (Ca = 7.6 mg/dL, NV = 8.8 - 10.2), upper limit phosphate levels (P = 4.6 mg/dL, NV = 2.3 - 4.6), high PTH levels (691 pg/mL), hypothyroidism and basal nucleus calcification at cranial CT scan.

The clinical diagnosis of PHP Ia was established and both mother and son genomic DNAs were submitted to molecular analysis. The *GNAS* coding region was directly sequenced and a novel heterozygous missense mutation I106T was identified in exon 5 in both patients. The codon 106 is located within the helical domain of *Gsα* protein which is functionally important for adenylyl cyclase activation. Moreover, another missense mutation was previously described in the same codon (I106S). Both patients were treated with thyroxin, vitamin D and calcium supplements, which led to control of symptoms and laboratorial testing.

Conclusion: In this family, clinical picture of the mother was the clue for the son's diagnosis. The molecular analysis of *GNAS* confirmed the diagnosis of PHP Ia in both patients and precocious diagnosis of the child was possible. Moreover, this novel missense substitution expands the spectrum of *GNAS* mutations associated with this disorder and allows a genetic counseling for this family.

Nothing to Disclose: MTAR, AC, CC, BBM, PHSC, RMM

P1-187

Novel PHEX Nonsense Mutation in a Patient with Familial Hypophosphatemic Rickets.

T Kienitz¹, M Ventz¹, E Kaminsky² and M Quinkler¹.

¹Charité Univ Med Berlin, Campus Mitte Berlin, Germany and ²Lab for Molecular Genetics Hamburg, Germany.

Introduction: The most common form of familial hypophosphatemic rickets is X-linked and caused by a defect in renal phosphate transport. PHEX has been identified as the gene defective in X-linked hypophosphatemic rickets. It is located on Xp22.1 and several inactivating mutations of PHEX are known so far. The defects lead to phosphate wasting, hypophosphatemia and inappropriate concentrations of 1,25-dihydroxyvitamin D in regard to hypophosphatemia. Clinical manifestations of the disease are skeletal deformities, short stature, osteomalacia, dental abscesses, bone pain, and loss of hearing.

Clinical characteristics: We report three cases of hypophosphatemic rickets. Two patients are female (55 and 24 years old), one is male (49 years old). Age at diagnose ranged from early childhood to the age of 35 years. One patient underwent hip replacement at the age of 41 due to secondary arthritis caused by coxa vara. Two patients have a mild calcification of kidney tissue. One patient suffers from loss of hearing since the age of 17. All three patients have been treated with phosphate supplements, two patients receive 1,25-dihydroxycholecalciferol for treatment of secondary hyperparathyroidism. Under this regimen blood levels of calcium, phosphate and parathyroid hormone are in normal range. In all patients at least one family member is affected by rickets, as well.

Genetical analysis: Genetic mutational analysis of the PHEX gene was performed in each patient. In both female patients known mutations were found: c.682delTC (exon 6, codon 228) and c.1952G>C (exon 19, codon 651, R651P). However, in one patient an unknown nonsense mutation was found in exon 7, codon 245 (c.735T>G, Tyr245Term, Y245X).

Conclusions: In hypophosphatemic rickets genetic testing of patients and family members is needed to guarantee early diagnosis and treatment. We report a novel nonsense mutation of PHEX that has not been identified so far.

Nothing to Disclose: TK, MV, EK, MQ

P1-188

Osteogenesis Imperfecta and Nephrolithiasis in a Young Adult.

NE Speil MD¹, P Jayaramaiah MD¹, S Gupta MD¹, CM Fratianni MD¹ and R Khardori MD, PhD¹.

¹Southern Illinois Univ Sch of Med Springfield, IL.

Osteogenesis Imperfecta (OI)- a heritable disorder of bone formation is characterized by low bone mass and bone fragility. At present eight subtypes have been identified and molecular diagnosis is possible through direct sequencing of Col1A1, Col1A2, CRTAP or LEPRE1 genes in majority of cases. However, a certain percentage of patients are classified/identified on the basis of clinical characteristics alone. Types IV and V OI fall in this latter category and patients are often not diagnosed because of a low index of suspicion. Several extraskeletal features suggest the diagnosis: blue sclera, dentinogenesis imperfecta, hyperlaxity of the skin, hypermobility of the joints. Calcitropic hormones, serum calcium and phosphate levels are often normal. Hypercalciuria and nephrolithiasis have been reported in 5 pediatric cases previously in the English literature and none in an adult as a primary event.

Clinical case: We describe herein a 22 year old female who was referred to our clinic for recurrent episodes of nephrolithiasis and fragility fractures since age 5.

Patient underwent a complete endocrine evaluation and all her hormonal studies were essentially normal. The patient has a younger brother who also has a history of nephrolithiasis. We have seen the patient suffer one fracture (metacarpal fracture) on hyperextension of her finger and she continues to have episodic nephrolithiasis. Her 24 hour urine calcium is 92mg/24 hours (normal up to 150). She has no evidence of hypovitaminosis D with 25OH D 50 ng/ml (normal 30-80); no hyperparathyroidism with iPTH 23 pg/ml (normal 11-50). There is no evidence of altered bone turnover with serum Osteocalcin 33 ng/ml (normal 11-50). No evidence of renal insufficiency or metabolic acidosis. Stone analysis revealed calcium oxalate stones.

Our final assessment leads us to believe that our patient has Type VI OI (Hyperosteoridosis) and bone biopsy would need to confirm any osteomalacia. Genotyping is likely to be negative for any mutation.

Conclusion: The association of nephrolithiasis with OI has not been previously reported in adults. Whether this is a primary abnormality associated with OI or an independent unrelated disorder remains to be characterized. Since association between OI and nephrolithiasis has been reported in children before, we are more inclined to associate it with the OI per se.

Nothing to Disclose: NES, PJ, SG, CMF, RK

P1-189

Siblings with Pseudohypoparathyroidism Type 1B.

Sangeetha Pillai M.D¹ and Pratima Kumar M.D¹.

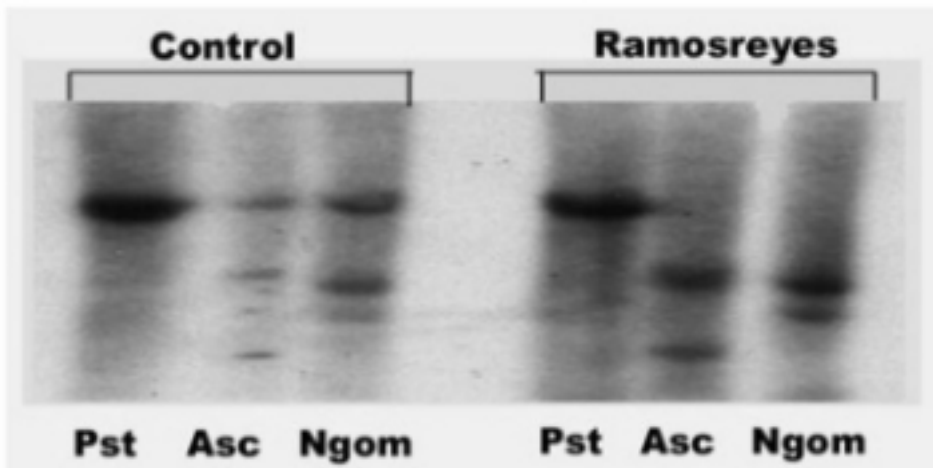
¹Univ of Texas Southwestern Austin Programs Austin, TX.

Background:

Although pseudohypoparathyroidism 1B (PHP1B) is rare, it should be suspected in young patients who present with seizures and hypocalcemia.

Clinical Case:

A 19 year-old girl presented with seizures and numbness in her hands. She had been on antiepileptics since age 11. Her corrected serum calcium was 5.3 (8.5-10.5 mg/dL), phosphorus was 6.7 (2.5- 4.6mg/dL), iPTH level was 126 (12-88 pg/mL) and TSH was 1.2 (0.34-5.60 mU/L). She had absence of AHO phenotype including short 4th metacarpals. She was treated with intravenous calcium and subsequently with calcitriol. Mutation testing for AHO was negative. Diagnosis of PHP1B was confirmed by Southern blot analysis using methylation sensitive restriction enzymes.



Her 23 year-old sister also informed us of her past seizure history. Her corrected serum calcium was 6.4 mg/dL, phosphorus was 6.7 mg/dL, iPTH was 190 pg/dL and TSH was 58 mU/L. She also lacked AHO phenotype with negative mutation testing thus suggesting PHP1B. The Southern blot analysis will confirm her diagnosis.

Discussion:

PHP1B is characterized by hypocalcemia and hyperphosphatemia due to renal PTH resistance without characteristic AHO phenotype. There is involvement of the α -subunit of stimulatory G-protein ($G_s\alpha$), a signaling protein necessary for PTH action. (1) $G_s\alpha$ is encoded by GNAS gene located on chromosome 20q13. In PHP1B, there is GNAS imprinting defect with loss of maternal imprinting (methylation) of exon 1A differentially methylated region (DMR) with both alleles having a paternal imprinting pattern. (2) Mild TSH resistance has also been noted in half of these patients. In familial PHP1B, microdeletions in STX16 gene located upstream of GNAS lead to exon 1A imprinting defect and PTH resistance when inherited maternally.

Conclusion:

It is important to assess patients who are diagnosed with seizures for hypocalcemia as calcium supplementation will be required to prevent seizures in the future.

If serum testing suggests pseudohypoparathyroidism, further history should be obtained regarding the patient's siblings. Mutation testing could then be considered for the patient and their first- degree relatives.

(1) Kozasa et al.1988. Isolation and characterization of the human Gs alpha gene. Proc Natl Acad Sci USA 85: 2081-2085

(2) Weinstein LS et al. 2001. Endocrine manifestations of stimulatory G protein α -subunit mutations and the role of genomic imprinting. Endoc Rev 22:675-705

Nothing to Disclose: SP, PK

P1-190

Management of Two Brazilian Cases of Gorham-Stout Disease.

D Iguchi¹, DVS Aranha¹, RM Martin¹ and PHS Correa¹.

¹Hosp das Clins, Univ of Sao Paulo Med Sch Sao Paulo, Brazil.

Gorham-Stout disease (GSD) is a rare disorder characterized by spontaneous and progressive osteolysis of one or more skeletal bones which is probably induced by abnormal lymphatic vessels proliferation. No standardized treatment of GSD has been established since the cases are scarce and its pathogenetic mechanisms are poorly understood. Bisphosphonates (BPs) are used based on evidence of enhanced pathological bone resorption. As GSD can have an unpredictable course, a marker to assess the disorder activity should be a useful tool for its management. Some authors consider that IL6 may have this role because it is a cytokine involved in formation, differentiation and activation of osteoclasts. Moreover, a previous report showed a GSD patient who had high IL6 levels that normalized after BP therapy followed by clinical and radiological stabilization of disease. **Case 1:** A 62-year man reported progressive mandibular bone loss, leading to difficulty in chewing and swallowing which started 12 yrs ago. Titanium dental implant and 3 bone grafting procedures were performed without improving or stopping the mandibular bone destruction. His dentist referred him to us because he considered BP treatment before a new dental implant. Although the patient was asymptomatic, he received IV zoledronic acid (ZA) but unfortunately he gave up underwent the procedure. **Case 2:** A 35-year woman informed a lower-right chest pain associated to chronic cough. The pain gradually increased in the last year when she noticed a nodule on the right lateral chest wall. Due to the possibility of being a malignant lesion, an oncological resection was done removing 8th, 9th and 10th ribs, without pain improvement. The histology of affected ribs was consistent with GSD. Bone scintigraphy revealed evolution of the disease to the upper ribs of her right hemithorax. Since GSD may be potentially lethal, especially if the disease is complicated by thoracic involvement and chylothorax, a recent infusion of ZA was administrated to her in order to stop the progression of lesions and to promote pain relief. In addition, IL6 was assessed in both patients. Patient 1 had normal IL6 levels which remains unchanged after BP therapy. On the contrary, patient 2 had normal IL6 levels despite the fact of active GSD. **Conclusion:** Even though IL6 may be considered as a potential marker of active GSD, the normal IL6 values in the patient 2 was not in accordance to expected and other markers can be considered.

Nothing to Disclose: DI, DVSA, RMM, PHSC

P1-191

Transient Elevation of Serum PTH in a Neonate with Mucopolidosis II; and Report of a Novel Mutation in the UDP-G1cNAc 1-Phosphotransferase Gene.

CA Leyva MD¹ and T Seeherunvong MD¹.

¹Univ of Miami Sch of Med Miami, FL.

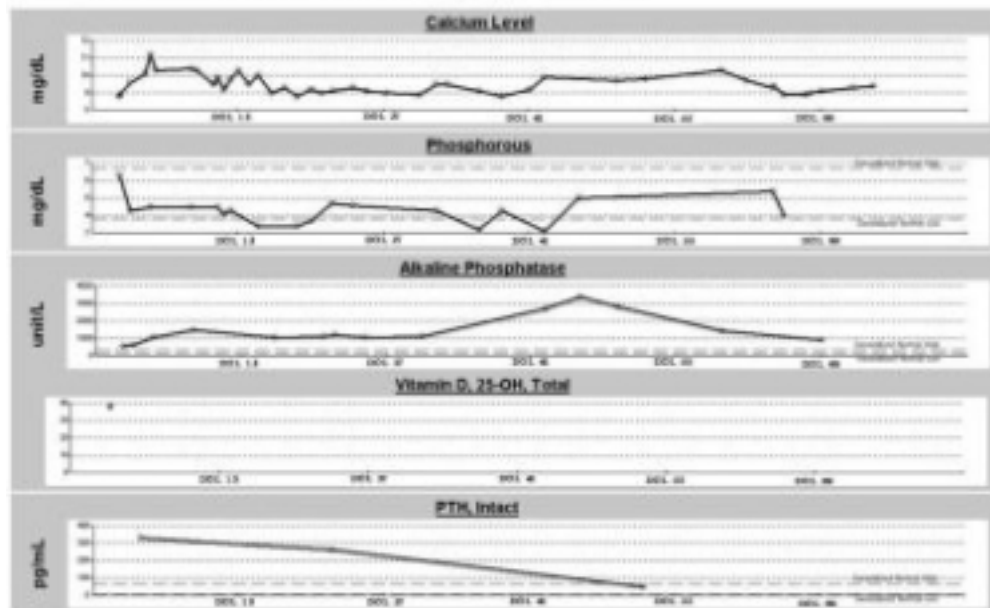
INTRODUCTION:

Mucopolidosis II alpha/beta (ML II, OMIM# 252500) is an autosomal recessive condition caused by a mutation in the UDP-G1cNAc 1-phosphotransferase gene. Most patients are ascertained in the newborn period because of typical Hurler-like physical features. In rare cases, hyperparathyroidism may also be present (1, 2).

CLINICAL CASE:

We present a male subject who was born at 30 weeks gestation with a birth weight that was appropriate for gestational age. The baby had gingival hyperplasia, low-set and posteriorly rotated ears, flat nasal bridge, decreased muscle tone and bilateral contracture of fingers and toes. Skeletal survey indicated severe demineralization of bones, brachycephaly, multiple fractures, metaphyseal irregularities in the long bones, punctuate calcifications in calcaneus and orbits, and hypoplasia of pubic bones. Figure 1 indicates the results of biochemical evaluation. Initially the patient had normal levels of serum calcium, but low-normal phosphorous. There was a marked elevation of serum alkaline phosphatase, but normal liver enzymes and serum creatinine. The serum level of intact PTH was abnormally elevated, but returned to normal by the seven week of life. Serum level of 25-hydroxyVitamin D was normal. Elevated plasma, but normal leukocyte levels of UDP-G1cNAc 1-phosphotransferase suggested a diagnosis of MLII. DNA sequence analysis of the GNPTAB gene identified two frameshift mutations including a novel mutation [c.2896delA (p.M966fsX1002)] in exon 14 and a mutation in exon 19 [c.3503_3504delTC (p.L1168fsX1172)] that has been reported. The patient died at 2 months of age from respiratory failure. Autopsy indicated collections of PAS-positive foamy histiocytes in multiple organs.

Figure 1: Biochemical evaluation of a subject with mucopolidosis II



CLINICAL LESSON:

MLII should be considered in neonates presenting with metabolic bone disease, marked elevated serum levels of serum intact PTH but normal serum calcium, and multiple dysmorphic features including gingival hyperplasia. Early diagnosis is important to identify patients for therapy and to provide genetic counseling to the family.

(1)Unger S, Paul DA, Nino MC, et al. Mucopolidosis II presenting as severe neonatal hyperparathyroidism. *Eur J Pediatr*. 2005;164(4):236-243.

(2)Sathasivam A, Garibaldi L, Murphy R, Ibrahim J. Transient neonatal hyperparathyroidism: a presenting feature of mucopolidosis type II. *J Pediatr Endocrinol Metab*. 2006;19(6):859-862.

Nothing to Disclose: CAL, TS

P1-192

A Novel K32X Mutation of the *GNAS* Gene in a Korean Family with Pseudohypoparathyroidism Type Ia.

ST Ji PhD¹, YB Sohn PhD¹, SW Park PhD¹, SJ Kim PhD¹ and DK Jin PhD¹.

¹Samsung Med Ctr, Sungkyunkwan Univ Sch of Med Seoul, Korea.

Background: Pseudohypoparathyroidism (PHP, OMIM 103580) consists of a heterogeneous group of endocrine disorders, with the common feature of resistance to PTH that manifests by hypocalcemia, hyperphosphatemia, and elevation of serum PTH despite normal renal function. Herein, we describe the first Korean cases with PHP-Ia, confirmed by clinical, biochemical and genetic studies.

Methods: A 6-year-old female and a 7-year-old male evaluated. The two patients had the typical signs of Albright hereditary osteodystrophy. We performed standard laboratory tests as well as DNA studies. Genomic DNA was isolated from peripheral blood leukocytes. The *GNAS* gene was amplified by PCR and bidirectional sequencing analysis was performed of all coding exons.

Results: The patients with suspected PHP-Ia, had characteristic clinical signs and radiological findings. Two *GNAS* mutations were found: c. 94A>T and c.344_345insT. Patient 1 had a nonsense mutation of c. 94A>T (p.K32X), which has not been previously described; the patient's mother also had the c.94A>T, and therefore was a familial mutation. Patient 2 had a known frame shift mutation for c.344_345insT (p.V117RfsX23); the family members of patient 2 had wild-type sequences.

Conclusions: Here we describe the first Korean patients confirmed to have PHP-Ia by genetic analysis, and identified a novel p.K32X mutation in the *GNAS* gene.

Nothing to Disclose: STJ, YBS, SWP, SJK, DKJ

P1-193

Vitamin D Dependent Rickets Type 1 Due to a Novel Mutation in CYP27B1 Gene in a 16-Month-Old Male Presenting with Severe Proximal Muscle Weakness, Renal Tubular Dysfunction and Failure to Thrive.

Yun Yan MD.¹, Elizabeth Fudge MD.¹, Ali Calikoglu MD.¹ and Nina Jain MD.¹.

¹Univ of North Carolina Chapel Hill, NC.

Vitamin D-dependent rickets type 1 (VDDR1) is an autosomal recessive disorder caused by an inactivating mutation of the 25-hydroxyvitamin D 1-alpha-hydroxylase (CYP27B1) gene. Clinical presentation is characterized by early onset of severe rickets with hypocalcemia. In addition to radiological findings of severe rickets, laboratory studies typically reveal normal or mildly elevated circulating 25(OH)-vitamin D3, low 1, 25(OH)-vitamin D3 levels, elevated parathyroid hormone and alkaline phosphatase, decreased serum calcium and phosphorous levels.

Here, we report a 16-month-old Caucasian male who presented with failure to thrive, severe muscle weakness, and renal tubular dysfunction with aminoaciduria and profound organic aciduria in addition to skeletal deformities, osteopenia and multiple fractures. At presentation, he had severe hypocalcemia 5.1 mg/dl(9.0-11.0), hypophosphatemia 3.7 mg/dl(4.5-6.7), high alkaline phosphatase 2459 U/L(145-320) and PTH levels 410 pg/ml(12-72). His 25(OH) vitamin D levels was normal 60 ng/ml(25-80) and 1,25(OH)₂ vitamin D level was in the normal low range 32 pg/ml(24-86) after 2 doses of calcitriol. He also had mild hyperchloremic metabolic acidosis [CO₂ 17 mmol/l(22-30) and chloride 112 mmol/l(98-107)]. Urine analysis showed very elevated glutaric and succinic acid levels and generalized aminoaciduria. DNA sequencing of CYP27B1 revealed a novel 15 base pair deletion in exon 2 (c.286_300del15) and a previously reported single base mutation in exon 7 (c.1166G>A). Once calcitriol therapy was initiated, the patient showed significant improvement in muscle strength and linear growth. Serum calcium, phosphorous, and alkaline phosphatase returned to normal range. Organic aciduria was resolved and aminoaciduria significantly improved 2 months after parathyroid hormone levels normalized.

While VDDR1 is a rare disease, it should be considered in patients presenting with clinical features of rickets but with a normal or elevated 25(OH)-Vitamin D level. Also, severe hyperparathyroidism secondary to hypocalcemia and defects in vitamin D action appear to cause reversible renal tubular dysfunction.

Nothing to Disclose: YY, EF, AC, NJ

P1-194

CRFR2 Modulation of Stress-Induced Behavioral Adaptations: Site-Specific Over-Expression of Urocortin 3 in the Limbic System.

A Neufeld-Cohen¹, A Chen¹, JR Seckl², W Vale³ and PM Jamieson².

¹Weizmann Inst of Sci Rehovot, Israel ; ²Queens Med Res Inst, Univ of Edinburgh Edinburgh, UK and ³Salk Inst for Biological Studies La Jolla, CA.

The CRF receptor type 2 (CRFR2) plays a role in regulating neuroendocrine and behavioral responses to stressors, and its close anatomical association with major terminal fields of Urocortin 3 (Ucn 3) in forebrain limbic structures suggests the involvement of Ucn 3 in these processes. To study the role of Ucn 3/CRFR2 in behavioral stress responses, we used mouse models overexpressing Ucn 3 broadly under the control of the ubiquitous murine ROSA26 promoter (UCN3+) or specifically in defined limbic structures using Ucn 3-expressing lentiviruses. UCN3+ mice overexpressed Ucn 3 extensively throughout the brain, as determined by ISH and RIA. For site-specific overexpression, we designed and generated Ucn 3 lentiviruses, and verified their production of Ucn 3 in vitro and in vivo using RIA, IHC and ISH. Ucn 3 or control (GFP-expressing) lentiviruses were delivered by bilateral intracerebral injection 2 weeks prior to behavioral analyses, which were carried out in group-housed adult male mice under basal conditions or immediately following an acute restraint stress. Under basal conditions, UCN3+ mice showed increased anxiety-like behavior in the elevated plus maze, with decreased open arm entries and time spent on the open arms of the maze as compared to wild-type littermates. In the tail suspension test, UCN3+ mice also showed increased immobility indicative of increased depressive-like behavior. To examine the potential contribution of specific CRFR2-expressing brain nuclei to this phenotype, the lateral septum (LS) or bed nucleus of the stria terminalis (BNST), limbic sites with high levels of CRFR2, were targeted by lentiviral overexpression of Ucn 3. In contrast to UCN3+ mice, mice overexpressing Ucn 3 in LS showed decreased depressive-like behaviors suggesting that the LS may not be the primary site of CRFR2 mediating altered stress-related behaviors in UCN3+ mice, or that chronic adaptations in these mice are responsible for their behavioural phenotype. Neither UCN3+ mice, nor mice overexpressing Ucn 3 in LS or BNST showed differences in stress-related behaviours following restraint, suggesting heightened anxiety in both control and Ucn 3 overexpressing mice following the stressor that may have reached a threshold masking the differences between the experimental groups. Further site-specific manipulation of the Ucn 3/CRFR2 system will enable us to further explore its contribution to the physiological and pathophysiological responses to stress.

Sources of Research Support: For PMJ, AC and JRS, work was supported by the Wellcome Trust. For AC, WV and PMJ work was supported by NIH Program Project Grant DK 026741 and in part by the Clayton Medical Research Foundation Inc. WV is a Senior CMRF Investigator.

Nothing to Disclose: AN-C, AC, JRS, WV, PMJ

P1-195

An Anxiety-Like Phenotype Is Present in Prkar1a Heterozygote Knock-Out Mice.

MF Keil MS, CRNP^{1,2}, N Gokarn BS¹, G Briassoulis MD¹, M Nesterova PhD¹, TJ Wu PhD² and CA Stratakis MD, DmSci¹.

¹NICHD, Natl Inst of Hlth Bethesda, MD and ²Uniformed Services Univ of the Hlth Scis Bethesda, MD.

The role of the cAMP/PKA signaling in the molecular pathways involved in fear and memory is well established. Prior studies in our lab reported that transgenic mice with a down-regulated Prkar1a gene (antisense transgene) (Griffin, 2004) exhibited behavioral abnormalities including anxiety (Batista, 2005) and depression (Batista, 2006). Several research groups have reported CNS effects in PKA knock-out(KO) mouse models for R1beta, R2alpha, and R2beta, as well as catalytic unit knockouts. A KO mouse heterozygous for a null allele of Prkar1a was developed in our lab as a model to investigate Carney complex (Kirschner, 2005). In the present study, we examined the role of altered PKA signaling on anxiety-like behaviors in these mice (KO) compared to wild-type (WT) littermates. The elevated plus maze (EPM) and marble bury (MB) tests were used to assess anxiety-like behavior. The hotplate test was performed to evaluate analgesia. The MB test is widely used to evaluate anxiolytic compounds and has also been characterized as a model of compulsive behavior. The EPM is widely used to assess anxiety-like behavior in rodents as well as motor activity in a novel environment. Results for the MB test showed a significant effect, with increased anxiety-like behavior in both male ($p < 0.05$) and female ($p < 0.05$) KO mice, compared to same sex WT littermates. MANOVA analysis was performed to investigate genotype differences in anxiety-like behavior in the EPM (dependent variables: open arm, closed arm, & open to-total time ratio). A significant difference was found between KO and WT on combined dependent variables ($F(3,633)$, $p = 0.019$; Wilks' $\lambda = 0.827$; partial $\eta^2 = 0.173$). There was also a difference ($p < 0.04$) between KO and WT mice for open arm time in both males (11.8 ± 7 vs. 26 ± 7 sec., respectively) and females (7 ± 9 vs. 24 ± 7 sec., respectively), and a trend noted for increased ($p < 0.07$) closed arm time in KO mice when compared to WT of their respective gender. Results of hotplate testing showed no genotype effect however; the expected sex difference was noted, with females showing a longer latency ($p < 0.05$) to paw-lick than males. We conclude that alterations in PKA signaling, as those that have been described in the Prkar1a mouse model, result in specific neurobiological modifications of behaviors involved in anxiety and fear sensitization in mice.

Griffin KJ, et al., *Endocr Res.* 2004,(4):903-11.

Batista DL, et al., *Endocrine Society Abstracts*, 2005.

Batista DL, et al., *Endocrine Society Abstracts*, 2006.

Kirschner LS, et al., *Cancer Res.* 2005,65(11):4506-14.

Nothing to Disclose: MFK, NG, GB, MN, TJW, CAS

P1-196

Corticotrophin-Releasing Factor Mediates the Hypophagia after Adrenalectomy through an Increased Satiety-Related Responses.

ET Uchoa MSc¹, J Antunes-Rodrigues MD, PhD¹ and LLK Elias MD, PhD¹.

¹Univ of Sao Paulo, Sch of Med of Ribeirao Preto Ribeirao Preto, Brazil.

Adrenalectomy-induced hypophagia is associated with an increased satiety-related responses, which involves neuronal activation of nucleus of the solitary tract (NTS) in the brainstem. Besides its effects on pituitary-adrenal axis, corticotrophin-releasing factor (CRF) has been shown to play an important role in the feeding behavior, possessing anorectic effects. Thus, in the present study, we evaluated the effect of intracerebroventricular (icv) pretreatment with CRF₂-receptor antagonist, antisauvagine 30 (AS30), on food intake and activation of NTS neurons in response to feeding in adrenalectomized (ADX) rats with and without corticosterone (B) replacement. Seven days before the experiments, anesthetized male Wistar rats (200-250g, n=5-8 per group) had a stainless cannula implanted in the lateral ventricle and were subjected to ADX or sham surgery. ADX animals received 0.9% NaCl in the drinking water, and half of them received corticosterone in the drinking water (B: 25mg/l, ADX+B). Six days after surgery, animals were fasted for 16 hours and they were pretreated with icv injection of AS30 (5 µg/5 µl) or vehicle (0.9% NaCl/ 5µl). Food intake was determined 2h after icv injections. Another set of each group of animals, was transcatheterically perfused, before or 2 hours after refeeding for brain tissue collection and immunohistochemical localization of Fos and double-labeled for tyrosine hydroxylase (TH) to identify catecholaminergic cells in the NTS. ADX animals pretreated with vehicle showed lower food intake, compared with sham and ADX+B groups. Pretreatment with AS30 did not affect the amount of food intake in sham and ADX+B groups; however, remarkably it reversed the hypophagia in the ADX group. In fasted animals, the number of Fos and Fos/TH neurons were similar in all vehicle and AS30 groups. In vehicle pretreated animals, refeeding increased ($P<0.05$) Fos expression and catecholaminergic neuronal activation in the NTS in sham, ADX and ADX+B animals, with higher ($P<0.05$) Fos expression and Fos/TH labeled neurons in the ADX group. Similar to its effect on food intake, pretreatment with AS30 in the ADX group also reversed the increased activation of NTS neurons induced by refeeding. In conclusion, these data demonstrated that CRF₂-receptor antagonist abolishes the increased activation of NTS induced by feeding in ADX animals, indicating that hypothalamic CRF neurons contribute to the higher satiety-related responses induced by adrenalectomy.

Sources of Research Support: FAPESP, CNPq, FAEPA.

Nothing to Disclose: ETU, JA-R, LLKE

P1-197

Neonatal LPS Injection Alters the Body Weight Regulation Systems of Rats under Non-Stress and Immune Stress Conditions.

T Matsuzaki MD, PhD¹, T Iwasa MD, PhD¹, S Fujisawa MD¹, R Kinouchi MD¹ and M Irahara MD, PhD¹.

¹The Univ of Tokushima Graduate Sch Tokushima, Japan.

OBJECTIVES

It has been reported that prenatal immune stress induced by lipopolysaccharides or cytokines increases food intake and leads to obesity and other features of metabolic syndrome in adulthood. Using Sprague-Dawley rats, we evaluated whether neonatal LPS injection altered their body weight regulation systems under non-stress and immune stress conditions.

METHODS AND RESULTS

On Day 10 after birth, all pups were injected with LPS (100 µg/kg, i.p.) (PND₁₀LPS) or saline (PND₁₀Saline). After weaning, body weight was significantly elevated in PND₁₀LPS compared with PND₁₀Saline. Thereafter, the rats were injected with LPS (100µg/kg, i.p.) or saline (used as a basal condition) from 7-8 weeks of age. Under basal conditions, cumulative food intake were significantly higher, serum leptin concentration was significantly increased, and hypothalamic NPY mRNA expression was significantly decreased in PND₁₀LPS compared with PND₁₀Saline. Under adult-LPS injected conditions, body weight gain and cumulative food intake were suppressed in both the PND₁₀LPS and PND₁₀Saline groups compared with those observed under basal adult saline-injected conditions. The suppressive effects induced by adult LPS injection were less evident in the PND₁₀LPS group than in the PND₁₀Saline group. Adult LPS injection increased the serum leptin concentration in the PND₁₀Saline rats, but not in the PND₁₀LPS rats. In addition, adult LPS injection increased the mRNA expression of anorexigenic factors (IL-1β, and TNF-α), and decreased that of the orexigenic factor NPY in both groups. However, the influence of adult LPS injection upon these factors was less evident in the PND₁₀LPS group than in the PND₁₀Saline group.

CONCLUSION

These results suggest that neonatal LPS injection alters body weight regulation under both non-stress and immune stress conditions in male rats. Changes in the endocrine, neuropeptide, and cytokine regulation systems might be involved in these alterations.

Nothing to Disclose: TM, TI, SF, RK, MI

P1-198

Pasireotide (SOM 230) Does Not Change the Stress Hyperglycemic Response to Immobilization Stress.

J Guimaraes¹, A Anjos¹, GF Nascimento¹, WC Silveira¹, BA Mariano¹, PHS Amaral¹, JROL Schweizer MD¹, RB Foscolo MD, PhD¹, CC Coimbra PhD¹ and A Jr Ribeiro-Oliveira, Jr MD, PhD¹.

¹Fed Univ of Minas Gerais Belo Horizonte, Brazil.

Pasireotide (SOM230) is a multi-receptor ligand somatostatin analogue which potentially suppresses growth hormone and corticotrophin (ACTH) secretion, indicating potential promising efficacy in acromegaly and Cushing's disease. In addition, previous studies suggest a possible hyperglycemic effect of Pasireotide. Immobilization stress, on the other hand, is accompanied by hyperglycemia and ACTH secretion, and it mimics the common social and psychological stresses faced in daily life. This study aims to investigate whether a chronic treatment with Pasireotide modifies the stress hyperglycemic response in rats submitted to immobilization stress. Male Wistar rats of 7-8 weeks were treated with Pasireotide 10 mcg/kg BID or vehicle for 2 weeks. Another group of rats were treated the same way with Pasireotide and received saline infusion as controls. Two days before the experiments, silastic catheters were implanted through the external jugular vein. Blood samples were withdrawn at 0, 5, 10, 20, 30 and 60min after immobilization stress. Glucose was determined in duplicate by glucose oxidase assays. Samples before and after stress were compared by paired Student T-test. The differences among groups were evaluated by repeated measures ANOVA followed by Bonferroni. The area under glucose curves were calculated by the trapezoidal rule. There were no significant differences in baseline glucose levels among groups. Both stressed groups of rats showed significant hyperglycemia which was already significant at 5min for vehicle- and at 10min for pasireotide-treated group, and hyperglycemia persisted throughout the experiment. However, no significant differences in blood glucose levels could be detected for the stressed groups. These data show, for the first time, that chronic treatment with Pasireotide does not change either baseline or hyperglycemic stress response in a common investigated stress model.

Sources of Research Support: CNPq; Novartis.

Disclosures: AR-O: Clinical Researcher, Novartis Pharmaceuticals.

Nothing to Disclose: JG, AA, GFN, WCS, BAM, PHSA, JROLS, RBF, CCC

P1-199

Effect of Chronic Stress on Hormone Regulation To Reduce Feeding and Body Weight in the Ovariectomized Female Rat.

DO Larco BS¹, DF Cruthirds CRNA, MAJ, AN¹, MJ Weiser PhD², RJ Handa PhD² and TJ Wu PhD¹.

¹Uniformed Services Univ Bethesda, MD and ²Colorado State Univ Fort Collins, CO.

Menopausal women have reduced estrogen levels increasing their risk for developing central obesity and certain mood disorders. The ovariectomized female rat has been used as a model to study estrogen effects on body weight. Treatment with 17 β -estradiol (E2) attenuates postovariectomy weight gain through activation of the estrogen receptor (ER)- α , and not ER β . ER α may enhance leptin or leptin receptor action in the medial basal hypothalamus (MBH) to alter energy expenditure and feeding. The objective of this study was to delineate the roles played by each ER in body weight regulation. Adult Sprague-Dawley rats were ovariectomized and implanted with an osmotic-pumps containing a hormone treatment: vehicle (VEH), E2, propylpyrazoletriol (PPT;ER α agonist), or diarylpropionitrile (DPN;ER β agonist). The effect of chronic stress on body weight was tested by dividing each hormone group into nonstress and stress subgroups. Stressed animals were restrained 60 min/day for 22 days. E2 and PPT treatment decreased ($p < .05$) body weight throughout the study. Food intake transiently decreased, however, by week 3 of hormone treatment E2 and PPT groups consumed amounts indistinguishable from VEH and DPN treated animals. Stress reduced ($p < .05$) body weight with VEH, PPT, and DPN treatment without changing food consumption. Interestingly, serum leptin was reduced with E2 and PPT treatment but stressed reversed this diminution to levels observed in VEH and DPN treated animals. E2 and PPT treatment increased ($p < .05$) serum insulin while stress significantly reduced ($p < .05$) these levels. CORT was reduced ($p < .05$) in all stressed groups regardless of hormone treatment. Western blot analysis of the MBH showed E2 increased ($p < .05$) leptin receptor and stressed decreased expression with E2 ($p < .1$), PPT ($p < .05$), and DPN ($p < .1$) treatment. Interestingly, pSTAT3, a common marker used for leptin signaling, decreased in stressed rats administered VEH ($p < .05$) and E2 ($p < .1$) while PPT and DPN treatment attenuated this reduction. SOCS3, inhibitor of STAT3, increased with E2 ($p > .05$) and DPN ($p > .1$) treatment but stress inhibited this increase only in E2 treated animals. PPT and DPN treatment ($p < .05$) increased SOCS3 with chronic stress. Additionally, PPT and DPN increased ($p < .05$) pAKT expression in the MBH relative to VEH and E2 treatment. In conclusion, hormone induced changes in leptin and insulin signaling are altered under chronic stress conditions in the MBH and peripherally.

Sources of Research Support: National Science Foundation, Flight Attendants Medical Research Institute.

Nothing to Disclose: DOL, DFC, MJW, RJH, TJW

P1-200

GABA Regulates the Levels of Immunoreactive Corticotropin Releasing Hormone in the Paraventricular Nucleus of Newborn Female but Not Male Mice.

M Stratton BS, MS¹, M Schow¹, C Dennison BS, MS¹, JG Knoll BS, MS¹ and SA Tobet BS, MS, PhD¹.

¹Colorado State Univ Fort Collins, CO.

The paraventricular nucleus of the hypothalamus (PVN) is a major regulator of the stress response via release of corticotropin releasing hormone (CRH) at the median eminence to reach the pituitary gland. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is characteristic of individuals with Major Depressive Disorder (MDD), a condition that affects more females than males. Immunohistochemical analysis revealed increased amounts of CRH in the PVN of newborn female mice with disruption of the GABA-B R1 subunit, and thereby lacking GABA-B receptor function. To test the hypothesis that more cells express CRH in the knockout animals compared to wild type littermates, two concentrations of primary antibody were used. With the CRH antiserum diluted at 1:50K there was no difference in the number of strongly immunoreactive cells. However when run at the more dilute concentration of 1:100K, knockout female mice had twice as many strongly immunoreactive cells in the PVN ($p < 0.05$). This suggests that there is no difference in the number of cells that could synthesize CRH, but rather a difference in the amount of immunoreactive CRH peptide within cells. There was no statistically significant difference seen in males. Ongoing qPCR studies will determine changes at the level of mRNA. Prior studies have shown GABA regulation of the HPA axis and potential sex differences in stress responses. The current study shows GABA-B receptor mediated regulation of CRH levels and potential sex differences in the regulation of CRH in early development. It will be important to determine potential mechanisms by which CRH levels might be regulated differently in males and females. It is surprising that GABA might regulate CRH prior to the development of significant PVN circuitry. Therefore, it will also be important to determine if the influence of GABA-B receptor stimulation on CRH in the PVN is dependent upon stages in the lifespan, and in particular whether the influence remains the same when the HPA circuitry is either less or more fully developed.

Sources of Research Support: NIH Grant MH082679 (co-PI SAT) and NSF training grant DGE-0841259 (co-PI SAT).

Nothing to Disclose: MS, MS, CD, JGK, SAT

P1-201

Influence of Maternal Separation on Weight Gain of Rats Submitted to a High Carbohydrates Diet.

DRC Foscolo MD, Master¹, RB Foscolo MD, PhD¹, JB Guimaraes Master¹, DC Lima Master¹, GV Rodvalho PhD¹ and CC Coimbra PhD¹.

¹UFMG Belo Horizonte, Brazil.

Previous studies showed that neonatal maternal separation causes impair of physical development, especially growth and weight gain in male rats. In order to investigate the effects of early life events on food consumption, weight gain, adipose tissue distribution and leptin secretion, we evaluated the effect of palatable food on male rats submitted to periodic maternal separation (MS) (180 minutes/day) during the first 2 weeks of life. Weaning male Wistar rats (4 weeks of age) were housed in individual cages. Just after weaning, the animals were separated at random into four experimental groups: CC group (n=8) was fed a regular rat chow (NUVILAB-CR, PR, Brazil), DC group (n=8) was fed with a high carbohydrates diet (made of chow 33%, sweetened condensed milk 33%, sugar 7%, and water 8.6%), CS group (n=8) maternally separated rats fed with regular chow and DS group (n=7) maternally separated rats fed with palatable diet for the next 9 weeks. Body weight and calorie intake were measured once a week throughout the experimental period. The Lee index was used to evaluate the development of obesity. The epididimal, retroperitoneal, inguinal and mesenteric white adipose tissues and interscapular brown adipose tissue were dissected and weighted. Leptin was measured by radioimmunoassay.

Our results showed that neonatal maternal separation cause a decrease of body weight gain already seen on second week of high carbohydrates diet (DS group) and remained lower through all experimental period ($p < 0,05$), when compared to control group (CS). DC showed an increase on weight gain only on ninth week of diet. Neonatal maternal separation caused a decrease on brown tissue when associated with high carbohydrate diet compared with CS and DC groups ($p < 0.01$). The evaluation of adipose tissue also showed that high carbohydrates diet caused an alteration of fat distribution, increasing epididimal, retroperitoneal, inguinal, mesenteric white adipose tissue and interscapular brown adipose tissue on DC group ($p < 0.05$). Plasma leptin was higher in rats submitted to maternal separation in both groups: diet and regular chow ($P < 0,01$).

This data indicated that maternal separation affects the energy homeostasis, including leptin secretion, the pattern of fat distribution and weight gain caused by high carbohydrates diet especially on brown adipose tissue, which suggest a higher activation of sympathetic nervous system.

Sources of Research Support: Fapemig, CNPq.

Nothing to Disclose: DRCF, RBF, JBG, DCL, GVR, CCC

P1-202

Relaxin-3 Linked to Depression: Evidence from Relaxin-3 KO Mice.

Steven W Sutton BA¹, James Shoblock PhD¹, Jonathan Shelton PhD¹, Natalie Welty BA¹, Sujin Yun BA¹, Christine Dugovic PhD¹, Pascal Bonaventure PhD¹ and Timothy Lovenberg PhD¹.

¹Johnson & Johnson Pharmaceutical R&D, LLC San Diego, CA.

Relaxin-3(1) and its receptor(2), the former orphan GPCR-135 (RXFP3), are recently characterized modulators of neural and neuroendocrine systems. Relaxin-3 is predominantly expressed in the pontine nucleus incertus, which due to its afferent and efferent connections is believed to contribute to learning, memory, attentive states, emotional processing and stress responses(3-6). Recent data have confirmed relaxin-3 and its receptor modulate and/or are modulated by alterations in these functions. For instance, neurons of the nucleus incertus have been shown to modulate hippocampal theta and spatial memory via relaxin-3 and its receptor(7). Stress or icv injection of CRF increases relaxin-3 and c-FOS expression in the n. incertus(8, 9), which expresses CRF-R1(10). Injection of relaxin-3 into the rat PVN has been shown to increase plasma ACTH and corticosterone(11). Injection of an RXFP3 agonist (icv) causes behavioral changes such as hyperphagia and hyperlocomotion in rats(12, 13).

Recent studies using C57/Bl6 knockout mice (KO) are suggestive of an anhedonic state. Acute feeding studies using a highly palatable, hi fat diet indicate C57/Bl6 relaxin-3 knockout mice eat less than congenic (WT) controls when food is available ad libitum, but after an 18 hour fast there is no significant difference between food consumption in KO and WT mice. Dosing with naloxone reduces consumption of the palatable diet in C57/Bl6 mice, but has no significant effect on similarly treated relaxin-3 KO mice. Similarly, C57/Bl6 relaxin-3 KO mice do not show a conditioned place aversion response to naloxone, which is seen in control mice. In a tail suspension test, the KO mice do not show the same improvement following SSRI or desipramine treatment that is seen in control mice. In sleep EEG studies, REM sleep episode frequency and total REM sleep time are increased in relaxin-3 KO mice, which is consistent with a depressive phenotype. Similar sleep EEG changes occur in rats following icv dosing with a RXFP3 antagonist. These data from relaxin-3 KO mice and RXFP3 antagonist dosed rats are consistent with a key role for relaxin-3 and RXFP3 in depression.

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Disclosures: SWS: Researcher, Johnson & Johnson. JS: Researcher, Johnson & Johnson. JS: Researcher, Johnson & Johnson. NW: Employee, Johnson & Johnson. SY: Employee, Johnson & Johnson. CD: Researcher, Johnson & Johnson. PB: Principal Investigator, Johnson & Johnson. TL: Principal Investigator, Johnson & Johnson.

P1-203

Selective Estrogen Receptor Modulator Treatment in Postmenopausal Women with Schizophrenia.

SR Davis MBBS, PhD¹, C Gurvich BA,BSc(hons), DPsych¹, S Lee BA(hons), DPsych¹, H Gilbert RN¹, E Gavrilidis BApSci¹, A deCastella BEd, BA, MApSci¹, M Berk MBCh, PhD², S Dodd BSc, DipEd, MSc, PhD², PB Fitzgerald MBBS, MPM, PhD¹ and J Kulkarni MBBS, PhD¹.

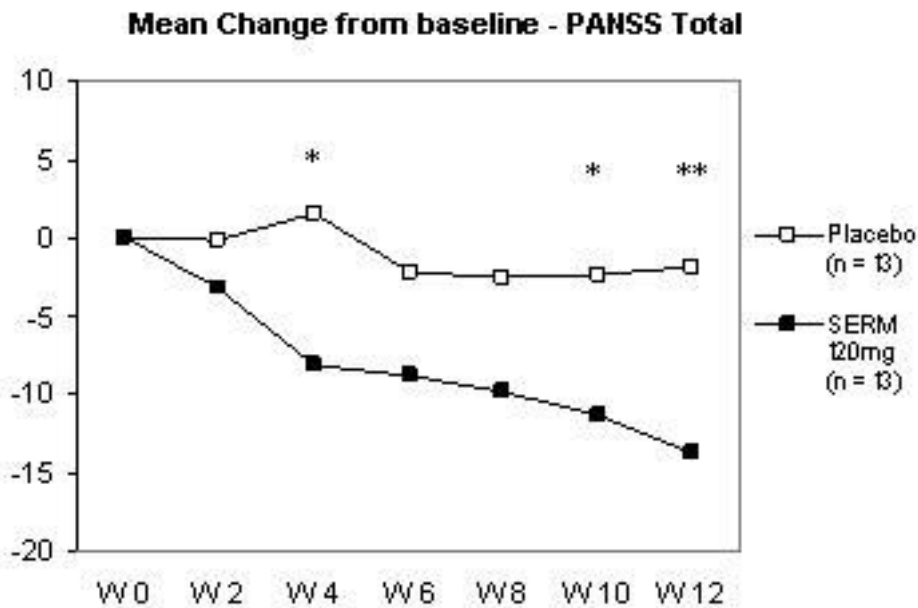
¹Monash Univ Prahran, Australia and ²Melbourne Univ Geelong, Australia.

Introduction: Estrogen treatment may enhance the recovery of schizophrenia in women. However, adverse effects on uterine and breast tissue and other physical side effects may limit the long-term therapeutic use of estrogen. Raloxifene hydrochloride (RLX) is a selective estrogen receptor modulator (SERM) that activates the estrogen receptors with different estrogenic and antiestrogenic tissue-specific effects. It acts as an estrogen antagonist in breast tissue and may have agonistic actions in the brain, potentially offering mental health benefits with few estrogenic side effects.

Design: A double blind placebo controlled trial of adjunctive 120mg/day oral RLX (n = 13) versus oral placebo (n = 13). **Participants:** Women aged 45 years or older who met DSM-IV criteria for schizophrenia, schizoaffective disorder or schizophreniform disorder and were currently acutely unwell as determined by a score of 60 or higher, on the Positive and Negative Syndrome Scale (PANSS). The diagnosis was made by their psychiatrist and confirmed by researchers using the Structured Clinical Interview for DSM-IV Disorders.

Main outcome measures: PANSS, a 30-item clinician rated measure of positive, negative and general symptoms of schizophrenia and the Montgomery-Asperg Depression Rating Scale (MADRS), a 10-item clinician-rated depression measure.

Results: Mean change from baseline for total PANSS scores are presented in Fig. 1, demonstrating that a trend for RLX superiority (after correcting for multiple comparisons) was evident by week 4, $t(24) = -2.68$, $p = 0.01$, and significant RLX superiority evident at week 12, $t(24) = -3.53$, $p = 0.002$. For general PANSS scores significant RLX superiority was evident at week 12, $t(24) = -3.07$, $p = 0.005$. No significant group differences were found for MADRS scores at any time points.



RLX superior to placebo at * $p < .05$ ** $p < .01$

Conclusion: The demonstrated benefit of adjunctive treatment with 120mg RLX offers support for the potential role of this SERM in treating postmenopausal women with schizophrenia.

Sources of Research Support: Stanley Medical Research Institute (SMRI), Washington, USA. (Grant ID: 03T-422); National Health and Medical Research Council (NHMRC), Canberra, Australia (Grant ID: 546084).

Nothing to Disclose: SRD, CG, SL, HG, EG, Ad, MB, SD, PBF, JK

P1-204

Neuron-Specific PPAR γ Deletion Causes Fertility Defects in C57BL/6 Mice.

SHWETA Sharma Ph.D.¹, Jerold Olefsky MD¹ and Nicholas Webster Ph.D.¹.

¹Univ of California, San Diego La Jolla, CA.

In the recent years, proliferator-activated receptor gamma (PPAR γ) ligands have been effectively used for the treatment of insulin resistance in a variety of pathological conditions, including type 2 diabetes and polycystic ovary syndrome (PCOS). With respect to reproduction, treatment with PPAR γ ligands, such as the thiazolidenedione (TZD) drugs, has led to improvement in ovulation rates and overall fertility in PCOS women. Particularly, TZD treatment women decreased GnRH-stimulated LH secretion suggesting the effect is central rather than ovarian. In mice, deletion of PPAR γ using progesterone receptor (PR)-cre caused a reduction in the number of eggs released upon superovulation and a decrease in litter size. However, the deletion was not specific to the ovary as PR is expressed in many other tissues. Taken together, these observations led us to hypothesize that PPAR γ may modulate hypothalamic GnRH secretion and alter GnRH neuron responsiveness. We tested this hypothesis using neuron-specific PPAR γ knockouts. We created neuron PPAR γ knock out mice using synapsin-cre. Female PPAR γ flox/+ :synapsin-cre mice were crossed with male PPAR γ flox/flox mice. We bred PPAR γ brain knock out (BKO) pairs and the control cre- littermates for a period of six months. We found that the BKO pairs have a significantly reduced litter size (5.8 vs 8.4) and number of litters (4.4 vs 6.6) as compared to the control pairs. Additionally, there was a delay of 42 days before the first litter was born to the BKO pairs as compared to 30 days after which the control pairs began delivering litters. To further understand whether the observed fertility defects in KO mice were due to the male or female BKO, we bred male BKO with a control female littermate and female BKO with a control male. Interestingly, both breeding pairs had significantly reduced litter sizes compared to the control breeding pairs. There was no statistical difference in the litter sizes of any breeding pair involving a BKO mouse. The mechanism for the fertility defects in BKO mice is under further investigation including analysis of basal gonadotropin levels, as well as sperm count and/or motility in male BKO mice and defects in estrous cycles, number of ovulated oocytes or defects in folliculogenesis in female mice. Taken together, our findings indicate that PPAR γ can modulate the hypothalamic regulation of reproduction and that deletion of PPAR γ in neurons causes fertility defects in male and female mice.

Sources of Research Support: NIH grants U54HD12303 and R01HD047400.

Nothing to Disclose: SS, JO, NW

P1-205

Transcriptional Regulation of the Human *EAP1* Gene.

Johanna Mueller¹, Katrin Tefs¹, Ines Koch², Wieland Kiess², Thomas Danne⁴, Sergio Ojeda³ and Sabine Heger^{1,4}.

¹Inst of Clin Biochem, Hanover Med Sch Hanover, Germany ; ²Univ Hosp for Children and Adolescents, Univ of Leipzig Leipzig, Germany ; ³Div of Neuroscience, ONPRC, OHSU Beaverton, OR and ⁴Children's Hosp "Auf der Bult" Hanover, Germany.

Mammalian puberty is initiated by an increased pulsatile release of gonadotropin-releasing hormone (GnRH) from specialized neurons located in the hypothalamus. GnRH neuronal activity is controlled by neuronal and glial networks, which are thought to be coordinated via transcriptional regulation. One of the transcriptional regulators is the recently described gene Enhanced at Puberty 1 (*EAP1*). Because *EAP1* has dual transcriptional activity, its role in controlling puberty may also be dual. In this study we used gene reporter assay to examine the hypothesis that *EAP1* expression is controlled by transcriptional regulators earlier postulated to serve as central nodes of a gene network involved in the neuroendocrine control of puberty. The transcriptional regulators include Thyroid Transcription Factor 1 (TTF1), Yin Yang 1 (YY1) and CUTL1, in addition to *EAP1* itself. While TTF1 has been shown to facilitate the advent of puberty, YY1 (a zinc finger protein component of the Polycomb silencing complex) may play a repressive role. The precise role of CUTL1 in this context is not known, but like *EAP1*, CUTL1 can either activate or repress gene transcription. DNA segments of two different lengths (998 and 2744 bp) derived from the 5'flanking region of the human *EAP1* gene were cloned into a luciferase reporter plasmid (pGL4). The constructs (500 ng) were then cotransfected into human embryonic kidney (HEK) cells with different concentrations (250 - 500 ng) of expression plasmids containing the coding regions of TTF1, YY1, CUTL1 and *EAP1*, in addition to a plasmid expressing renilla luciferase used for normalization purposes. Forty-eight h after transfection the cells were collected and analyzed for luciferase activity. The results showed that while TTF1 (n=15) stimulates *EAP1* transcription, YY1 (n=12), CUTL1 (n=12) and *EAP1* itself (n=12), behave as transcriptional repressors. No significant differences in response were observed between the two promoter constructs. These observations suggest that *EAP1* gene expression is under dual transcriptional regulation imposed by a trans-activator (TTF1) and two repressors (YY1 and CUTL1) previously postulated to control a gene network involved in the regulation of puberty. In addition, *EAP1* itself appears to control its own expression via a negative autofeedback loop mechanism. Further studies are needed to determine if the occupancy of the *EAP1* promoter by these regulatory factors changes at the time of puberty.

Sources of Research Support: German Research Foundation grant HE3151/3-1 and HE3151/4-1, NIH grant HD25123,U54 HD18185 and R000163.

Nothing to Disclose: JM, KT, IK, WK, TD, SO, SH

P1-206

Spatially Selective Pruning of Primary Gonadotropin Releasing-Hormone (GnRH) Neuronal Dendrites Occurs during Postnatal Development in Male Rats.

N. Ybarra Ph.D.¹, P.J. Hemond B.S.¹, M.P. O'Boyle M.S.¹ and K.J. Suter Ph.D.¹.

¹The Univ of Texas at San Antonio San Antonio, TX.

Adult GnRH neurons exhibit a stereotypic morphology with a small soma, single axon and single dendrite with limited branching arising from the soma. The adult morphology of GnRH neurons in mice reflects an anatomical consolidation of dendrites over postnatal development. We examined this issue in rat GnRH neurons identified by green fluorescent protein. Live hypothalamic slices (300 μm) were prepared from males at prepubertal ages [PPA (12.9 \pm 0.3 days of age; 29 animals)] or as adult litter mates (71.7 \pm 2.3 days of age; 25 animals). After biocytin-filling of GnRH neurons, slices were placed in 4% paraformaldehyde. Twenty four hours later, biocytin-containing neurons were recovered with avidin-Cascade Blue (1:100). Neurons were then determined to be immuno-positive for GnRH (HU 11B at 1:12.5). Biocytin-containing GnRH neurons (69 GnRH neurons in PPA and 36 GnRH neurons in adults) were reacted with avidin-biotin-horseradish peroxidase and 3,3' diaminobenzidine with nickel enhancement followed by anatomical reconstruction. Somatic area (248.5 \pm 29.4 vs. 145.4 \pm 9.8 μm^2 ; $p=0.01$) and total dendritic length (523.4 \pm 57.7 vs. 320.4 \pm 41.6 μm ; $p=0.02$) were significantly greater in PPA males than in adults. Moreover, total numbers of dendrite branches were greater in PPA males as compared to adults (10.3 \pm 1.4 vs. 1.06 \pm 0.06; $p<0.001$). Further analysis showed the number of 1st, 2nd, 3rd, 4th, and 5th order branches were greater in PPA males than adults (2.2 \pm 0.2 vs. 1.0 \pm 0.0; 2.6 \pm 0.3 vs. 0.1 \pm 0.07; 2.6 \pm 0.4 vs. 0.0; 1.5 \pm 0.3 vs. 0.0; 0.8 \pm 0.2 vs. 0.0 branches; $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$; respectively). The average lengths of 2nd, 3rd, 4th, and 5th order branches were significantly greater in PPA males than adults (28.9 \pm 4.3 vs. 4.9 \pm 4.7; 23.4 \pm 3.9 vs. 0.0; 17.5 \pm 4.1 vs. 0.0; 8.9 \pm 2.4 vs. 0.0 μm ; $p<0.001$, $p<0.001$, $p<0.001$, $p=0.01$; respectively). Most interestingly, in adults a single dendrite arose from the somata. In contrast, PPA animals had an average of 2.2 \pm 0.2 primary dendrites arising from somata (range 1-7 primary dendrites). Based on polar plot comparisons, angles relative to the axon at which dendrites in PPA animals emanated from the GnRH somata were highly variable. Thus, the conserved geometric orientation of 180° between the axon and single dendrite in adults suggests a spatially selective pruning of primary dendrites of GnRH neurons occurs during postnatal development in rats.

Sources of Research Support: HD-060818.

Nothing to Disclose: NY, PJH, MPO, KJS

P1-207

Dynamic and Homeostatic Mechanisms of Testosterone-Mediated Inhibition of Gonadotropin Releasing-Hormone (GnRH) Neuronal Activity.

K.J. Suter Ph.D.¹, A.D. Coop Ph.D.¹, M.P. O'Boyle M.S.¹ and P.J. Hemond B.S.¹.

¹The Univ of Texas at San Antonio San Antonio, TX.

The regulation of GnRH secretion from the GnRH-containing neurons of the hypothalamus exemplifies classical negative feedback control. We examined neuronal control exerted steroid negative feedback in male mice by studying alterations in long-term electrophysiological activity in GnRH neurons using single and dual somatic recordings. Live hypothalamic slices (300 μ m) were prepared from gonadally-intact adult males (57 neurons) and in males following bilateral castration after 24 hrs-3 days (13 neurons), 7 days (10 neurons), 14-18 days (23 neurons) and 50 days or later (65 neurons). Single recordings were analyzed for changes in instantaneous frequency (frequency), burst index and entropy (mean \pm standard error). Simultaneously recorded pairs of GnRH neurons (77 pairs) were analyzed for coordinated firing within 1.0 second bins. Neurons from intact males had a mean instantaneous frequency of 3.4 (\pm 0.29) Hz. Frequency shifted to 5.4 \pm 0.68 Hz by 50 days (or greater) following castration. The burst index in intact males was 15.7 (\pm 5.43). By 14-18 days post-castration, the burst index peaked (39.12 \pm 17.3) and was comparable to that observed in long-term castrates (42.95 \pm 18.8). Entropy, or the orderliness of the discharge pattern, did not change with long-term castration. In intact animals, 69% of pairs (20/29) exhibited coordinated firing. Castration reduced coordinated firing within 24 hrs-3 days (50% of pairs). Coordinated firing was exhibited by 38.8% of pairs at 50 days (or longer) after castration. Our findings suggest that testosterone-mediated inhibition of GnRH neuronal activity operates through temporally distinct parameters. Dynamically, testosterone appears to rapidly alter patterns of firing in individual neurons (as determined by burst index) and coordination between at least pairs of GnRH neurons. On a longer time frame, testosterone controls action potential frequency. Finally, orderliness of firing as assessed by entropy appears to be homeostatically maintained even in the face of a changing steroid milieu and neuronal activity.

Sources of Research Support: HD-049664.

Nothing to Disclose: KJS, ADC, MPO, PJH

P1-208

Differential Expression of *NELF* Splice Variants in Human and Mouse Immortalized GnRH Neurons.

LY Goldberg¹, HG Kim¹, LP Chorich¹ and LC Layman¹.

¹Med Coll of Georgia Augusta, GA.

Idiopathic hypogonadotropic hypogonadism (IHH) results from defective gonadotropin releasing hormone (GnRH) secretion or action, and provides a useful human model to study mechanisms of puberty and reproduction. Kallmann Syndrome (KS) is due to the failure of migration of GnRH and olfactory neurons during development, resulting in IHH with anosmia. Patients with IHH or KS similarly present with impaired or absent pubertal development and subsequent infertility. Nasal embryonic LHRH factor (NELF) is an attractive candidate gene for KS since it was first differentially isolated from migratory GnRH neurons, and was therefore hypothesized to play a role in the migration of GnRH neurons during development. NELF, a nuclear protein, was more highly expressed in migratory than post-migratory immortalized mouse GnRH neurons, although *Nelf* mRNA expression did not differ. In addition, our NELF-specific antibody yielded different sized bands by western blot analysis. We hypothesize that the mRNA/protein expression discordance and protein expression patterns could be due to the variable expression of different splice variants, which are known to occur for NELF. We therefore sought to determine which *NELF* splice variants are present in immortalized GnRH neuronal cell lines from mice (migratory GN11 and NLT cells; postmigratory GT1-7 cells), and immortalized human embryonic GnRH olfactory neuroblasts (FNCB4-hTERT cells). Total RNA was extracted from each cell type and subjected to RT-PCR, subsequent cloning, denaturing gradient gel electrophoresis (DGGE), and DNA sequencing. Preliminary DNA sequencing results indicate that human FNCB4-hTERT cells predominantly express variant 2, with minimal expression of variant 4. In all three immortalized mouse GnRH neuronal cells, variants 1 and 2 were expressed at similar levels. However, DGGE findings suggest the presence of one or two additional variants—a total of three for NLT and GT1-7 cells, four for GN11 cells, and three for human FNCB4-hTERT cells. We are currently studying additional colonies and will identify and clone the full length cDNAs of each expressed splice variant. Our findings indicate that at least two *NELF* splice variants are expressed in immortalized GnRH neurons in both human and mouse, which could explain our previous observations demonstrating bands of different molecular weights by western blot analysis and the mRNA/protein discordance.

Nothing to Disclose: LYG, HGK, LPC, LCL

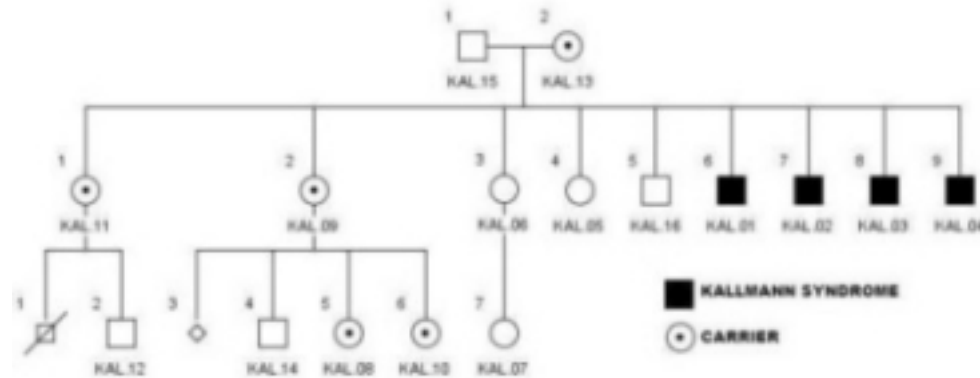
P1-209

A Novel Nonsense Mutation of the *KAL1* Gene (Trp204Stop) in Kallmann Syndrome.

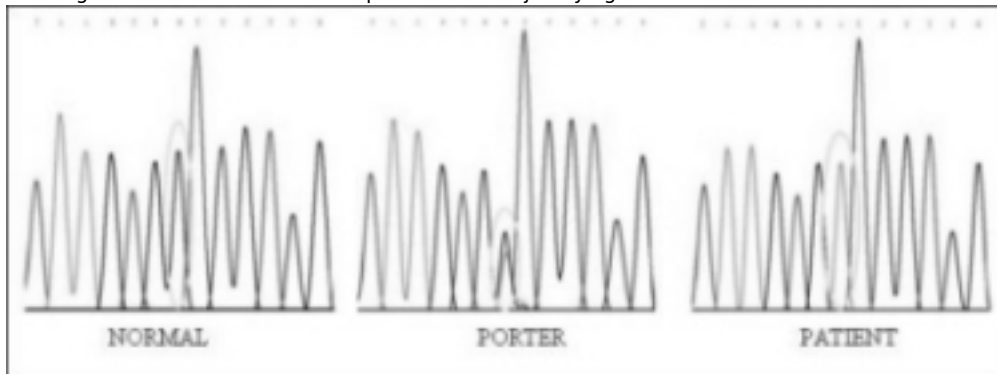
AS El-Husny MD^{1,2}, MR Moraes MD¹, AKC Ribeiro-dos-Santos PhD¹ and MC Fernandes-Caldato PhD^{2,3}.

¹Fed Univ of Pará Belém, Brazil ; ²Univ of the State of Pará Belém, Brazil and ³Ctr Univ da Pará Belém, Brazil.

Kallmann syndrome (KS) combines hypogonadism with any sense of smell deficiency (anosmia/hyposmia). It is a very heterogenous genetic disorder prevalent in 1:10.000 men. The genetics of the X-linked form of KS is more clearly understood, in general, there is an alteration in one of the fourteen exons of *KAL1* gene, located in Xp22.3. Many *KAL1* mutations have already been described, all of them were able to alter function or formation of anosmin-1 which is the protein involved in olfactory and gonadotropin releasing hormone neurons migration. **Objective:** To elucidate the KS molecular basis in four brothers with the pathology. **Methods:** It was done DNA extraction from venous blood of patients and their relatives in a total of sixteen individuals.



The material was conducted to polymerase chain reaction for amplification of exons 5, 6 and 9, then to automatic sequencing. These exons correspond to the three with more mutations described in the literature. **Results:** Molecular analysis found the 762G>A tranversion in the exon 5, mutation still not identified in any studied group of Kallmann syndrome patients. This alteration determines a change of a tryptophan codon for a stop codon at 204 position, resulting in the truncate anosmin-1 production and justifying the KS in molecular level.



It was identified five asymptomatic mutation carriers among relatives, pointing to the great importance of genetic counseling in this family.

Sources of Research Support: FADESP E CNPq.

Nothing to Disclose: ASE-H, MRM, AKCR-d-S, MCF-C

P1-210

Mutations in the Gene Encoding Fibroblast Growth Factor 8, *FGF8*, Are Associated with Complex Midline and Hypothalamo-Pituitary Defects.

MJ McCabe PhD¹, V Tziaferi MBBS¹, C Gaston-Massuet PhD¹, LC Gregory BSc¹, J Walker MD², PS Tsai PhD³, N Pitteloud MD, PhD⁴, JP Martinez-Barbera PhD¹ and MT Dattani MD¹.

¹Inst of Child Hlth London, UK ; ²Queen Alexandra Hosp Portsmouth, UK ; ³Univ of Colorado Boulder, CO and ⁴Harvard Reproductive Scis Ctr Boston, MA.

FGF8 mediates the development of GnRH neurons and is believed to play a critical role during normal hypothalamo-pituitary development. Loss-of-function mutations in *FGF8* in humans have been associated with Kallmann syndrome (KS), which is characterised by anosmia and hypogonadotrophic hypogonadism. Other phenotypic features include cleft lip/palate and sensori-neural hearing loss. Hypomorphic *Fgf8* mutant mice demonstrate poor telencephalic development with deletions of midbrain tissue, absence of olfactory bulbs and optic chiasm, and holoprosencephaly (HPE) with an abnormal corpus callosum. We therefore aimed to investigate the role of *FGF8* in the formation of midline forebrain and craniofacial structures in humans.

600 patients with congenital hypopituitarism and midline forebrain/craniofacial defects were screened by direct sequencing for mutations in *FGF8*.

Two novel missense mutations were identified in *FGF8* in regions that are highly conserved across species: a homozygous change p.R189H and a heterozygous change p.Q216E. The p.R189H mutation was identified in a female patient with semi-lobar HPE, diabetes insipidus and ACTH insufficiency born to consanguineous parents. The heterozygous parents were phenotypically unaffected. The p.Q216E mutation was identified in a female patient with an absent corpus callosum, hypoplastic optic nerves and Moebius syndrome. Functional analysis of these mutations is currently underway.

Expression studies of *FGF8* in human embryonic tissue (Carnegie stages 16 and 22) using in situ hybridization revealed the presence of transcripts in the ventral diencephalon and anterior forebrain, but not in the developing Rathke's pouch. A similar expression pattern has been previously described in the mouse.

To conclude, we demonstrate for the first time a recessive *FGF8* mutation in a patient with HPE, a condition that has previously been associated with heterozygous mutations in *SIX3* and the *Sonic Hedgehog* signalling pathway. Our data suggest a role for *Fgf8/FGF8* in forebrain and hypothalamic development in humans, suggesting an overlap in pathogenesis between KS and complex midline defects with hypopituitarism. Furthermore, this is the first report to implicate *FGF8* mutations in Moebius syndrome.

Sources of Research Support: Birth Defects Foundation, UK.

Nothing to Disclose: MJM, VT, CG-M, LCG, JW, PST, NP, JPM-B, MTD

P1-211

Perifornical Urocortin 3 Mediates the Link between Stress-Induced Mood and Energy Homeostasis.

Y Kuperman MSc¹, O Issler MSc¹, L Regev MSc¹, I Musseri MSc¹, A Neufeld-Cohen MSc¹ and A Chen PhD¹.

¹Weizmann Inst of science Rehovot, Israel.

In modern western societies, the high stress load correlates with increasing incidence of both obesity and metabolic syndrome which have reached epidemic proportions over the past decades. Understanding the mechanisms that mediate the reciprocal relationships between stress-related behaviors and metabolic homeostasis may provide novel insights into the pathophysiology of both mental and metabolic conditions. Studies conducted using corticotropin-releasing factor receptor type-2 (CRFR2) -null mice provide evidence that in addition to its role in mediating stress-related behavior, central CRFR2 is important in modulating metabolic rate, appetite and feeding behaviors. Urocortin 3 (Ucn3), a selective CRFR2 ligand, expressed by the perifornical area (PFA) projects to both the lateral septum and the ventromedial hypothalamus. These brain nuclei express high levels of CRFR2 and are considered as behavioral and metabolic related nuclei, respectively. Therefore, PFA-Ucn3 is a potential modulator of the behavioral stress response and its metabolic consequences. In order to study the involvement of this PFA-Ucn3-CRFR2 pathway in mediating stress-induced behavioral and metabolic changes, we generated transgenic mice model that over-express Ucn3 in an inducible manner using the Tet-On system. Transgenic mice, carrying the tetracycline responsive element sequence upstream to the Ucn3 coding sequence, were injected with lentiviruses expressing the reverse tetracycline transactivator protein, specifically into the PFA. These mice were tested, for variety of stress-related behavioral performances and metabolic parameters with or without the presence of the inducer doxycycline. Ucn3 over-expressing mice showed an increase in anxiety-like behavior, as measured by the light/dark transfer and the open field tests. Indirect calorimetry revealed augmentation in oxygen consumption, carbon dioxide production and heat production, indices of whole body energy expenditure. In addition, though PFA Ucn3 over-expressing mice have normal glucose tolerance, they demonstrate a reduction in insulin sensitivity. Our results support a regulatory role for PFA Ucn3 in anxiety-like behaviors, energy expenditure and glucose metabolism. We suggest that PFA Ucn3 may mediate the link between stress-induced mood and energy homeostasis.

Nothing to Disclose: YK, OI, LR, IM, AN-C, AC

P1-212

Central Administration of Neuropeptide W Stimulates Adrenocorticotropin Release Via Corticotropin-Releasing Factor but Not Via Arginine Vasopressin.

K Yogo M.D.¹, Y Oki M.D., Ph.D.¹, K Iino M.D., Ph.D.¹, M Yamashita M.D., Ph.D.¹, D Nakahara Ph.D.¹ and H Nakamura M.D., Ph.D.¹.

¹Hamamatsu Univ Sch of Med Hamamatsu, Japan.

Neuropeptide W (NPW) was isolated and sequenced as an endogenous ligand for GPR7 and GPR8, orphan G protein-coupled receptors. It has been reported that central administration of NPW stimulates corticosterone secretion in rats. In this study, we investigated how NPW activates the hypothalamic-pituitary-adrenal (HPA) axis. [Methods] For culture study, rat adrenal fasciculate-reticularis cells and pituitary cells were prepared with collagenase and cultured in 95% air - 5% CO₂ atmosphere at 37°C. For in vivo study, a plastic catheter was inserted into jugular vein for blood sampling and a stainless-steel single cannula was planted into the lateral cerebroventricle to administrate NPW. Corticosterone and ACTH were measured by radioimmunoassay. [Results] Firstly, in primary cultured cells, NPW at 1 pM - 10 nM did not alter the basal and ACTH-induced corticosterone secretion from cultured rat adrenal fasciculata-reticularis cells, nor the basal and corticotropin-releasing factor (CRF)-induced ACTH release from cultured rat anterior pituitary cells. Secondly, in conscious and unrestrained male rats, the periphery administration of 2.5 and 25 nmol NPW didn't alter plasma ACTH levels, but the central administration of 2.5 and 5.0 nmol NPW increased plasma ACTH levels remarkably at 15 and 30 min (at 2.5 nmol NPW: 168 ± 110 pg/ml at 0 min, 1170 ± 360 pg/ml at 15 min, 981 ± 329 pg/ml at 30 min, p<0.0001; at 5.0 nmol NPW: 108 ± 38 pg/ml at 0 min, 1803 ± 615 pg/ml at 15 min, 1635 ± 508 pg/ml at 30 min, p<0.0001). Finally, the pretreatment with astressin, CRF receptor antagonist, blocked plasma ACTH levels stimulated by central administration of 2.5 nmol NPW at 15 and 30 min (p<0.01 by ANOVA), but not by V1a/V1b receptor antagonist (p>0.05), (1532 ± 841 v.s. 453 ± 432 v.s. 1244 ± 599 pg/ml at 15 min, vehicle v.s. astressin v.s. V1a/V1b receptor antagonist, respectively). [Summary and Conclusion] The peripheral administration of NPW did not influence the HPA axis, but the icv administration of NPW activated the HPA axis. In addition, the increase in plasma ACTH levels by icv NPW was blocked by the pretreatment with CRF receptor antagonist, but not by the pretreatment with V1a/V1b receptor antagonist. These results suggest that central NPW may have an important role in activating the HPA axis through CRF.

Nothing to Disclose: KY, YO, KI, MY, DN, HN

P1-213

Decreased α Melanocyte Stimulating Hormone (α MSH) Expression in the Hypothalamic Infundibular Nucleus of Subjects with Type 2 Diabetes.

A Alkemade PhD^{1,2}, M Harakalova MSc², DF Swaab MD, PhD², E Fliers MD, PhD¹ and A Kalsbeek PhD^{1,2}.

¹Academic Med Ctr of the Univ of Amsterdam Amsterdam, Netherlands and ²Netherlands Inst for Neuroscience, an Inst of the Royal Netherlands Academy of Arts and Sci Amsterdam, Netherlands.

Context. Rodent data have shown that altered hypothalamic signaling contributes to the development of obesity and insulin resistance.

Objective. Our objective was to identify possible differences in expression levels of hypothalamic neuropeptides between subjects with type 2 diabetes and control subjects.

Design. Post-mortem human brain material was studied by means of quantitative immunocytochemistry.

Subjects. We studied hypothalamus specimens of n=8 subjects with type 2 diabetes, and of n=8 matched controls.

Main Outcome Measure(s) Total melanocyte stimulating hormone (α MSH) immunoreactivity, neuropeptide Y (NPY) and Agouti Related Protein (AgRP) peptide expression levels in the infundibular nucleus (IFN) and suppressor of cytokine signaling (SOCS) 3 expression in the paraventricular and supraoptic nucleus (PVN and SON, respectively).

Results. Total α MSH immunoreactivity in the IFN was significantly decreased in subjects with type 2 diabetes as compared to controls. By contrast, NPY, AgRP and SOCS3 expression were not affected by type 2 diabetes. A highly significant positive correlation was observed between AgRP and NPY expression in the infundibular nucleus. Subjects with type 2 diabetes, but not the control subjects, showed a positive correlation between SOCS3 immunoreactivity in the PVN and NPY/AgRP expression in the IFN.

Conclusions. α MSH expression is decreased in the IFN in type 2 diabetes. Furthermore, NPY/AgRP expression in the IFN correlates with SOCS3 immunoreactivity in the PVN of subjects with type 2 diabetes, but not controls. These observations may have functional implications for the pathogenesis of type 2 diabetes.

Sources of Research Support: Veni-grant of The Netherlands Organization for Health Research and Development, and the Dutch Diabetes Foundation.

Nothing to Disclose: AA, MH, DFS, EF, AK

P1-214

Immunohistochemical Evidence That Argilin, a Product of the ECRG4 Gene, Is a Novel Neurohypophyseal Neuroendocrine Peptide.

AM Gonzalez PhD¹, A Robertson MD^{1,2}, S Podvin PhD², SY Lin PhD², W Leadbeater PhD¹, R Coimbra MD, PhD², MC Miller BSc³, J Chukwueke BSc^{2,3}, E Stopa MD³, C Johanson PhD³, BP Eliceiri PhD² and A Baird PhD².

¹Univ of Birmingham Edgbaston, UK ; ²Univ of California San Diego San Diego, CA and ³Brown Univ Providence, RI.

Genes that encode neuropeptides are highly conserved across species, trafficked in cells by secretory signal peptides and processed at consensus proteolytic cleavage sites to neuroactive peptides. One such gene is esophageal cancer-related gene-4 (ECRG4), which was first identified as a potential tumor suppressor gene down-regulated in esophageal cancer. Augurin, the 14kDa product of the ECRG4 gene, has the features of a neuroendocrine precursor and generates argilin, an 8kDa C-terminal peptide. We therefore evaluated the distribution of ECRG4 in the rat and human brain, anterior and posterior pituitary to determine whether it localizes to known neuroendocrine loci.

In the hypothalamus, immunoreactivity (ir) is observed in paraventricular (PVN) and supraoptic (SON) neurons, a recognized source of vasopressin (AVP) and oxytocin (OT). Confocal microscopy reveals that few cells contain both immunoreactive ECRG4 and AVP but a large majority of ECRG4 positive neurons, but not all, are both ECRG4 and OT positive. In the median eminence, ir-ECRG4 is restricted to axonal fibers that are either OT or AVP positive although some axons in the inner band of the median eminence are only ECRG4 positive.

In the neurohypophysis, ECRG4 is evenly distributed throughout the lobe where it localizes to the herring bodies that contain secretory granules responsible for the release neuropeptides into the pituitary circulation. Confocal studies show that ECRG4 positive structures co-localize either with OT or AVP. Immunoblotting of neurohypophyseal extracts reveal a strong band at 8kDa which corresponds to the predicted c-terminal domain of ECRG4 that we call argilin, the "RG-peptide" of EC"RG"4. The possibility that the RG-peptide derived from ECRG4 is a neuropeptide is supported by the fact that when it is displayed on phage, the RG-peptide enables targeting and internalization of phage into HEK cells suggesting that it encodes a ligand, possibly for kidney cells. Furthermore, profound effects of edema are observed when ECRG4 gene expression is knocked down in developing zebrafish embryo. ECRG4 gene knock outs in mice are reported unsuccessful. Accordingly, while the physiological activities of the RG-peptide argilin remain to be determined, its distribution points to functions associated with OT neurons of the PVN, SON and neurohypophysis; for example fluid homeostasis and edema and/or the control of emotion and behavior. Characterization of the RG-receptor is underway.

Sources of Research Support: NIH support through NIGMS Grant 078421 (AB), NEI Grant 018479 (AB), NIHHLB 73396 (BPE) and American Recovery and Reinvestment Act (ARRA) competitive supplement (AB) and studentship (AB) funding.

Nothing to Disclose: AMG, AR, SP, SYL, WL, RC, MCM, JC, ES, CJ, BPE, AB

P1-215

Ovarian Hormones Regulate the Connectivity of Oxytocin (OT)-Labeled Synaptic Elements in the Fiber Plexus Lateral to the Hypothalamic Ventromedial Nucleus (VMH) in Female Rats.

S.L. Ferri-Kolwicz M.A.¹, G.D. Griffin PHD¹, B.A.S. Reyes PHD², E.J. Van Bockstaele PHD² and L.M. Flanagan-Cato PHD¹.

¹Univ of Pennsylvania Philadelphia, PA and ²Thomas Jefferson Univ Philadelphia, PA.

The neuropeptide OT is a critical modulator of social and ingestive behaviors, with several sites of brain action, including the VMH. In female rats, estradiol and progesterone regulate the extension of dendrites from the VMH toward the OT-labeled axons lateral to the VMH, based on Golgi analysis (Griffin and Flanagan-Cato, 2008). Likewise, electron microscopic analysis revealed that treatment with estradiol decreased the density of dendritic profiles in the lateral fiber plexus compared with the vehicle- and estradiol plus progesterone-treated groups, in ovariectomized adult Sprague-Dawley rats. It remains unclear whether the estradiol-induced reduction in dendrite availability is associated with a concomitant rearrangement in synaptic input. The present study tested the hypothesis that ovarian hormones selectively modify the pattern of synaptic input to dendrites in this region. Animals were randomly assigned to vehicle, estradiol alone, or estradiol plus progesterone treatment (n=3 per group). The fiber plexus lateral to the VMH was immunohistochemically labeled for OT and prepared for electron microscopy. Across the three groups, 1,744 dendritic profiles were analyzed. The number of synaptic contacts per dendritic element was quantified, noting whether or not the dendrite was labeled for OT. For the 86% dendritic profiles that were not OT-labeled in the VMH fiber plexus, estradiol treatment decreased their density by 29%. Those non-OT-labeled dendrites that remained after estradiol treatment displayed no change in their average number of synaptic inputs. In contrast, for the 14% dendritic profiles that were labeled with OT, estradiol treatment did not change their density. However, estradiol treatment increased the number of synaptic contacts per dendritic profile on OT-labeled dendrites by approximately 66% ($p < 0.05$). The effects of estradiol on both dendrite density and synaptic contacts per dendrite were reversed by concomitant treatment with progesterone. These results support the hypothesis that estradiol treatment induces a specific synaptic rearrangement in the fiber plexus lateral to the VMH. Ongoing analysis is assessing whether the OT-labeled pre-synaptic terminals are differentially associated with OT- versus non-OT labeled dendrites. This additional ultrastructural analysis will provide further insight into the physiological significance of OT in dendrites in this region.

Sources of Research Support: R01-MH64371 and TG-MH017168.

Nothing to Disclose: SLF-K, GDG, BASR, EJV, LMF-C

P1-216**TRPV4^{P19S} Dominant Negative Nonsynonymous Single Nucleotide Polymorphism (SNP) Is Not Associated with the Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH) in a Male Infant.**MR Benson MD¹, J Wang MD¹, JE Sylvester PhD¹, A Tolaymat MD² and JA Canas MD¹.¹Nemours Children's Clin Jacksonville, FL and ²Univ of Florida Coll of Med Jacksonville, FL.

Background: SIADH in children is usually transient and often associated with central nervous system (CNS) lesions or drug use. Congenital forms of SIADH without CNS lesions are rare. *TRPV4* is a candidate osmosensor in the CNS and a SNP P19S has been reported in human hyponatremia.

Clinical case: A 13 month old male presented with failure to thrive and chronic hyponatremia. His height was 65.1 cm (-3.8 SD), weight 6.5 kg (- 4.7 SD), pulse 133 bpm and BP 109/78 mm/Hg. Growth velocity was 2 cm/yr. At 6 weeks of age thyroid and adrenal functions were normal.

Laboratory Summary

| Age (months) | Normal Range | 2 | 13 | 17 | 25 |
|---------------------|--------------|------|------|------|------|
| S Na (meQ/L) | 134-146 | 122 | 126 | 130 | 133 |
| S K (meQ/L) | 3.4-4.7 | 6* | 5 | 5.1 | 5 |
| BUN (mg/dL) | 8-23 | 19 | 15 | 20 | 20 |
| Cr (mg/dL) | 0.3-0.7 | 0.4 | 0.3 | 0.3 | 0.2 |
| S Osm (mOsm/kg) | 285-293 | 273 | 255 | 277 | |
| U Osm (mOsm/kg) | N/A | 202 | | 386 | |
| U Na (meQ/L) | N/A | | | 81 | |
| U Cr (mg/dL) | N/A | | | 10.2 | |
| FE Na (%) | N/A | 0.41 | | 1.8 | |
| Renin (ng/dL/hr) | < 15 | 3.26 | | | |
| Aldosterone (ng/dL) | 2-70 | 33.9 | | | |
| IGF-1 (ng/mL) | 30-122 | | | < 25 | < 25 |
| IGFBP-3 (mg/L) | 0.8-3 | | | 1 | |
| ADH (pg/mL) | 0.7-3.8 | | 16.5 | | 8.3 |

* Hemolysis Present

MRI of the brain showed no midline defects and a normal posterior pituitary. During water deprivation he was able to concentrate his urine to 576 mOsm/kg with a weight loss of 3.6%. During water loading he was able to dilute to 58 mOsm/kg excreting > 90% in four hours. These features are typical of the Reset Osmostat Syndrome (SIADH Type C). Random ADH levels have been inappropriately elevated suggesting SIADH Type A. He has been treated with salt supplementation and fluid restriction and his sodium and growth has improved (15 cm/yr). A trial of Urea (max dose 0.8 g/kg/day) worsened his hyponatremia and increased natriuresis.

Methods: We isolated DNA from our patient and his parents and performed RFLP using SMA 1 restriction enzyme. The *TRPV4* exon was PCR amplified using primers bracketing the *TRPV4* P^{19S} polymorphism (rs3742030) that results in amino acid substitution at residue 19 (Pro to Ser). All 3 samples were shown to be homozygous CC (Pro19) by PCR-RFLP (192bp, 97bp and 21bp).

Conclusion: This is a unique case of congenital SIADH with impressive failure to thrive. He excretes a water load and concentrates his urine as Type C but he has inappropriately elevated ADH levels as seen in Type A. He does not have the P19S SNP in *TRPV4* gene which was recently reported to associate with hyponatremia in adults.

(1) Tian W et al., Proc Natl Acad Sci USA, 2009. 106(33): p. 14034-9.

Nothing to Disclose: MRB, JW, JES, AT, JAC

P1-217

Estradiol Potentiates the Activation of Magnocellular Vasopressin Neurons and Vasopressin Secretion in Hemorrhagic Shock.

AS Mecawi¹, IG Araujo¹, LC Reis PhD², LLK Elias MD, PhD¹ and J Antunes-Rodrigues MD, PhD¹.

¹Univ of Sao Paulo, Sch of Med of Ribeirao Preto Ribeirao Preto, Brazil and ²Fed Rural Univ of Rio de Janeiro Seropedica, Brazil.

The aim of this study was to investigate the role of estradiol on neuroendocrine control during acute hypovolemia induced by hemorrhage. Female Wistar rats (~220g) were ovariectomized and treated with corn oil (OVX) or 17-beta-estradiol (OVX-E2 40µg/kg sc) for 8 days. On the 7th day, the femoral artery was cannulated for blood withdrawal (1.5 ml/100g over 1min). Hemorrhage was performed 24h after the cannulation and blood was collected by decapitation 5, 15 or 30 minutes after the hemorrhage to determine angiotensin II, atrial natriuretic peptide (ANP) and vasopressin plasma levels. We also evaluated the activation pattern of supraoptic (SON) and paraventricular (PVN) magnocellular vasopressin neurons by immunohistochemistry for Fos protein expression. Plasma angiotensin II levels increased (~100%, p<0.05) and ANP decreased (~40%, p<0.05) 5, 15 and 30 minutes after hemorrhage, when compared with baseline, with no differences between OVX and OVX-E2. Plasma vasopressin levels enhanced ~100% (p<0.05) after hemorrhage in OVX rats and this response was potentiated by estradiol, which increased it to ~170% (p<0.05) in 5 minutes. Likewise, hemorrhage induced intense activation of vasopressinergic neurons in all groups. Treatment with estrogen enhanced the activation of vasopressinergic neurons of the SON in relation to oil-treated rats (80.2 ± 5.1% vs. 57.5 ± 4.9%, p<0,001). The medial magnocellular region (MM) of PVN showed a similar pattern of response. Estrogen-treated rats also showed the potentiation of vasopressinergic neuron activation of the MM in the PVN in relation to oil-treated rats (46.5 ± 5.4% vs. 17.5 ± 2.4%, p<0,001). In the lateral magnocellular region (LM) of PVN, estrogen potentiates vasopressin neurons activation (59,9 ± 5,8% vs. 33,7 ± 5,3%, p<0,001). These results indicate that estrogen potentiates the vasopressin magnocellular neuron activation and vasopressin secretion in response to acute hypovolemia induced by hemorrhage in ovariectomized rats.

Sources of Research Support: FAPESP, CNPq, FAEPA.

Nothing to Disclose: ASM, IGA, LCR, LLKE, JA-R

P1-218

Suppressor of Cytokine Signaling 3 (SOCS3) Expression in the Post-Mortem Human Hypothalamus and Its Relationship with Pre-Mortem Serum Leptin.

A Alkemade PhD^{1,2}, UA Unmehopa BSc², EVS Hessel MSc^{1,2}, DF Swaab MD, PhD², A Kalsbeek PhD^{1,2} and E Fliers MD, PhD¹.

¹Academic Med Ctr of the Univ of Amsterdam Amsterdam, Netherlands and ²Netherlands Inst for Neuroscience, an Inst of the Royal Netherlands Academy of Arts and Sci Amsterdam, Netherlands.

Context. In rodents, the mediobasal hypothalamus and the hypothalamic paraventricular nucleus (PVN) are implicated in leptin signaling, whereas surprisingly little data is available on the human hypothalamus.

Objective. To study the expression of α melanocyte stimulating hormone (α MSH), agouti related protein (AgRP) and suppressor of cytokine signaling 3 (SOCS3) in the human infundibular nucleus (IFN) and PVN, and to investigate the relationship between these neuropeptide expressions and serum leptin concentrations in a blood sample taken just before death.

Design. Post-mortem human brain material was studied by means of quantitative immunocytochemistry.

Subjects. We determined post mortem hypothalamic tissues of patients, whose serum leptin concentrations had been assessed in a sample taken <24h before death.

Main Outcome Measure(s). We studied the expression of α MSH, AgRP and SOCS3 in the IFN and PVN. Total protein expression was correlated with serum leptin. In addition, we assessed correlations with neuropeptide Y (NPY) and thyrotropin releasing hormone (TRH) gene expression assessed previously in the same brain specimens.

Results. SOCS3 immunoreactivity was widely distributed throughout the hypothalamus, and most prominent in the PVN. Surprisingly, SOCS3 expression in the PVN was inversely related to serum leptin. A highly significant positive correlation was observed between AgRP and NPY expression in the IFN.

Conclusions. Although the pattern of SOCS3 expression in the human hypothalamus is in general agreement with that in rodents, the inverse correlation between SOCS3 expression in the PVN and serum leptin was unexpected. It may be related to fatal illness rather than to nutritional status.

Sources of Research Support: Veni-grant of The Netherlands Organization for Health Research and Development, and the Dutch Diabetes Foundation.

Nothing to Disclose: AA, UAU, EVSH, DFS, AK, EF

P1-219

Interrelationship between Metabolic and High Bone Mass Phenotype in a Mouse Model of Adult-Onset, Isolated, GH-Deficiency (AOiGHD).

JW Bracey MD¹, RA Skinner¹, NS Akel¹, AB Reddy^{2,3}, J Cordoba-Chacon⁴, Q Lin^{2,3}, PV Subbaiah³, RM Luque MD⁴, D Gaddy PhD¹, RD Kineman PhD^{2,3} and LJ Suva PhD¹.

¹UAMS Little Rock, AR ; ²Jesse Brown VA Med Ctr and Univ of Illinois at Chicago Chicago, IL ; ³Univ of Illinois at Chicago Chicago, IL and ⁴Univ of Córdoba; Inst Maimónides de Invest Biomed (IMIBIC), and CIBER Fisiopatología de la Obesidad y Nutrición Córdoba, Spain.

Growth hormone (GH) secretion by pituitary somatotrophs has anabolic and lipolytic properties, while the actions of insulin-like growth factor I (IGF-I) can mimic those of insulin. Normally, circulating GH/IGF-I levels steadily rise after birth, plateau at puberty, and decline thereafter. Therefore, the fall in GH that occurs with age and obesity may contribute to derangements in adult metabolic function. To determine the effects of specific GH deletion on adult metabolism, we utilized a mouse model of adult-onset, isolated GHD (AOiGHD). Rat GH promoter driven Cre recombinase transgenic mice were crossed with inducible monkey diphtheria toxin receptor (iDTR) mice, generating a model of AOiGHD (DT-treated, rGhp-Cre/iDTR). AOiGHD and GH-INTACT control mice were fed a high fat diet (HF, 45% kcal from fat), and compared to mice fed a low fat diet (LF, 10%). AOiGHD mice are more insulin sensitive than GH-INTACT, independent of diet, with lower insulin levels. In addition, whole body and fat depot weights, as well as the response to GTT, did not differ between LF-fed AOiGHD and GH-INTACT. In contrast, HF-fed AOiGHD mice had decreased body weight, increased relative fat mass, impaired glucose clearance, and inappropriately low insulin levels, compared to HF-fed GH-INTACT. Surprisingly, HF-fed AOiGHD but not LF-fed AOiGHD liver weights and triglyceride content were lower than GH-INTACT, without changes in circulating triglycerides, NEFA, or cholesterol. In the context of this hormonal/metabolic milieu, the femurs of HF-fed AOiGHD were shorter, and both femurs and tibias exhibited a high bone mass phenotype with increased total cross sectional area and periosteal perimeter. Despite these changes, biomechanical strength was not different. In addition, marrow fat content was significantly increased in HF-fed AOiGHD, compared to HF-fed GH-INTACT. Taken together these results present the possibility that the decline in circulating GH in adults, may in fact serve a protective mechanism to maintain systemic insulin sensitivity. This in turn protects the liver from excessive lipid accumulation, thereby shifting excess nutrients to storage depots. Consistent with this idea, the skeleton also appears to serve as a storage depot and is another source of increased adipocytes and osteoblasts. It remains to be determined if changes in circulating GH directly or indirectly, via alterations in insulin sensitivity, mediate these changes in bone mass and strength.

Sources of Research Support: VA Merit and AG031465 (to RDK), BFU2008-01136; RYC-2007-00186; JC2008-00220 (to RML), FI06/00804 (to JCC); DK74024 (to DG); HL68585 and DK78165 (to PVS); Carl L. Nelson Chair in Orthopaedic Creativity (to LJS).

Nothing to Disclose: JWB, RAS, NSA, ABR, JC-C, QL, PVS, RML, DG, RDK, LJS

P1-220

O-Linked Glycosylation of Growth Hormone Enhances Its Plasma Half-Life and *In Vivo* Activity.

S Okada PhD¹, J Xu PhD¹, S Sankaran MS¹, MJ Kieliszewski PhD¹ and JJ Kopchick PhD¹.

¹Ohio Univ Athens, OH.

Growth hormone (GH) is a potent regulator of growth and metabolism. Recombinant human (rh)GH is approved for GH deficiency (GHD) as well as conditions associated with growth impairment in the absence of GHD. hGH has a short plasma half-life (~25 min) mainly due to rapid renal clearance and susceptibility to serum proteases. This greatly reduces the efficacy as a therapeutic agent. Also, frequent injections of rhGH are required and can negatively influence patients' compliance. These problems can be overcome by increasing its molecular size (thus extending its biological half-life) through chemical derivatization with polyethylene glycol (PEGylation) or by expressing it as a fusion protein with serum albumin or a polypeptide designated XTEN(1,2,3). All of these modifications, however, reduce bioactivity. We have expressed a biologically active rhGH with increased plasma half-life when synthesized as an arabinogalactan-protein (AGP) in tobacco BY-2 cells. This rhGH analog is expressed with 10 repeats of the AGP glycomodule Ser-Hyp (SO) at the C-terminus (rhGH-(SO)₁₀). These repeats direct Hyp-O- glycosylation and increase the molecular mass of rhGH from 22 kDa to ~50 kDa. rhGH-(SO)₁₀ exhibits the same binding affinity to cell surface GH receptors (R) as wild type hGH, indicating the bulky glyco units on the C-terminus do not interfere with its binding abilities. Also, rhGH-(SO)₁₀ stimulates tyrosine phosphorylation of Stat5 to the same extent as wild type hGH in mouse fibroblasts expressing the mGHR. Daily injections of rhGH-(SO)₁₀ at pharmacological doses into mice yielded both an increase in serum IGF-1 and enhancement of whole body growth similar to that observed with hGH injections. Importantly, with 1mg GH equivalent/g body weight/day in a once daily injection for 5 days, the effect of rhGH-(SO)₁₀ was greater than wild type hGH in terms of the amplitude and the duration of the IGF-1 increase. This increase in IGF-1 may be due to the enhanced serum half-life of rhGH-(SO)₁₀. We have compared the plasma half-life by direct venous injection of rhGH-(SO)₁₀ and subsequent sampling of plasma rhGH-(SO)₁₀. We have found it to be detected 24 hrs after the single injection and still detectable 4 days post-injection. This clearly shows that the plasma half-life of rhGH-(SO)₁₀ is greatly enhanced compared to wild type hGH. These results demonstrate the feasibility of this approach for producing long-acting, biologically active rhGH and other therapeutic proteins.

(1) Clark R et al., J Biol Chem 1996:21969

(2) Osborn BL et al., Eur J Pharmacol 2002 456:149

(3) Schellenberger V et al., Nat Biotechnol 2009 27:1186

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll; by funds from the NIH (grant DK075436-01 and AG019899-06 for JJK); support from the Diabetes Research Initiative at Ohio University; by funds from the Provost's Undergraduate Research Fund at Ohio University, and a generous gift from AMVETS.

Nothing to Disclose: SO, JX, SS, MJK, JJK

P1-221

Immunogenicity, Toxicology, Pharmacokinetics and Pharmacodynamics of Growth Hormone Ligand-Receptor Fusions.

E Ferrandis¹, SL Pradhananga², C Touvay¹, C Kinoshita³, IR Wilkinson², K Stafford³, Z Wu⁴, CJ Strasburger⁴, JR Sayers², PJ Artymiuk² and RJ Ross².

¹Ipsen Les Ulis, France ; ²Univ of Sheffield Sheffield, UK ; ³Ipsen Biomeasure Milford, MA and ⁴Campus Mitte Charite-Univ Med Berlin, Germany.

We have recently demonstrated that a ligand-receptor fusion (LR-fusion) of growth hormone generates a potent long-acting agonist (1). A fundamental concern for all new biologicals is the possibility of inducing an immune response. This is specifically the case where a recombinant protein is a fusion, contains non-native sequence or is presented in a novel conformation. To address this concern we have designed molecules to reduce potential immunogenicity and undertaken pharmacokinetic and pharmacodynamic studies in rats and toxicology studies in Cynomolgus monkeys. Two variants of the LR-fusion; one with a flexible linker (GH-LRv2) and the other without (GH-LRv3), were tested for biological activity in rats and then toxicology performed in monkeys. Comparison was made with both E. coli derived and mammalian expressed native human GH. GH-LRv2 and GH-LRv3 demonstrated similar pharmacokinetics in rats with reduced clearance compared to native GH and potent agonist activity with respect to body weight gain in a hypophysectomised rat model. In Cynomolgus monkeys a low level of antibodies to GH-LRv2 was found in one sample but there was no other evidence of any immunogenic response to any molecule. There were no toxic effects and specifically no changes on histology at injection sites after two repeated administrations. The pharmacokinetic profile confirmed an exceptionally delayed clearance for both GH-LRv2 and GH-LRv3. In conclusion, repeated administration of a GH LR-fusion was safe, non-toxic and the pharmacokinetic profile suggests that once monthly administration is a potential therapeutic regimen for humans.

(1) Wilkinson IR et al., Nature Medicine 2007; 13:1108

Disclosures: EF: Collaborator, Ipsen. SLP: Coinvestigator, Asterion. CT: Collaborator, Ipsen. CK: Collaborator, Ipsen. IRW: Coinvestigator, Asterion. KS: Collaborator, Ipsen. JRS: Coinvestigator, Asterion. PJA: Coinvestigator, Asterion. RJR: Director, Asterion Ltd, Diurnal Ltd.

Nothing to Disclose: ZW, CJS

P1-222

A Novel Y332C Missense Mutation in the Intracellular Domain of the Human Growth Hormone Receptor (GHR) Does Not Alter STAT5b Signaling: Redundancy of GHR Intracellular Tyrosines Involved in STAT5b Signaling.

MA Derr MS¹, P Fang PhD¹, SK Sinha MD², S Ten MD², V Hwa PhD¹ and RG Rosenfeld MD¹.

¹Oregon Hlth & Sci Univ Portland, OR and ²State Univ of New York Downstate Children's Hosp & Maimonides Infant's & Children's Hosp of Brooklyn Brooklyn, NY.

The human growth hormone receptor (GHR), upon binding with GH, induces JAK2-mediated phosphorylation of GHR intracellular tyrosines, which then recruit components of multiple signaling pathways, including STAT5b, with subsequent regulation of the growth promoting insulin-like growth factor (IGF)-1. Aberrancies in STAT5b signaling, due to mutations in the *GHR* or *STAT5b* genes, results in poor response to GH, pronounced IGF-I deficiency (IGFD) and severe short stature. Of the more than 70 *GHR* mutations identified to date in over 250 IGFD cases, mutations within the GHR intracellular domain have been rare, often involving small DNA insertions or deletions.

Objective: A novel Y332C mutation, identified in a patient with modest short stature, and the seven intracellular tyrosines of the human GHR (prepeptide: Y332, Y436, Y487, Y534, Y566, Y595, Y627), were evaluated in the GHR-STAT5b signaling process.

Results: Human GHR constructs carrying Y332C or single Y->F changes for each of the seven intracellular tyrosines, did not alter GH-induced GHR-STAT5b signaling in HEK293 reconstitution studies. However, GH-induced STAT5b activation (tyrosyl-phosphorylation of STAT5b, and Luciferase reporter activities) was specifically abrogated in an hGHR variant in which all seven tyrosines were inactivated. When hGHR variants carrying single intracellular tyrosines were evaluated, STAT5b activation was comparable to that of wild-type hGHR only with variants carrying Y534, Y566 or Y627. Activation of the STAT-1, -3 and ERK1/2 pathways, in contrast, remained normal and GH-inducible, suggesting that the intracellular GHR tyrosines were necessary and redundant only for STAT5b signaling. The role(s) of the remaining four intracellular tyrosines in STAT5b signaling is currently being investigated; preliminary data suggests that Y487 may be involved in down-regulating STAT5b activation.

Conclusion: We provide evidence for the first time that in the human GHR, three intracellular tyrosines (Y534, Y566, and Y627) are critical and redundant in the GH-induced STAT5b signaling process. This redundancy may explain why an Y332C variant, identified in a short statured patient, did not alter STAT5b signaling, and may explain why GHR intracellular mutations identified to date involve significant DNA aberrations that abrogated these three critical tyrosines. Identification of missense variants in the human GHR intracellular domain should be interpreted with caution and rigorously analyzed.

Sources of Research Support: March of Dimes Foundation (RGR); Tercica, Inc (RGR).

Nothing to Disclose: MAD, PF, SKS, ST, VH, RGR

P1-223

Longitudinal Analysis of Plasma Proteome of Bovine Growth Hormone Transgenic Mice.

Juan Ding Ph.D.¹ and John J. Kopchick Ph.D.^{1,2}.

¹Edison Biotech Inst Athens, OH and ²Dept of BioMed Scis Athens, OH.

Attenuation of the growth hormone (GH)/insulin-like growth factor-1 (IGF-I) axis results in extended lifespan in many organisms including mice. Consistent with this, bovine (b) GH transgenic mice die prematurely at ~14 months versus ~24 months of age for wild type (WT) mice. We have followed bGH mice (n=9) and their WT littermates (n=8) longitudinally and found several interesting age-related changes. In contrast to WT mice which gained fat mass, had normal fasting glucose and slightly elevated insulin levels during aging, bGH mice lost fat mass, became hypoglycemic and showed lower insulin levels at older ages (12-16 months) despite being hyperinsulinemic when young (3-5 months). To examine potential abnormalities in plasma protein levels in bGH mice that may be indicators of their premature aging, we analyzed plasma proteins at 2, 4, 8, 12 and 16 months of age by two-dimensional electrophoresis (2-DE) followed by mass spectrometry (MS) and MS/MS. Several plasma proteins differed (up to 22-fold) between genotypes. Specifically, apolipoprotein E (APOE), haptoglobin (HP) and mannose-binding protein-C (MBP-C) increased significantly ($p < 0.01$) in bGH mice versus controls at all ages. Likewise, transthyretin (TTR) and apolipoprotein A-1 (APOA1) decreased significantly ($p < 0.01$). Many proteins existed as multiple isoforms, presumably due to post-translational modifications (PTMs). Alpha-2 macroglobulin (A2M) exhibited isoform-specific changes in bGH mice, suggesting GH regulation of this protein at the PTM level. Importantly, three isoforms of HP and two isoforms of clusterin (CLU) exhibited a significant interaction between genotype and age, increasing markedly as bGH mice aged but not in WT mice. Thus, specific isoforms of these two proteins (CLU and HP) reflect an age-dependent difference in bGH mice. In terms of their biological functions, HP, MBP-C and A2M are induced (acute phase proteins) and TTR is suppressed by inflammation. APOE is primarily associated with very low-density lipoprotein (VLDL) and LDL, whereas APOA1 and CLU are associated with HDL. In conclusion, identification of these plasma proteins revealed that chronic GH excess resulted in an increased inflammatory state and altered lipid metabolism, possibly contributing to their diminished life expectancy. In addition, several of these proteins have never been reported to be associated with GH action and represent novel biomarkers of GH action especially in case of GH doping.

Sources of Research Support: World State of Ohio's Eminent Scholars Program that includes a gift by Milton and Lawrence Goll; by NIH Grants DK075436-01, AG019899-06 and 1P01AG03173601A; by The World Anti-Doping Agency (WADA); by AMVETS, and by the Diabetes Research Initiative and BioMolecular Innovation and Technology Partnership at Ohio University.

Nothing to Disclose: JD, JJK

P1-224

The Production of Recombinant Growth Hormone (GH) and GH Antagonists and the Development of Three In Vitro GH Activity Assays.

ES Gosney¹, D Cruz Topete¹ and JJ Kopchick¹.

¹Ohio Univ Athens, OH.

Our laboratory has previously reported the construction of *E. coli* expression vectors for human growth hormone (hGH), mouse growth hormone (mGH), hGH receptor antagonist (hGHA) and mGH receptor antagonist (mGHA) (1). Our recombinant GH and GHA protein production and purification protocols have been completed and optimized. To test the activity of these molecules, we have developed three in vitro assays that can be used to determine their biological activities. The first assay utilizes mouse L-cells that have previously been stably transfected with a mGH receptor minigene (2), designated E6 cells. Confluent E6 cells were deprived of serum for 24 hours and treated with GH and/or GHA for 10 minutes. The level of GH signaling was measured by quantification of STAT5b phosphorylation via Western Blotting. Treatment of cells with 10 nM GH induced phosphorylation of STAT5b, which was largely abrogated by co-treatment with 100 nM GHA. The second assay measures biological activity of GH in human B-lymphoblast-derived IM-9 cells, which endogenously express GH receptor. This cell line has previously been shown to proliferate in response to GH treatment (3). Cells were grown in media containing 10% fetal bovine serum (FBS) to a concentration of 1×10^6 cells/ml. Cells were collected and resuspended to a concentration of 2×10^5 cells/ml in media containing 3% FBS. After 48 hours, cells were treated with GH and/or GHA and incubated for 24 hours. Cell proliferation was measured. Treatment of cells with 1 nM GH increased cell proliferation compared to controls. The proliferative effect of GH was inhibited by co-treatment with 10 nM GHA. The third assay utilizes 3T3-L1 mouse fibroblast cells. 3T3-L1 cells have been shown to undergo adipogenesis upon GH stimulation (4). Differentiation was induced by treatment with a cocktail containing dexamethasone, insulin and IBMX in the presence or absence of GH and/or GHA. Treatment with cocktail containing 2 nM GH resulted in increased triglyceride accumulation, as indicated by Oil Red staining. Glycerol 3-phosphate dehydrogenase (GPDH) activity was measured as a secondary indicator of adipogenesis. Cells induced with cocktail containing 2 nM GH showed high GPDH activity, which was completely absent in the untreated control. In summary, we have established a quick and efficient GH and GHA production protocol and optimized three in vitro GH activity assays that can be used to measure the potency of the various preparations.

(1) Gosney E, et al., Poster P3-141; 90th Annual Meeting of the Endocrine Society; San Francisco 2008

(2) Wang X, et al., PNAS 1994; 91:13911

(3) Suzuki K, et al., J Bio Chem 1990; 265:11320

(4) Tominaga S, et al., J Biochem 2002; 132:881

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by grants from the Diabetes Research Initiative at Ohio University, the AMVETS organization, NIH grants DK075436, AG19899 and 1P01AG031736.

Nothing to Disclose: ESG, DCT, JJK

P1-225

Seasonal Regulation of the GH/IGF-I Axis in the American Black Bear (*Ursus Americanus*) Modulates Energy Metabolism and Prevents Excessive Ketogenesis in Winter Denning.

Stanley Blumenthal MD¹, Rebecca Morgan-Boyd PhD², Ralph Nelson MD, PhD² and Terry Unterman MD¹.

¹Univ of Illinois at Chicago and Jesse Brown VA Chicago, IL and ²Univ of Illinois and Carle Res Foundation Urbana, IL.

The American black bear, *Ursus americanus*, exhibits an extraordinary capacity to maintain lean body mass despite prolonged (4 months) starvation during winter denning. From late summer until December, the bear consumes up to 20,000 kcal/d and accumulates an enormous store of fat, its sole source of energy and water (through metabolism) once winter sleep begins. We asked whether changes in the GH/IGF-I axis may contribute to this remarkable adaptation to prolonged starvation.

In initial studies, we measured IGF-I levels by RIA in serum samples from wild and captive male black bears after acid-solid phase extraction to remove binding proteins (ursine GH was not detected in assays for human or bovine GH). IGF-I levels followed a highly reproducible pattern of seasonal regulation in captive bears studied for 3 consecutive years. IGF-I levels were highest in spring and summer (496 ± 16 ng/mL), then fell in autumn (279 ± 24) when bears were hyperphagic in preparation for denning. IGF-I levels fell further during early denning (168 ± 8) but then rose to exceed pre-denning levels despite continued starvation (343 ± 17 , $P < 0.001$ vs. autumn levels). A similar pattern of seasonal regulation of IGF-I levels was observed in black bears living in the wild.

To explore the role that regulation of activity in the GH/IGF-I axis may play in winter denning, we administered GH (0.1 mg/kg/d x 5d) to 3 black bears in December. GH treatment increased IGF-I levels and decreased blood urea nitrogen, indicating that bears remain responsive to GH during starvation and that GH/IGF action has protein-sparing effects. At the same time, GH treatment accelerated weight loss, indicating that increased GH also had catabolic effects, possibly due to accelerated fatty acid (FA) metabolism. Serum studies revealed that GH induced a significant ketoacidosis (bicarbonate decreased from 25 to 15 mEq/L and the anion gap rose from 12 to 26 mEq/L, while levels of beta-hydroxybutyrate increased 10-fold [0.67 to 6.8mM] reflecting increased free FA levels) which was reversed after GH was discontinued. These results demonstrate seasonal regulation of GH/IGF-I axis activity in black bears. Diminished GH secretion/action may serve to promote fat storage in preparation for denning and prevent excessive ketogenesis and premature exhaustion of fat stores, the principal source of energy, during the winter den.

Nothing to Disclose: SB, RM-B, RN, TU

P1-226

Exercise and Fasting Activate Growth Hormone Dependent Myocellular STAT5b Phosphorylation and IGF-I mRNA Expression in Humans.

MH Vendelbo MD¹, N Jessen MD¹, JO Jorgensen MD¹ and N Moller MD¹.

¹Århus Univ Hosp NBG Århus, Denmark.

Physiologically growth hormone (GH) secretion is stimulated by stress, such as fasting and exercise. In humans, high levels of GH activate the GH receptor leading to phosphorylation of signal transducer and activator of transcription (STAT)5. Phosphorylated STAT5 dimerizes and translocates to the nucleus, where a STAT5b binding site has been characterized in the IGF-I gene promoter region.

The present study was consequently designed to test the hypothesis that fasting and exercise would lead to increased GH levels and subsequently to increased STAT5b phosphorylation and increased IGF-I mRNA levels in human skeletal muscle tissue.

Methods: The participants were eight healthy men (25.5 ± 4.3 yr, 82.9 ± 8.8 kg body weight, 23.8 ± 1.6 kg/m² body mass index, 4189 ± 465.5 ml/min VO₂max), which in a randomized cross-over design were examined on 3 days separated by at least 1 month: 1) in the postabsorptive state after an overnight fast of 10 h, 2) after 72 h fasting and 3) after 1 h ergometer cycling at 65% VO₂max. The experimental day was divided in a 4 h basal and 2 h hyperinsulinemic euglycemic clamp period. At t = 30 and 270 min, a muscle biopsy was obtained from vastus lateralis.

Results: During exercise GH concentrations rapidly increased to a peak of $10,61 \pm 2,22$ ng/ml and remained elevated for > 1 h ($p < 0.001$). During fasting more sporadic GH bursts occurred, leading to an overall increase in AUC_{t=0-270}; control (195 ± 45) vs fasting (633 ± 98) ($p < 0,001$). At t = 30 min after exercise we observed a 400 percent increase in STAT5 phosphorylation ($p < 0,001$), whereas 72 h fasting did not significantly increase STAT5 phosphorylation ($p = 0,62$). IGF-1 mRNA expression was significantly increased both after exercise and fasting ($p = 0.026$ and 0.008 , respectively).

Conclusion: These results demonstrate that exercise and fasting are associated with increased GH levels and increased IGF-1 mRNA expression in muscle, whereas only exercise with an orderly and well defined GH surge increased STAT5 phosphorylation. Our data are compatible with the notion that endogeneous GH secretion during physiological exercise and fasting activates the intracellular GH signal pathway in a manner similar to administration of exogenous GH. The GH peak during exercise promotes higher IGF-1 mRNA expression through upregulation of the STAT5 pathway, and suggests that the same mechanism is present during fasting.

Nothing to Disclose: MHV, NJ, JOJ, NM

P1-227

The Prolactin Receptor Is Expressed in Macrophages within Human Carotid Atherosclerotic Plaques; a Role for Prolactin in Atherogenesis?.

A.Q. Reuwer MD¹, M. Eijk PhD¹, F.M. Houttuijn-Bloemendaal¹, C.M. van der Loos PhD¹, N. Claessen¹, P. Teeling¹, J.J. Kastelein MD PhD¹, J. Hamann PhD¹, V. Goffin PhD², J. Aten PhD¹, J. von der von der Thusen MD PhD¹ and Th.B. Twickler MD PhD¹.

¹Academic Med Ctr Amsterdam, Netherlands and ²Fac of Med, Necker Site Paris, France.

Background: Atherosclerotic vascular disease is the consequence of a chronic inflammatory process¹ and prolactin has been shown to be a component of the inflammatory response².

Materials and methods: Human carotid atherosclerotic plaques were obtained from 25 patients by endarterectomy. We evaluated the presence of the prolactin receptor therein using quantitative real time PCR, in situ hybridization, immunohistochemistry and immuno-electron microscopy.

Results: The mRNA of prolactin receptor, but not of prolactin, was detected in atherosclerotic plaques by quantitative real time PCR. In situ hybridization confirmed the expression of prolactin receptor in mononuclear cells. Analysis at the protein level using immunohistochemistry and immuno-electron microscopy revealed that the prolactin receptor was abundantly present in macrophages near the lipid core and shoulder regions of the plaque.

Conclusion: Our findings demonstrate that the prolactin receptor is present in macrophages of the atherosclerotic plaque at sites of most prominent inflammation. We propose that prolactin receptor signalling contributes to the local inflammatory response within the atherosclerotic plaque and therefore to atherogenesis.

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Sources of Research Support: Dr. Th.B. Twickler is recipient of a grant from the Netherlands Organization of Health and Science (NWO-ZONMW).

Nothing to Disclose: AQR, ME, FMH-B, CMvdL, NC, PT, JJK, JH, VG, JA, JvdT, TBT

P1-228

Differential Expression of microRNAs in Craniopharyngiomas: Potential Oncogenic Functions.

ML Campanini MD¹, TPF Cabral PhD¹, BMC Paixao PhD¹, AC Moreira MD, PhD¹, SR Antonini MD, PhD¹ and M Castro MD, PhD¹.

¹Sch of Med of Ribeirao Preto of Univ of Sao Paulo Ribeirao Preto, Brazil.

The pathogenesis of craniopharyngiomas (CP) is poorly understood. CP could arise from neoplastic transformation of embryonic squamous cell rests of the involuted craniopharyngeal duct or they could result from metaplasia of adenohypophyseal cells in the pituitary stalk or gland. Mutations of the glycogen synthase kinase 3b (GSK3b) binding domain of β -catenin gene has been detected in adamantinomatous CP. MicroRNAs (miRNAs) are small non-coding RNAs and contribute to cancer development/progression. We analyzed the expression, by real-time PCR, of a panel of miRNAs in 16 patients with adamantinomatous CP (9 children/ 7 adults), previously screened for β -catenin mutations (8 positive and 8 negative), and 15 normal pituitaries. We also verified whether the presence of β -catenin mutations was associated with age of patients, tumor size or different miRNA expression. Relative quantification of miRNA expression was calculated using the 2^{-DDCt} method. Fold change of the expression of each miRNA was determined by the median of 2^{-DDCt} values of CP related to median of 2^{-DDCt} values of normal pituitaries. We observed hyperexpression of miR-150 (1.7-fold; $p=0.01$); no different expression of miR-16-1, miR-21 and miR23a; and an underexpression of miR-141 (-7.5-fold; $p<0.0001$), Let-7a (-7.4-fold; $p<0.001$), miR-16 (-7.2-fold; $p<0.0001$), miR-449 (-6.6-fold; $p=0.007$), miR-145 (-5.4-fold; $p=0.002$), miR-143 (-3.8-fold; $p=0.01$), miR-23b (-3.2-fold; $p=0.02$), miR-15a (-2.5-fold; $p=0.009$), and miR-24-2 (-2.5-fold; $p=0.01$) in CP compared to normal pituitaries. Younger patients presented more frequently β -catenin mutation (7/9 patients) than adults (1/7) ($p=0.01$). On the other hand, there was no association between tumor size or the expression of each miRNA and the presence of β -catenin mutation. In conclusion, our data suggest that β -catenin mutation can be related to the pathogenesis of CP in children. In addition, we demonstrated, for the first time, a hyperexpression of miR-150, an oncogenic miRNA, and an underexpression of Let-7a, miR-16, miR-15a, miR-23b, miR-24-2, miR-141, miR-143, miR-145, and miR-449 in CP, which are tumor suppressive. These data indicate that these miRNAs might potentially target genes encode proteins with potential oncogenic functions which could have a role in the pathogenesis of CP. Further studies are needed to predict miRNA target genes for either downregulated or upregulated miRNAs in CP.

Sources of Research Support: Sao Paulo Research Foundation Grant 07/58365-3.

Nothing to Disclose: MLC, TPFC, BMCP, ACM, SRA, MC

P1-229

Persistent Doxycycline-Induced HA-V12Ras/pMAPK Diminishes the Transformed Phenotype of GH4 Pituitary Somatotrope Tumor Cells in a Checkpoint-Dependent Manner.

A Gutierrez-Hartmann MD¹ and T Trudeau BA¹.

¹Univ of Colorado Denver Aurora, CO.

Neuroendocrine tumors are typically benign adenomas, and they rarely progress to frank carcinoma. Despite significant efforts, our understanding of clinically relevant mechanisms underlying human pituitary tumorigenesis remains ashamedly meager. Prolactinomas are the most common pituitary tumor and they respond well to dopamine agonists. The growth factor/Ras/MAPK pathway appears to contribute to prolactinoma formation, but it remains unknown whether the timing and/or levels of MAPK activation dictate outcome, and if extra hits are required in combination with activated pMAPK. To address the role of Ras/MAPK in pituitary adenoma formation and to better understand the molecular mechanisms by which neuroendocrine cells fail to progress to carcinoma, we generated clonal GH4 somatotrope cell lines expressing a tightly-regulated, Doxycycline (Dox)-inducible HA-V12Ras. Using two such lines, clones #10 and #20, we show a stringent Dox-dose dependent induction of HA-V12Ras expression, with maximal induction occurring at 1 µg/ml Dox. Western blot analysis over a 6-day time-course study revealed that, at a maximal Dox-dose of 2 µg/ml, expression of HA-V12Ras and pMAPK persists at high levels for the first 4 days, but then decreases in the last 2 days post-Dox. Marker gene expression revealed a similar pattern for PRL expression, whereas GH, Pit-1 and cMyc showed minimal changes in expression initially, but their expression was reduced in the last 3-4 days of Dox. Menin was unchanged and p21Cip1 showed less consistent changes. Persistently-activated HA-V12Ras/pMAPK caused a 60% reduction in soft agar colony number and a 70% decrease in xenograft tumor size in nude mice. Tumor IHC analyses revealed persistent expression of HA-V12Ras at 30 days only in the Dox-treated mice, yet BrdU incorporation was equivalent ± Dox. Similarly, persistent HA-V12Ras/pMAPK induction had no effect on the in vitro proliferation rate, but knockdown of p21Cip1 or p27Kip1 checkpoint did increase GH4 cell proliferation, but only in the setting of Dox-induced pMAPK. Taken together, these data reveal that GH4 pituitary cells are resistant to an enhanced tumorigenic phenotype induced by persistent V12Ras/pMAPK expression, via a checkpoint-dependent mechanism; but persistent pMAPK, combined with checkpoint knockdown, promotes tumorigenesis.

Sources of Research Support: NIH R01 DK46868.

Nothing to Disclose: AG-H, TT

P1-230

MicroRNA-200c Is Downregulated in Sparsely-Granulated Growth Hormone Pituitary Tumors and Regulates ZEB1 Expression in GH-Derived Cells.

Deborah L Fretwell B.S.¹, Aaron J. Knox B.S.¹, Mei Xu B.S.¹, Margaret E. Wierman M.D.², Kevin O. Lillehei M.D.¹, Bette K. Kleinschmidt-DeMasters M.D.¹, Aik-Choon Tan Ph.D.¹ and Janice M. Kerr M.D.¹.

¹Univ of Colorado, Denver Aurora, CO and ²VA Med Ctr Denver, CO.

MicroRNAs (miRs) are a newly-discovered class of non-coding RNAs that regulate gene expression by the post-transcriptional repression of target mRNAs. Dysregulation of microRNAs is implicated in several tumorigenic processes, including cell proliferation and migration. The down-regulation of miR-200c, in particular, has been linked to more aggressive breast and endometrial tumor phenotypes, by the loss of the transcription factor, Zinc-finger E-Box-binding homeobox 1 (ZEB1), and its repressive effects on E-cadherin. The potential role of microRNAs in pituitary tumorigenesis is not well studied to date. The 'microRNome' of sparsely-granulated growth hormone (GH)-secreting pituitary tumors, a GH tumor sub-type that is classically large and invasive at time of diagnosis, was investigated using a Dharmacon microRNA expression profile (of 900 human microRNAs from the Sanger 11.0 database) that compared 3 sparsely-granulated growth hormone adenomas with age- and gender-matched normal pituitary glands. Forty microRNAs were downregulated and 23 microRNAs were upregulated (≥ 2 -fold change, P-value < 0.05). The miR-200 family members, including: 200c, 200a, 200b, 141 and 429 were the most significantly changed at 31, 22, 12, 28, 5-fold decreased levels, respectively. Confirmatory qRT-PCR studies, using 8 sparsely-granulated GH tumors, showed a 36-fold decreased level of miR-200c, when compared to 6 densely-granulated GH tumors and to 7 normal pituitaries. To determine the functional significance of miR-200c in pituitary cells, transient miR-200c knock-downs in GH₃ cells, a rat somatotrope cell line that expresses miR-200c at a level comparable to the normal human pituitary gland, were performed. A $>95\%$ transient silencing of miR-200c was associated with ~ 2.0 -fold increased ZEB1 mRNA and protein expression. This finding was corroborated by qRT-PCR in 6 human sparsely-granulated GH tumors, which showed a 2-fold increased ZEB1 mRNA expression (p = 0.04), compared to densely-granulated GH tumors. Thus, miR-200c is downregulated in sparsely-granulated GH tumors, compared to densely-granulated growth hormone tumors or normal pituitary, and modulates ZEB1 expression in GH-derived cells.

Sources of Research Support: NIH(K08DK069511).

Nothing to Disclose: DLF, AJK, MX, MEW, KOL, BKK-D, A-CT, JMK

P1-231

Vascular Density and Nestin Expression in Human Pituitary Adenomas.

C Cristina PhD^{1,2}, MI Perez Millan MS¹, G Luque Miss¹, SI Berner MD³ and D Becu PhD¹.

¹IBYME Buenos Aires, Argentina ; ²Univ Natl del Noroeste de la Prov de Buenos Aires Junín, Argentina and ³Sanatorio Santa Isabel Buenos Aires, Argentina.

Angiogenesis has been extensively studied in malignant tumors but its role is controversial and less understood in pituitary adenomas. We studied its relation to the expression of nestin, a marker of progenitor cells that has recently been reported in the pituitary but whose localization and function are yet to be explored.

Vascularization of normal and tumoral pituitaries was evaluated by immunohistochemistry using CD31 and CD34 as markers of endothelial cells. We also analyzed by immunohistochemistry, the existence of pituitary stem cells assessing the expression of nestin. The proliferative activity of the tumors was tested by the Ki-67 index. Adenomas (all macroadenomas) were classified as GHomas (GH, N=9), Non Functioning adenomas (NF, no hormone produced, N=27) and Prolactinomas (PRL, N=10).

In 51 samples analyzed, we detected a lower vascular area (% of area occupied by vessels) in pituitary tumors when compared to normal tissue (N= 5), $P < 0,05$ when determined both by CD31 and CD34 immunoreactivity. Interestingly,

pituitary adenomas had predominantly small vessels (area less than $100 \mu\text{m}^2$) whereas normal pituitaries showed mainly large vessels ($P < 0,05$ for the relation % small vessels / % large vessels for both CD34+ and CD31+ vessels). In tumoral samples several but not all capillaries were positive for nestin whereas practically no or scarce staining was detected in normal glands. Significant differences were found between controls and tumors when the % of area nestin+ was quantified ($P < 0,05$ control vs. adenomas).

Ki-67 correlated neither to vascular area nor to the % of area stained for nestin in the different pituitary adenomas.

In conclusion, our results indicate that angiogenesis is active in human pituitary adenomas in which small vessels are abundant, and nestin expression in progenitor endothelial cells is evident. On the other hand we determined that the highly vascular normal pituitary gland has mostly large blood vessels with quiescent endothelia where no or sparsely nestin immunopositivity was detected. The low grade of angiogenesis that characterizes pituitary macroadenomas may play a role in the usually slow growth of these tumors and the rare cases of metastasis found. Nevertheless, understanding the role of angiogenesis on the development of these tumors may facilitate therapeutical decisions in the cases of adenomas that are unable to be controlled by conventional therapy.

Nothing to Disclose: CC, MIPM, GL, SIB, DB

P1-232

Transforming Growth Factor beta (TGF β) Signalling Is Inhibited in Anterior Pituitary Tumours in a Mouse Model of Multiple Endocrine Neoplasia Type 1 (MEN1).

GV Walls BA(Hons), MB BS¹, MA Nesbit BA(Hons), PhD², J Jeyabalan BSc(Hons), DPhil¹, PJ Newey BSc(Hons), MB ChB¹, M Javid MA, MB BS¹, HE Schulz PhD², N Huebner MD² and RV Thakker MD¹.

¹Univ of Oxford Oxford, UK and ²Max-Delbrueck Ctr for Molecular Med Berlin, Germany.

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the combined occurrence of tumours of the parathyroids, pancreatic islets, and anterior pituitary. The gene causing MEN1 encodes a 610 amino acid protein, menin, which has roles in transcription regulation, genome stability and cell proliferation. For example, menin is known to interact with the transcription factor Smad3, which is an effector of the antiproliferative signalling ligands transforming growth factor beta (TGF β) and activin, and menin has been shown to be required *in vitro* for Smad3-mediated growth inhibiting properties in the anterior pituitary. To further investigate the *in vivo* role of menin in this pathway, we compared the cDNA expression profiles of somatolactotrophinomas from *Men1*^{+/-} mice, to that of normal pituitaries from *Men1*^{+/+} mice (n=5 in each group). RNA was extracted and biotin-labelled cRNA hybridised onto Affymetrix Mouse Genome 430 v2.0 arrays. Of the 45101 transcripts, 3.6% were differentially expressed between *Men1*^{+/-} pituitary tumours and *Men1*^{+/+} pituitaries, with a false discovery rate significance of <0.05. Alterations in TGF β signalling pathway components were found in the *Men1*^{+/-} pituitary tumours. Thus, chordin and noggin, inhibitors of bone morphogenic protein (BMP) signalling, and follistatin-like 5, an inhibitor of activin signalling, were up-regulated (+1.9, +2.9 and +3.3 fold, respectively); whilst the ligands BMP6 and TGF β 2, its type II receptor TGF β R2 and the signalling effectors Smad1, Smad3 and Smad7 were all down-regulated (-2.6, -2.1, -2.0, -1.9, -1.9 and -1.6 fold, respectively). Furthermore, inhibitor of DNA binding 4 (Id4), mitogen-activated protein kinase 8 (Mapk8 or JNK), p27 (Cdkn1b) and cAMP responsive element binding protein 1 (Creb1), TGF β pathway targets, were down-regulated (-3.4, -1.5, -1.4 and -1.4 fold, respectively). The findings for the ligand TGF β 2, its receptor TGF β R2 and BMP pathway target Id4 were confirmed by quantitative reverse transcriptase PCR (-2.9, -2.4 and -3.6 fold, *P*<0.02, Student's *t*-test). Protein expression of Smad7 and p21 (Cdkn1a), assessed by immunohistochemistry, was decreased in *Men1*^{+/-} pituitary tumours compared to *Men1*^{+/+} pituitaries. In conclusion, the TGF β signalling pathway is down-regulated in mouse MEN1-associated anterior pituitary tumours suggesting that modulation of TGF β , TGF β R2, p21 and Id4 may be potentially useful in the treatment of these endocrine tumours.

Sources of Research Support: Medical Research Council Clinical Research Training Fellowships (G0501780, G0601423), UK awarded to GVW and PJN; Wellcome Trust Clinical Training Fellowship (087332/Z/08/Z), UK awarded to MJ; Medical Research Council Programme Grant (G9825289, 2004), UK awarded to RVT.

Nothing to Disclose: GVW, MAN, JJ, PJN, MJ, HES, NH, RVT

P1-233

Expression of Survivin in Different Types of Pituitary Adenomas.

Magdalena Jaskula MD PhD¹, Ryszard Wasko MD PhD Professor¹, Joanna Waligorska-Stachura MD PhD¹, Anna Jankowska MD PhD¹, Malgorzata Kotwicka MD PhD¹, Wlodzimierz Liebert MD PhD Professor¹, Marek Bolanowski MD PhD Professor² and Jerzy Sowinski MD PhD Professor¹.

¹Univ of Med Scis Poznan Poland Poznan, Poland ; ²Univ of Med Scis Poznan Poland Poznan, Poland ; ³Univ of Med Scis Poznan Poland Poznan, Poland and ⁴Med Univ Wroclaw Wroclaw, Poland.

Introduction: Survivin is a protein which belongs to the family of apoptosis inhibitors. Its expression was detected in the majority of human neoplasms. The level of survivin expression shows a positive correlation with the fast tumour progression. It has been proposed a negative prognostic factor for numerous neoplasms. Our study was designed to localize survivin peptide in different types of pituitary tumors and to correlate its expression with the ability of the cells to proliferate.

The **aim** of our study was to evaluate the expression and localization of survivin gene on the protein level in different types of pituitary tumors and normal pituitary. The coexpression of survivin and proliferating cell nuclear antigen - PCNA in pituitary was also examined.

Material and Methods: the studied tissues consisted of 32 pituitary tumors obtained during the transsphenoidal surgery and included 23 invasive and 9 non-invasive tumors. There were 20 somatotroph adenomas (obtained from patients treated with octreotide LAR or lanreotide Autogel before the surgery), 2 prolactinomas, 3 adrenocorticotropinomas and 7 non-functioning adenomas. As a control for the study, normal pituitary tissue obtained at autopsy was used. The evaluation of survivin and PCNA expression was performed with the use of immunohistochemical staining with antibodies against the study antigens.

Results: The presence of survivin peptide was demonstrated in all examined pituitary tumours. The immunohistochemical localization of survivin in the pituitary tumours tissue showed that the protein is localised mainly in cell nucleus, however some cytoplasmic staining within a few cells was observed. Survivin was also found in single cells of normal pituitary. The analysis of pituitary tumor cells proliferation index, based on PCNA reactivity, showed that survivin and PCNA are coexpressed especially in case of invasive tumors.

Conclusions: Survivin plays an important regulatory function in both normal and neoplastic cells of the pituitary. The higher expression of survivin gene in pituitary tumors compared with the normal pituitary suggests that the presence of survivin might be one of the factors of neoplastic transformation in the pituitary gland. The co-expression of survivin and proliferating cell nuclear antigen (PCNA) in pituitary tumours, clearly increased in the case of the invasive tumours, seems to confirm the correlation between survivin and cell proliferation.

Nothing to Disclose: MJ, RW, JW-S, AJ, MK, WL, MB, JS

P1-234

A Subset of Non Functioning Pituitary Adenoma with Immune Positivity for Anterior Pituitary Glycoprotein Hormones Presents under Expression of DLK1 and miR-143 and Overexpression of GSTP1 on Its Molecular Pathogenesis.

BMC Paixao PhD¹, DS Lima PhD¹, F Saggiaro MD¹, AC Moreira MD, PhD¹, HR Machado MD PhD¹ and M Castro MD, PhD¹.

¹Sch of Med of Ribeirao Preto of Univ of Sao Paulo Ribeirao Preto, Brazil.

The pathogenesis underlying non functioning pituitary adenoma (NFPA) has not been well established. MicroRNAs (miRNAs) might contribute to cancer development/progression. **Objective:** To generate the first serial analysis of gene expression (SAGE) library and validate genes and a panel of miRNAs differentially expressed in NFPA; to verify whether miRNAs correlate with target genes, tumor size, clinical control after surgery, and immunohistochemistry findings in NFPA. **Material and Methods:** MiRNAs and Genes differentially expressed were validated by real time PCR in 29 NFPA and in 15 normal pituitaries. Relative quantification of genes and miRNA expression was calculated by 2^{-DDCt} method. **Results:** We observed no expression of DLK1, an underexpression of EGR1 (-16.6-fold; p<0.0001), an over-expression of GSTP1 (4.8-fold; p=0.0009), NFkBIA (3.1-fold; p=0.0004), and APP (2.8-fold; p= 0.01) in NFPA. We observed no expression of miR-21 and miR-145 and underexpression of miR-141 (-33.3-fold; p=0.002), miR-16 (-14.3-fold; p=0.76), let-7a (-11.1-fold; p<0.0001), miR133a (-7.1-fold; p=0.001), miR-150 (-6.6-fold; p=0.0008), and miR-143 (-4.0-fold; p<0.0001) in NFPA. There was an association between bigger tumors and immune positive staining to anterior pituitary hormones (p=0.02) and a trend of positive immunohistochemistry and a worse chance to achieve clinical control (p=0.07). Higher expression of GSTP1 was associated with positive staining on immunohistochemistry (p=0.05). There was no association between the expression of let-7a, miR-21, miR-133a miR-141 and miR-145 and tumor size or immunohistochemistry. The lower expression of miR-16 was associated with bigger tumors (p=0.01) and the lower miR-143 expression associated with positive staining on immunohistochemistry (p=0.03). We observed a negative correlation of APP gene and miR16 in normal pituitaries and tumors (r=-0.68;p=0.004; r=-0.58; p=0.001). **Conclusion:** we report the first SAGE library for NFPA. The association of miR-16/APP and miR150/GSTP1 suggests a role of these miRNAs in modulating these genes. The higher expression of the *GSTP1* and under expression of miR-143 seem to be involved in pathogenesis of a subset of NFPA with positive staining for anterior pituitary hormones, which tumors were bigger and had worse chance to achieve clinical control. Our data contribute to elucidate the molecular pathogenesis of NFPA, improving the future classification of its histotypes.

Nothing to Disclose: BMCP, DSL, FS, ACM, HRM, MC

P1-235

TGF β -1 in a Prolactinoma Experimental Model: The Dopamine Receptor D2 Knock out Mouse (*Drd2*^{-/-}).

MV Recouvreux¹, MC Guida¹, D Becu-Villalobos¹ and G Diz-Torga¹.

¹IBYME-CONICET Buenos Aires, Argentina.

Prolactinomas are the most frequent pituitary tumors. Dopamine type 2 receptor (*Drd2*) mediates dopamine inhibition of prolactin on lactotropes. Therefore, female *Drd2*^{-/-} mice develop prolactinomas, chronic hyperprolactinemia and lactotrope hyperplasia. TGF β 1 is a potent cytokine that regulates many biological functions such as growth and apoptosis in various cell types. TGF β 1 must undergo a highly regulated activation process to be functional. Activation consists in dissociation of the cytokine from its large latent complex that sequesters and attaches it to the extracellular matrix. TGF β 1 is locally synthesized by lactotropes where it inhibits cell proliferation and prolactin synthesis. It was suggested that TGF β 1 might mediate, in part, dopamine inhibitory action on lactotropes in rats.

On this basis, the aim of this study was to evaluate total and active TGF β 1 and its regulation by dopamine and estradiol, in pituitary glands from *Drd2*^{-/-} and wild-type (wt) mice.

Active or total TGF β 1 content in pituitary homogenates was quantified by ELISA with a TGF β 1 Emax

ImmunoAssay-System (PROMEGA). Significantly lower pituitary active TGF β 1 was found in both male and female *Drd2*^{-/-} mice when compared with wt mice ($p < 0.0001$). Females of both genotypes showed lower active TGF β 1 levels than males ($p = 0.0003$). However, we did not find significant differences in total pituitary TGF β 1 between genotype or sex. On the other hand, we found that ip sulpiride (10mg/kg, 24hs) lowered active pituitary TGF β 1 in wt females but not in males ($p = 0.01$), although it increased serum prolactin levels in both sexes. Cabergoline treatment (2mg/kg, 24hs) did not affect active pituitary TGF β 1 content in either sex, in spite of its lowering effect on serum prolactin. Surprisingly, estradiol treatment (0.2mg/kg, sc. 24hs) increased active TGF β 1 in *Drd2*^{-/-} pituitaries from female mice ($p = 0.013$), without affecting total TGF β 1 in either sex or genotype.

In summary, we demonstrate that active but not total pituitary TGF β 1 content is reduced by disruption of the *Drd2*. Our results suggest a dopaminergic and estrogenic control on cytokine activation process at the pituitary level. We also found a sexually differentiated dopaminergic and estrogenic control on pituitary active TGF β 1 expression. The lower active TGF β 1 in *Drd2*^{-/-} pituitaries could contribute to prolactinoma development, positioning TGF β 1 as a possible target for therapeutic treatment.

Sources of Research Support: CONICET; ANPCYT.

Nothing to Disclose: MVR, MCG, DB-V, GD-T

P1-236

A Correlation of Endocrine and Anticancer Effects of GHRH Antagonists.

Magdolna Kovacs MD., PhD.^{1,2}, Andrew V. Schally PhD.^{2,3}, Florian Hohla MD.^{2,3,4}, Ferenc G. Rick MD.^{2,3}, Éva Pozsgai MD.^{2,3}, Luca Szalontay MD.^{2,3}, József J. Varga PhD.^{2,3} and Márta Zarandi PhD.^{2,3,5}.

¹Univ of Pécs, Med Sch Pécs, Hungary ; ²Univ of Miami Miller Sch of Med Miami, FL ; ³Veterans Affairs Med Ctr and South Florida Veterans Affairs Foundation for Res and Education Miami, FL ; ⁴Paracelsus Med Univ Salzburg, Austria and ⁵ Szeged Univ, Albert Szent-Györgyi Med Ctr Szeged, Hungary.

GHRH receptor antagonists inhibit growth and metastasis of a large number of experimental tumors expressing the pituitary GHRH receptor (pGHRH-R) and its major splice variant SV1. In this study, using Western-blot, we demonstrated that DBTRG-05 and U-87MG human glioblastoma cell lines express pGHRH-R at levels 6-15 times higher than SV1. To reveal a correlation between the anticancer potency and the endocrine potency on inhibition of GH release, we compared the antitumor effect of GHRH antagonists JV-1-63 and MZJ-7-138 on growth of DBTRG-05 human glioblastomas grafted into athymic nude mice with their inhibitory potency on GH release. JV-1-63 strongly suppressed the stimulated GH secretion induced by clonidine in rats and inhibited the exogenous GHRH-induced GH surge by 88-99% *in vivo* and *in vitro*. MZJ-7-138 decreased the stimulated GH secretion by 58% *in vitro* and showed only a tendency to inhibit GH secretion *in vivo*. The strong inhibitor of GH release JV-1-63 reduced tumor growth of DBTRG-05 glioblastomas in nude mice by 46%, while the weak GH release suppressor MZJ-7-138 did not have an effect. Exposure of DBTRG-05 cells to the GHRH antagonists *in vitro* caused an upregulation of mRNA expression for pGHRH-R and a downregulation of SV1 expression, with JV-1-63 having significantly greater effects than MZJ-7-138. Our results demonstrate that a positive correlation exists between the endocrine potency and the antiproliferative efficacy of GHRH antagonists in tumors strongly expressing pGHRH-R.

Sources of Research Support: Medical Research Service of the Veterans Affairs Department, South Florida Veterans Affairs Foundation for Research and Education and Departments of Pathology and Medicine, Division of Haematology/Oncology Sylvester Comprehensive Cancer Center of the Miller Medical School, University of Miami (all to A.V.S.) and the Hungarian Research Grant of University of Pécs, Medical School (AOK-KA 34039-8-2009 to M.K.).

Nothing to Disclose: MK, AVS, FH, FGR, EP, LS, JJV, MZ

P1-237

The Role of the Transcriptional Co-Repressor CtBP in Pituitary Oncogenesis.

Katie Dorman¹, Zhongyi Shen¹, Shereen Ezzat¹ and Sylvia Asa¹.

¹Ontario Cancer Inst Toronto, Canada.

The C-terminal Binding Protein (CtBP) proteins are ubiquitously expressed transcriptional co-repressors that have been implicated as key factors in mammalian development and tumorigenesis. CtBP1-deficient mice are small and nearly a quarter die by postnatal day 20, while CtBP2-deficient mice are embryonic lethal. CtBP proteins have been shown to contribute to tumorigenesis through promotion of epithelial-mesenchymal transition (EMT), repression of tumor suppressor genes, and anti-apoptotic activity. The physiological roles of CtBP have been well-explored in the immune system and in several developmental programs; however its potential role in pituitary development or tumorigenesis has yet to be examined.

It was previously demonstrated that CtBP interacts with the DNA-binding transcription factor Ikaros both *in vitro* and *in vivo* in immune tissue. This interaction proved to be important in Ikaros' ability to repress transcription of the tk and AdML promoters. We have previously identified Ikaros as a necessary factor in pituitary cell development, differentiation, proliferation, and oncogenic transformation. As such, CtBP may play an important role in the pituitary through its co-repressive effects with Ikaros.

We show that CtBP interacts with wild-type Ikaros (Ik1) and a dominant negative isoform (Ik6) in both AtT20 mouse corticotroph and GH4 rat mammosomatotroph pituitary tumor cells. To identify common promoter targets of repression by Ikaros and CtBP, we have performed chromatin immunoprecipitation (ChIP) followed by NimbleGen's promoter array screen.

CtBP activity is enhanced by high levels of free NADH that are characteristic of hypoxic environments. As such, CtBP has been identified as a key modulator of hypoxia-induced tumor cell migration through repression of adhesion genes such as E-cadherin. Consistent with this prediction, Ikaros levels rise dramatically in GH4 pituitary tumor cells exposed to graded hypoxia. Thus we examined the rate of dissociation between Ikaros and CtBP under these conditions and their distinct roles in pituitary proliferation, cell-cycle progression, and apoptosis.

These studies provide new insights into the exclusive and overlapping functions of two critical members of the chromatin remodelling network in pituitary tumor cells.

Sources of Research Support: Canadian Institutes of Health Research (CIHR).

Nothing to Disclose: KD, ZS, SE, SA

P1-238

Modulation of cAMP- and Calcium-Signalling in GH-Secreting Pituitary Adenomas through Subtype Specific Activation of Somatostatin Receptors.

B Mayr¹, R Buslei¹, M Buchfelder¹ and C Schofl¹.

¹Friedrich-Alexander-Univ Erlangen-Nuremberg Erlangen, Germany.

Introduction: The cAMP- and calcium pathways play crucial roles in growth hormone (GH) secreting pituitary cells. Activation of the cAMP signaling pathway is a frequent cause of pituitary adenomas, while the calcium pathway is pivotal for GH secretion. Both pathways can be modulated by activation of somatostatin receptors (SstRs) with SstR agonists such as octreotide, which are used to treat acromegaly. SstR agonists are clinically effective in 50% of patients and thought to mainly act through SstR-2 in vivo. We studied the expression of SstR subtypes and the in vitro effects of octreotide and SRIF-14 on cAMP- and calcium-signaling in a series of 22 GH-secreting pituitary adenomas.

Methods: Cells isolated from 22 GH-secreting pituitary adenomas were cultured for 3-5 days. Using quantitative RT-PCR to quantify the cAMP target gene ICER and fura-2 microfluorometry to measure intracellular free calcium ($[Ca^{2+}]_i$) we assessed the response of these pathways to octreotide and SRIF-14. Expression of SstR subtypes was determined by immunohistochemistry from paraffin embedded surgical tissues using a panel of antisera against subtypes 1, 2, 3, 4 and 5.

Results: All adenomas responded to octreotide or SRIF-14 treatment with a drop in ICER levels to an average of $30\% \pm 4.4\%$ (mean \pm SEM, $p < 0.0001$) and $34\% \pm 4.6\%$ ($p < 0.0001$) of control levels respectively. Cells from 19 of 22 adenoma samples showed a significant decrease in basal $[Ca^{2+}]_i$ from 114 ± 7 to 81 ± 4 nM in response to 100 nM octreotide (mean \pm SEM, $p < 0.0001$) and from 99 ± 4 to 80 ± 3 nM after treatment with 100 nM SRIF 14 ($p < 0.0002$). All adenomas insensitive to octreotide were also resistant to SRIF-14. Neither octreotide nor SRIF-14 inhibited the K^+ (45 mM)-depolarization induced increase in $[Ca^{2+}]_i$. Membrane staining for SstR-1, 2, 3 and 5 was detected in 86%, 14%, 9% and 36% of samples whereas cytoplasmic staining was detected in 100%, 100%, 50% and 55% respectively. SstR-4 staining was not detectable in any tumour sample. There was no significant correlation with the in vitro sensitivity of the cAMP- or calcium pathway to octreotide or SRIF-14 for any of these groups.

Discussion: Spontaneous cAMP-signalling and cytosolic calcium activity was suppressible by octreotide and the SstR-panagonist SRIF-14 in the majority of GH-secreting pituitary adenomas. No sample showed selective sensitivity to octreotide or SRIF-14. There is no apparent correlation with the SstR subtype staining pattern in surgical specimens.

Nothing to Disclose: BM, RB, MB, CS

P1-239

Pituitary Transcription Factors and β -Catenin Gene Mutations in Adamantinomatous Craniopharyngiomas.

ML Campanini MD¹, SR Antonini MD, PhD¹, FC Amaral PhD¹, HR Machado MD, PhD¹, AC Moreira MD, PhD¹ and M Castro MD, PhD¹.

¹Sch of Med of Ribeirao Preto of Univ of Sao Paulo Ribeirao Preto, Brazil.

The pathogenesis of Craniopharyngiomas (CP) is poorly understood. CP may arise from embryonic remnants of Rathke's pouch, which is the primordium of the anterior and intermediate lobes of the pituitary gland. Several transcription factors play important role in pituitary lineage specification and cell-type-specific gene expression. In order to elucidate their contribution to CP tumorigenesis, we performed the molecular analysis of three homeodomain containing transcription factors, *HESX1*, *PROP1*, *POU1F1*, involved in anterior lobe of the pituitary gland development, and the β -catenin gene, a co-regulator for transcription activity of *PROP1*. We studied 16 patients (8F/8M; 6-55years, median 15.5years) with adamantinomatous CP followed from 2005-2008. All patients underwent clinical, neurological and biochemical evaluation to diagnose hypothalamic and pituitary dysfunctions. Tumoral DNA/RNA were extracted by TRizol® and cDNA was synthesized (TaqMan RT reagents) from tumor tissues; the entire coding region of *HESX1*, *PROP1*, *POU1F1* genes and exon 3 of β -catenin gene were amplified by PCR, followed by automatic sequencing. For comparison, we sequenced 50 controls. No mutations were identified in *HESX1*, *PROP1*, *POU1F1* genes. However, we found four polymorphisms (SNPs) in *PROP1* gene, the IVS+3G>A at intron A, rs 7445271, and rs1135320 at exon 1 and rs1800197 at exon 3. All polymorphisms were in Hardy-Weinberg equilibrium. The allelic frequency of these polymorphisms in CP patients was similar to controls. We found seven different mutations in β -catenin gene in eight patients (50%), c.94G>A at codon 32 (Asp32Phe); c.122C>T at codon 41 (Thr41Ile); c.98C>G at codon 33 (Ser33Cys); c.98 C>T at codon 33 (Ser33Phe); c.101A>C at codon 34 (Gly34Glu); c.121A>G at codon 41 (Thr41Ala); and c.110G>A at codon 37 (Ser37Cys) in two patients. Mutations or SNPs in pituitary transcription factors involved in Rathke's pouch and anterior pituitary development are unlike to contribute to the adamantinomatous craniopharyngiomas pathogenesis. In addition, β -catenin gene mutations on exon 3 were observed in a high prevalence, confirming that this alteration is a hallmark of the adamantinomatous craniopharyngiomas variant also in Brazilian patients.

Sources of Research Support: Sao Paulo Research Foundation Grant 07/58365-3.

Nothing to Disclose: MLC, SRA, FCA, HRM, ACM, MC

P1-240

BCL2, BAX and CASP3 Apoptosis Related Genes Expression in Non-Functioning Pituitary Adenoma and Their Role as Potential Markers of Tumor Behavior.

VAS Cescato MD¹, EM Pinto², NR de Castro Musolino MD¹, SAC Siqueira MD¹ and MJ Teixeira MD¹.

¹Hosp das Clins, Univ of Sao Paulo Med Sch Sao Paulo, Brazil and ²Univ of Sao Paulo Med Sch Sao Paulo, Brazil.

Pituitary adenomas (PA) are slow-growing tumors and account for up to 15% of all intracranial tumors. Non-functioning PA (NFPA) is usually diagnosed due to neurological and ophthalmologic symptoms and account for around one third of all PA. Surgery, the gold standard treatment, is effective in relieving compressive symptoms but cure is uncommon. Despite benign in nature, NFPA usually show aggressive behavior. There are no reliable tumor markers for NFPA. Apoptosis-related genes, BCL2, BAX, and CASP3, were here studied in NFPA to assess their role as potential markers of tumor behavior. Out of 119 patients with PA, operated on from Aug 2008 to Jul 2009, 30 cases (median age 54.5y, 17 men) harboring NFPA were studied. Tumor dimensions and invasiveness were observed by MRI. Intra-operative information such as tumor invasion and consistence was also recorded. Histological examination by HE and immunohistochemistry (IHC) staining of pituitary hormones, Ki-67, P53, and BCL2 were performed. The molecular analysis of BCL2, BAX, and CASP3 genes was performed by RT-PCR in all tumor specimens collected during surgery and compared to a pool of normal pituitary tissues. All patients had macroadenomas diagnosed due to visual loss (87%), headache (53%), other neurological symptoms (17%) and one case was incidentally found. Hormonal deficits were seen in 92% of 26 cases. Median tumor volume was 11.6 cm³. Giant tumors (≥ 4 cm) were diagnosed in 40% and invasive ones in 67% of the patients. A transsphenoidal approach was used in all patients, except one who had pterional craniotomy. Soft tumors were observed in 87% of cases. Five patients presented post-operative complications: 3 had CSF leakage, 2 meningitis and 2 died. PA was confirmed in all cases, 60% were null cell and 40% showed a positive IHC for one or more hormones. IHC for P53 was negative in all cases; for Ki-67 was negative in 11, positive in less than 3% of the cells in 15 and positive in more than 3% of the cells in 4 cases; and for BCL2 was weakly positive only in 3 cases. Expression of BCL2, BAX and CASP3 by RT-PCR analysis revealed very low expression compared to normal pituitary tissues. There was found a positive correlation between the expressions of the three genes but no correlation between them and patient's age, tumor volume or invasion. The BCL2, BAX, and CASP3 expression analysis indicate that these genes are involved in tumorigenesis process but cannot be used as markers of tumor behavior in NFPA.

Nothing to Disclose: VASC, EMP, NRdCM, SACS, MJT

P1-241

Temozolomide as an Adjuvant Treatment for Aggressive Pituitary Tumors: Correlation of Clinical Outcomes with DNA Methylation and MGMT Expression.

ZM Bush MD¹, JA Longtine MD², T Cunningham BS¹, D Schiff MD¹, JA Jane, Jr MD¹, ML Vance MD¹, MO Thorner MD¹, ER Laws MD² and MBS Lopes MD, PhD¹.

¹Univ of Virginia Charlottesville, VA and ²Brigham and Women's Boston, MA.

The typically indolent behavior of pituitary tumors is juxtaposed with high rates of microinvasion into adjacent dural structures, and occasional aggressive behavior. Although clinically significant invasion and malignant transformation remain uncommon, there are limited treatment options available for the management of these aggressive tumors and morbidity and mortality can be high. Several recent case reports have reported clinical efficacy of temozolomide for the adjuvant treatment of aggressive pituitary tumors. We report a series of seven patients with aggressive pituitary tumors that have been treated with temozolomide. We compared *MGMT* promoter methylation and *MGMT* expression in fourteen surgical specimens from these seven patients, and correlated these molecular features with the clinical response to temozolomide. Clinically significant tumor regression was seen in two of seven patients (29%), a 20% reduction in tumor volume with subsequent stable tumor size was noted in one patient, arrest of tumor growth occurred in three patients, and progressive metastatic disease developed during temozolomide treatment in one patient. The DNA promoter site for *MGMT* was unmethylated in all 14 adequate specimens, and variable amounts of *MGMT* expression were seen in all 14 cases. There was no correlation between *MGMT* expression and clinical outcomes. We conclude that adjuvant medical therapy with temozolomide can be helpful in the management of life-threatening pituitary tumors that have failed to respond to conventional treatments. Clinically significant tumor volume regression occurred in two of seven patients, but complete remission was not achieved. Side effects were common and dose limiting in three patients. The optimal duration of treatment in patients with stabilization or reduction of tumor size has not been established, and long term follow up studies are needed.

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Nothing to Disclose: ZMB, JAL, TC, DS, JAJ, MLV, MOT, ERL, MBSL

P1-242

FOXO1 Expression Is Reduced in Human Pituitary Adenomas.

CL Farris B.S.¹, DO Jung B.S.¹, RV Lloyd M.D., Ph.D.² and BS Ellsworth Ph.D.¹.

¹Southern Illinois Univ Carbondale, IL and ²Mayo Clin Rochester, MN.

FOXO1 is a forkhead transcription factor that inhibits proliferation and promotes apoptosis. It regulates genes that are involved in many diverse processes, including regulation of cell proliferation and cancer. The goal of our research is to understand FOXO1 and the role that it plays in human pituitary adenomas. In order to characterize FOXO1's role in cell proliferation, we performed immunohistochemical staining with FOXO1 and bromodeoxyuridine (BrdU) on mouse pituitaries at various ages. We find that FOXO1 does not co-localize with actively proliferating cells labeled with BrdU in the mouse pituitary at ages e12.5, e14.5, and e16.5. Because of the role that FOXO1 plays as a tumor suppressor, we hypothesized that FOXO1 expression in human pituitary tumors would be at lower levels than in normal (non-tumor) human pituitary tissue. To determine FOXO1 expression levels in human pituitary adenomas, real time RT-PCR was performed. We examined two different pituitary adenoma types, gonadotropinoma and null cell adenoma. Null cell adenomas do not produce any hormones but they are believed to be related to gonadotropes. Real time RT-PCR data showed that FOXO1 levels are reduced by 16.5 fold in gonadotropinomas and 14 fold in null cell adenomas as compared to normal human pituitary tissue. These data suggest that FOXO1 may act as a tumor suppressor in pituitary gonadotrope and lactotrope cells.

Sources of Research Support: SIU-SOM start up.

Nothing to Disclose: CLF, DOJ, RVL, BSE

P1-243

Growth Hormone Receptor mRNA Expression and GH Production with and without Pegvisomant in Somatotroph Pituitary Adenomas.

s.j.c.m.m. Neggers MD¹, m. Bidlingmaier MD PhD², p.m. Koetsveld¹, a.m. Waaijers¹, d.m. Sprij-Mooij¹, a.j. van der Lely MD PhD¹ and I.j. Hofland¹.

¹Erasmus Univ Med Ctr Rotterdam Rotterdam, Netherlands and ²Ludwig Maximilians Univ Munich Munich, Germany.

Background: Of the currently available treatment regimes for acromegaly, pegvisomant (PEG-V) has the highest efficacy. During treatment with PEG-V, endogenous growth hormone (GH) serum levels increase. How this is regulated remains unclear. It might be explained by an ultra-short feedback loop via GH receptors in the pituitary.

Objective: To assess the expression of GH receptor (GHR) mRNA in a series of somatotroph adenomas and to evaluate whether PEG-V is able to increase GH secretion by primary cultures of somatotroph adenomas.

Design: In RNA extracted from 11 somatotroph adenomas and 3 samples of human liver tissue, quantitative PCR was performed to determine the mRNA expression level of the full length GHR. Somatotroph adenoma tissue (n=3) was dispersed into single cell suspensions and cultured as described in detail previously¹. GH secretion was assessed

without and with 24 and 72 h incubation with PEG-V, concentrations ranging from 10^{-6} to 10^{-10} M. GH concentration in the culture media was determined by a two-site immunofluorometric assay, which does not cross-react with PEG-V peptide at drug concentrations as high as 50,000 µg/liter^{2, 3}.

Results:

Full length GHR mRNA expression levels in the somatotroph adenomas were low, but detectable, in 7 out of 11 (64%) of cases and amounted only 1,4% of the expression level detected in human liver tissue. The addition of PEG-V (control, 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} M) did not induce significant changes in GH secretion by the cultured somatotroph adenomas after 24h and 72h, respectively

Conclusion: In human somatotroph pituitary adenomas the expression of GHR mRNA is low, compared to liver tissue. PEG-V did not change GH secretion by cultured somatotroph adenomas. Therefore, it seems unlikely that GHR on somatotroph adenomas are responsible for the raise in GH during PEG-V treatment.

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Nothing to Disclose: SJCMMN, MB, PMK, AMW, DMS-M, AjvdL, LJH

P1-244

AIP Mediates the Effects of Somatostatin Analogues in Acromegaly - Evidence from Clinical and Experimental Studies.

HS Chahal MBBS, MRCP, BMedSci¹, N Alband¹, O Ansorge MD², N Karavitaki MD², E Carlsen MD¹, JAH Wass MA, MD, FRCP³, AB Grossman BA, BSc, MD, FRCP¹ and M Korbonits MD, PhD¹.

¹Barts and the London Sch of Med, Queen Mary Univ of London London, UK ; ²John Radcliffe Hosp Oxford, UK and ³Churchill Hosp Oxford, UK.

Background: Recently, germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene have been found to occur in familial and sometimes in early-onset sporadic somatotroph adenomas. These tumors tend to respond less well to somatostatin analogues (SSA), are diagnosed at an earlier age, and behave more aggressively. It has been shown previously that AIP can up-regulate the transcription factor Zac1 in liver cells, and we were able to also demonstrate this in pituitary cells. On the other hand, Zac1 is itself up-regulated in response to SSA. Therefore, we have hypothesised that SSA might mediate their effects by a pathway involving both AIP and Zac1.

Aim: To study the effect of SSA on AIP and Zac1 expression in patients with sporadic acromegaly and in a rodent somato-mammotroph pituitary cell line (GH3).

Methods

Clinical study: Thirty-four patients with sporadic acromegaly were studied: 17 were treated with lanreotide 30mg weekly or fortnightly for a 16 week period prior to transsphenoidal surgery. They were matched (age, sex and tumor size) to 17 patients with no pretreatment prior to pituitary surgery. AIP protein expression was measured by immunohistochemistry.

Experimental study: GH3 rat pituitary cells were treated with 1nM and 100 nM octreotide and AIP and ZAC expression was measured by 'real time' PCR and immunoblotting.

Results: AIP immunostaining was significantly increased in the lanreotide group (60.3±19% positive cells) versus the control group (27.9±11.7%) in both sexes (P<0.001). In female patients there was a correlation between AIP staining and the reduction in IGF-1 levels after lanreotide treatment (R= -0.66, P<0.05). After treatment of GH3 cells with 1nM octreotide, both AIP and Zac1 mRNA expression were significantly increased at 6hr (P<0.02), while AIP protein expression was significantly increased at 9 and 12hr (P<0.01).

Conclusion: Our previous data on the lack of effect of SSA in patients with AIP mutations, together with the increased AIP protein expression in somatotrophinomas after SSA pretreatment, and the in vitro data on AIP and Zac1 up-regulation, suggest that AIP is an important element in mediating the effects of SSA in patients with sporadic acromegaly.

Sources of Research Support: HSC is supported by Medical Research Council.

Nothing to Disclose: HSC, NA, OA, NK, EC, JAHW, ABG, MK

P1-245

Influence of Growth Hormone Receptor (GHR) Exon 3 Genotype on Clinical, Metabolic and Hormonal Profiles in Untreated Acromegalic Patients.

RS Jallad MD, PhD¹, E Trarbach PhD¹, T Chile PhD¹, FHG Duarte MD¹, MD Bronstein MD, PhD¹ and AAL Jorge MD, PhD¹.

¹Hosp das Clins da Fa de Med da FMUSP Sao Paulo, Brazil.

Background: A polymorphism in the human GHR gene resulting from a genomic deletion of exon 3 (d3-GHR) has been associated with clinical presentation, biochemical measurements and response to therapies in acromegaly.

Objective: To evaluate the GH, IGF-I and IGFBP3 levels and the prevalence of comorbidities at diagnosis of acromegaly according to GHR-exon3 genotype

Patients and Methods: Records of 77 untreated acromegalic patients were retrospectively obtained. GHR-exon 3 genotypes (fl/fl, d3/d3 and d3/fl) were assessed by multiplex PCR.

Results: The distribution of patients in the three different genotypes was 43% for fl/fl, 49 % for fl/d3 and 9 % for d3/d3. Clinical characteristics, laboratorial measurements and prevalence of acromegaly-associated comorbidities did not differ significantly amongst the three genotypes groups. The results were expressed as mean \pm SD and range. IGF-I was expressed as SDS and % ULNR.

Table1 Clinical and laboratorial data in acromegalics patients

| | fl/fl | fl/d3 | d3/d3 |
|--------------------------|-------------------------|--------------------------|-------------------------|
| Patients (n 77) | 33 | 38 | 6 |
| Age (yr) | 42 \pm 16 | 46 \pm 13 | 36 \pm 17 |
| Females (%) | 18 | 42 | 33 |
| Genotype GHR (%) | 43 | 49 | 8 |
| IMC (kg/m ²) | 32 \pm 4 | 31 \pm 7 | 27 \pm 6 |
| Baseline GH (μ g/L) | 35 \pm 38 (4-153) | 67 \pm 147 (1-593) | 42 \pm 36 (2-99) |
| Nadir GH (μ g/L) | 23 \pm 27 (2-111) | 37 \pm 87 (2-452) | 59 \pm 48 (5-111) |
| % ULNR IGF-I* | 317 \pm 161 (128-698) | 381 \pm 186 (171-814) | 206 \pm 42 (169-287) |
| IGF-I SDS | 4.8 \pm 1.3 (1.8-6.9) | 4.7 \pm 2.5 (-7.6-7.7) | 4.1 \pm 1.0 (2.7-5.9) |
| IGFBP3 (mg/L) | 10 \pm 12.6 (4.8-7.6) | 7.5 \pm 1.5 (3.2-9.4) | 7.7 \pm 1.2 (6.1-9.3) |
| Diabetes Mellitus (%) | 45 | 34 | 33 |
| Hypertension (%) | 76 | 79 | 67 |
| Colonic polyps (%) | 42 | 42 | 50 |

*% ULNR IGF-I: Percentage of Upper Limit of the Normal age- and sex-matched Range of IGF-I

Discussion and Conclusion: The present data did not show any significant influence of GHR-exon 3 genotype on clinical and laboratorial features in acromegalics patients. This is a controversial issue (1-4). The higher GH level with an extremely wide variation, associated with limitation of statistical power, could be responsible for our results.

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2-Schmid C et al., Clin Chem 2007; 53:1484

3-Bianchi A et al., J Clin Endocrinol Metab 2009; 94:2015

4- Mercado M et al., J Clin Endocrinol Metab 2008; 93:3411

Nothing to Disclose: RSJ, ET, TC, FHGD, MDB, AALJ

P1-246

GH Receptor His49Arg Mutation Was Not Detected in Japanese Acromegalics.

J Yasufuku-Takano MD, PhD^{1,2}, K Takano MD, PhD¹, S Mori PhD¹, S Yamada MD, PhD³, A Teramoto MD, PhD⁴, T Sano MD, PhD⁵ and T Fujita MD, PhD¹.

¹Univ of Tokyo Fac of Med Tokyo, Japan ; ²Pharma & Med Devices Agency Tokyo, Japan ; ³Toranomon Hosp Tokyo, Japan ; ⁴Nippon Med Sch Tokyo, Japan and ⁵Inst of Hlth Bioscis, The Univ of Tokushima Graduate Sch Tokushima, Japan.

[Aim of study]Causes of sporadic GH-secreting pituitary adenomas remain elusive except for *gsp* mutations. Recently, Asa and colleagues reported that heterozygous somatic mutation in GH-receptor gene (His49Leu) is frequently found in sparsely granulated GH-adenomas (6 out of 14 adenomas), while no mutation was found in densely granulated GH-adenomas (0 out of 12 adenomas)(1). Sparsely granulated GH-adenomas are considered to be more aggressive than the densely granulated type with poorer prognosis. We conducted this study to evaluate the clinical significance of this mutation in Japanese Acromegalics. We also studied whether the presence of *gsp* mutation or ptd-FGFR4 have correlations with densely-granulated or sparsely-granulated GH-adenomas.

[Methods]GH-secreting pituitary adenomas from 45 Japanese acromegalic patients were used with patients' written informed consent. This study was approved by the Ethical Committee of University of Tokyo. Genomic DNAs extracted from these adenomas were amplified by PCR GH receptor with primers encompassing His49. We classified the adenomas to sparsely-granulated or densely-granulated by staining pattern for low-molecular weight cytokeratin (CAM5.2; Becton Dickinson, San Jose, CA, USA).

Gsp mutations and presence or absence of ptd-FGFR4 were examined in 44 and 34 out of 45 cases, respectively. (See ref. (2) and (3) for methods.)

[Results]We found no mutation at His 49 in these 45 adenomas. These included 10 sparsely-granulated GH-adenomas and one sparsely-granulated GH-adenoma with densely-granulated pattern of cytokeratin staining.

Within 9 sparsely-granulated adenomas, 6 harbored *gsp* mutation and 3 did not harbor *gsp* mutation. Ptd-FGFR4 was present in 2, absent in 5, and was not evaluated in 2 adenomas.

Within 25 densely-granulated adenomas, 16 harbored *gsp* mutation and 9 were without *gsp* mutation. Ptd-FGFR4 was present in 6, absent in 14, and was not evaluated in 5 adenomas.

[Conclusion]Our finding suggests that GHR His49Arg mutation is rare if not absent in Japanese acromegalic patients. It also suggests that this mutation does not account for sparsely granulated GH-adenoma in Japanese.

Gsp mutation and ptd-FGFR4 occurred similarly in sparsely-granulated and densely-granulated adenomas in this study population.

(1)Asa SL et al.: Cancer Res 67:7505, 2007

(2)Yasufuku-Takano J et al.: Clin Endocrinol (Oxf) 64:91, 2006

(3)Morita K et al.:Clin Endocrinol (Oxf) 68:435, 2008

Sources of Research Support: Grant from the Japan Society for the Promotion of Science; Grant from the Ministry of Health, Labour and Welfare.

Nothing to Disclose: JY-T, KT, SM, SY, AT, TS, TF

P1-247

Short Term Natural History of Pituitary Incidentalomas in Children.

SM Jackman MD¹, EV Jorgensen MD¹ and DI Shulman MD¹.

¹Univ of South Florida St Petersburg, FL.

Introduction:

Pituitary incidentalomas are incidentally discovered asymptomatic pituitary lesions. There is a lack of studies that document the natural history pituitary incidentalomas in children; thus decisions for pediatric patients are based on adult data. We reviewed our experience with pediatric pituitary incidentalomas referred to our pediatric endocrine division from 2002- 2008.

Patients and Methods:

The charts of 31 pediatric patients (ages 1.2 - 18 years) presenting with incidentally discovered pituitary lesions on MRI were retrospectively reviewed. There were 16 (11 female) patients with microincidentalomas (< 10mm), and 8 (5 female) patients with macroincidentalomas (> or equal to 10mm) who had follow up MRIs. All patients had some initial hormonal screening, including prolactin (90%), free T4 (90%), TSH (90%) , IGF-1 (77%), cortisol (77%), and FSH (74%), and LH (74%) levels. The final repeat MRIs of the microincidentalomas and macroincidentalomas were performed 16.1 months (range 2- 56 months) and 15.8 months (range 6- 36 months) after the original MRI study, respectively.

Results:

Of the microincidentalomas, 50% remained stable in size, 38% resolved, and 6% decreased in size. Of the macroincidentalomas, 62.5% remained stable in size, 12.5% resolved, and 12.5% decreased in size. Only 1 (3.6%) of the 28 patients who had prolactin levels drawn, had an elevated prolactin level (55 ng/ml) attributed to a microprolactinoma. All other tumors were felt to be clinically and biochemically non-secreting. One microincidentaloma and one macroincidentaloma increased in size over a 20 month and 58 month period of observation, respectively. Both enlarging lesions occurred in boys.

Conclusions:

The majority of pediatric patients with pituitary incidentalomas appear to have a favorable clinical course: tumor size remained the same or decreased in 94% of microincidentalomas and in 87.5% of macroincidentalomas. These favorable outcomes are similar to that reported in adults (Molitch, Endocrinology and Metabolism Clinics, 2008) (1). Although our series is small, and further longer term data is needed, it appears reasonable to apply adult algorithms in management of pediatric patients with pituitary incidentalomas.

(1)Molitch ME, Endocrinology and Metabolism Clinics 2008; 37:151-171

Nothing to Disclose: SMJ, EVJ, DIS

P1-248

Sheehan's Syndrome in Modern Times.

HL Kristjansdottir MD¹, SP Bodvarsdottir MD¹ and HA Sigurjonsdottir MD, PhD, Assoc.Prof.^{1,2}.

¹Landspítali Univ Hosp Reykjavik, Iceland and ²Univ of Iceland Reykjavik, Iceland.

Introduction

Sheehan's syndrome (SS) is a pituitary failure occurring in women after labour. Half a century ago the prevalence was 10-20 per 100.000 women. With better obstetric help the prevalence has decreased and SS received little attention. In developing countries with high incidence of home deliveries and where obstetric help is poor SS is a big health issue. Studies indicate that SS is more common in the Western world than was previously thought. The aim of this study was to estimate the prevalence of SS in modern times in Iceland.

Methods

This is a nation wide retrospective population based study. Patients were identified by interviewing all practising endocrinologists in Iceland and by scanning the electronic journal system of Landspítali University hospital which goes back to 1983. The Dept. of Endocrinology at this hospital is the only one in Iceland and the Obstetric clinic the most advanced. Information regarding obstetric care, clinical presentation and hormonal assays were collected.

Results

Eight women were identified with SS, thus the prevalence of SS was 5.1 per 100.000 women. The average age at inclusion in the study was 51.5 years (range 41-81). The oldest woman (born in 1928) was excluded for further evaluation because of lack of information. Mean age at delivery and diagnosis was 33.0 (range 21-39) and 36.6 (range 30-41) years respectively, resulting in a diagnostic delay (DD) of 2-240 months. The one with the longest DD (20 years) was diagnosed incidentally. Four women had low blood pressure during delivery and five had massive blood loss (>1000ml). Six had complicated deliveries. The most common clinical presentation was failure to lactate (six women) and failure to resume menstruation (six women). The patients had 3-5 failing pituitary axis. Six women were diagnosed with somatotrophic failure, five with corticotrophic failure, five with thyrotrophic failure, six with gonadotrophic failure and six with prolactin deficiency. No woman had vasopressin deficiency. No connection seemed to be between complications during delivery and DD or the multiplicity of hormonal deficiency.

Discussion

The low prevalence of SS in Iceland can be explained by modern obstetric care available. Long DD and incidental diagnosis indicates that women might be missing the correct diagnosis and treatment. As SS is easily diagnosed and treatable, but can be life threatening if unrecognised, doctors need to be aware of the disease.

Nothing to Disclose: HLK, SPB, HAS

P1-249

Clinical Features of the Patients with Sheehan's Syndrome.

I Fukuda MD, PhD¹, N Hizuka MD, PhD¹, M Kurimoto MD, PhD¹, J Morita MD, PhD¹, S Tanaka MD¹, Y Yamakado MD¹, T Muraoka MD¹, A Yoshida MD¹ and K Takano MD, PhD¹.

¹Inst of Clin Endocrinology, Tokyo Women's Med Univ Tokyo, Japan.

Recently, with the better obstetric care, the incidence of newly diagnosed Sheehan's syndrome has decreased. However, 13% of hypopituitarism in Japanese women is still due to this syndrome. In this study, we analyzed clinical features of patients with Sheehan syndrome that we have followed at our department. The duration between postpartum event and an occurrence of hypopituitarism, clinical manifestations, pituitary hormone deficiency and co-morbidities in 16 patients with Sheehan's syndrome (age at diagnosis: 29 - 69 years old) were investigated using medical records. The patients had a history of delivery with massive bleeding or severe hypotension from 1 to 38 years (median: 9.5 years) before the diagnosis of hypopituitarism. CT and/or MRI scan of sella revealed empty sella in all patients. All patients had more than three pituitary hormone deficiencies in various combination, and thyrotropin, corticotropin, gonadotropin, GH, prolactin and vasopressin were deficient in 100, 94, 94, 88, 81 and 0% of the patients, respectively. Amenorrhea, general fatigue and failure of lactation were the most common clinical manifestations. Bone mineral density of lumbar ranged from 59 to 109% of young adult mean data and 4 patients were receiving the medicine for osteoporosis. Six of ten patients who received blood transfusion had a history of viral hepatitis. These data suggested that Sheehan's syndrome developed either in a short period or many years after the episode of postpartum hemorrhage or hypovolemia. Regardless of the period between postpartum hemorrhage and the occurrence of hypopituitarism, most patients with Sheehan's syndrome have multiple pituitary hormone deficiency when diagnosed. Lifelong care for co-morbidities as well as an appropriate hormone replacement therapy might be important for improving QOL in these patients.

Nothing to Disclose: IF, NH, MK, JM, ST, YY, TM, AY, KT

P1-250

The GH/IGF-I Axis and Pituitary Function and Size in Adults with Prader-Willi Syndrome.

IC van Nieuwpoort¹, M Sinnema², JA Castelijns¹, JWR Twisk³, LMG Curfs² and ML Drent¹.

¹VU Univ Med Ctr Amsterdam, Netherlands ; ²Academic Hosp Maastricht, Maastricht Univ Maastricht, Netherlands and ³ VU Univ Med Ctr and Inst of Hlth Scis, VU Univ Amsterdam, Netherlands.

Context: In adults with PWS, limited information is available about pituitary function, more specific the prevalence of growth hormone deficiency.

Objective: To gain more insight in endocrine function, with emphasis on growth hormone secretion, in PWS adults.

Design: Measurements of basal pituitary hormone levels and a combined GHRH-Arginine test were performed. Size of the pituitary gland was measured on MRI images.

Setting: The study was conducted in the clinical research unit of the VU University Medical Center.

Patients: Fifteen randomly selected adult PWS individuals were included and 14 healthy brothers and sisters served as a control group.

Main outcome measures: IGF-I and IGFBP-3 levels and peak GH level after a combined GHRH-Arginine test were measured. GHD was defined by standard criteria for GHD and BMI-related cut off points. Other pituitary hormone deficits are diagnosed based on serum levels of the concerning hormones.

Results: In adult PWS subjects, IGF-I levels were low when compared to healthy controls and IGFBP-3 levels were normal. GHD was diagnosed in 23-38 % of the PWS patients depending on the criteria being used. Peak GH level was strongly correlated with weight, BMI, waist and fat mass. Hypogonadism was present in 87 % of the patients. Hypothyroidism and adrenal insufficiency could also be demonstrated. Anterior pituitary size was lower in PWS individuals when compared to healthy controls.

Conclusions: GHD and other pituitary hormone deficits are demonstrated in a considerable amount of adult PWS patients. Early detection and treatment of pituitary hormone deficits in adult PWS individuals can have major therapeutic consequences.

Sources of Research Support: Partly funded by an independent grant from Pfizer Inc. - Netherlands.

Nothing to Disclose: ICvN, MS, JAC, JWRT, LMGC, MLD

P1-251

Risk Factors of Metabolic Complication in Korean Young Adults with Childhood-Onset Growth Hormone Deficiency.

HH Lim MD¹, IS Yun MD¹, MJ Kang MD¹, SH Lee MD¹, JH Kim MD¹, YA Lee MD¹, CH Shin MD, PhD¹ and SW Yang MD, PhD¹.

¹Coll of Med, Seoul Natl Univ Seoul, Republic of Korea.

Background : The growth hormone(GH) replacement in patients with GH deficiency(GHD) needs to prevent metabolic derangements. Increased incidence of metabolic mortality has been reported in GHD. But, it remained unclear if childhood-onset adult GHD(COAGHD) is associated with metabolic consequences independent GH replacement. This study was designed to analysis of risk factors of metabolic complication in Korean COAGHD. **Methods :** In retrospective, cross-sectional study, 36 patients(22 males aged 18.6-37.6 years, 14 females aged 16.6-28.8 years) with COAGHD were reviewed. The metabolic syndrome was defined as National Cholesterol Education Program : Adult Treatment Panel III(NCEP:ATP III) and International Diabetes Federation 2006. **Results :** A prevalence of metabolic syndrome in COAGHD was 8.3%. Waist circumference(WC) was 90.1±9.4 cm and 81.5±15.3 cm, systolic blood pressure(BP) was 117.5±12.4 mmHg and 111.6±12.4 mmHg, diastolic BP was 67.3±9.4 mmHg and 63.6±12.7 mmHg, serum triglyceride was 115.6±60.1 mg/dL and 140.1±82.1 mg/dL, high density lipoprotein(HDL) cholesterol was 50.5±14.6 mg/dL and 47.0±10.4 mg/dL, and fasting blood sugar(FBS) was 78.5±14.0 mg/dL and 79.6±8.7 mg/dL in male and female, respectively. The correlations between NCEP:ATP III-based metabolic score and parameters were as followings: WC($P=0.005$); systolic BP($P=0.571$); diastolic BP($P=0.120$); triglyceride($P=0.020$); HDL cholesterol($P=0.002$); FBS($P=0.687$). The metabolic syndrome is associated with body mass index($P=0.031$), waist/hip ratio($P=0.027$), total cholesterol($P=0.386$), low density lipoprotein cholesterol($P=0.114$), fasting insulin($P=0.013$), HOMA-IR($P=0.005$), C-reactive protein($P=0.225$), serum uric acid($P=0.043$), aspartic transaminase($P=0.386$), alanine transaminase($P=0.090$), and total bilirubin($P=0.528$). There was no correlation between duration of GHD and GH replacement($P=0.922$), onset of COAGHD and adult-dose GH replacement($P=0.945$), and metabolic syndrome in GH treated COAGHD. **Conclusions:** The risk factors of metabolic complication in COAGHD were central obesity(WC, waist/hip ratio), insulin resistance(fasting insulin, HOMA-IR), and dyslipidemia(hypertriglyceridemia, low HDL cholesterol), but further studies including more patients were needed.

Nothing to Disclose: HHL, ISY, MJK, SHL, JHK, YAL, CHS, SWY

P1-252

TRH-Stimulated TSH and Prolactin Levels in Patients with PROP1 Mutations: Model for Exclusive Congenital Pituitary Deficiency.

FA Correa MD¹, LR Carvalho PhD¹, VN Brito PhD¹, MY Nishi PhD¹, IJP Arnhold PhD¹ and BB Mendonca PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

Introduction: Congenital hypopituitarism can be idiopathic or result from mutations in genes responsible for the development of the pituitary gland or hypothalamus. *PROP1* is a transcription factor expressed exclusively during pituitary development. Therefore, it is expected that patients with *PROP1* mutations reveal a "pituitary type" response to the TRH test.

Objectives: To present the TSH and PRL responses to the TRH test in patients with proven *PROP1* mutations and establish the range of values expected in this exclusive pituitary transcription factor deficiency.

Material and Methods: A retrospective analysis of the serum TSH and PRL responses in 14 TRH tests in 10 different patients with *PROP1* mutations and in 4 patients with GHRH receptor mutations. Serum TSH and PRL were measured before and 15, 30, 45, 60 and 90 min after iv administration of 200 mcg TRH. The hormone measurements were made by RIA until 1993, by ICMA from 1993 to 1998 and by FIA after 1998. Statistical analysis: data are presented as mean \pm SD and range. The TSH response was compared to that of 4 patients with isolated GH deficiency due to *GHRH* receptor mutations by the Student's t-test. Statistical significance was set at $p < 0.05$.

Results: Fourteen TRH stimulation tests from 10 patients with *PROP1* mutations revealed basal TSH of 1.78 ± 0.54 U/L (range 0.9 to 2.44) and peak of 3.70 ± 1.39 U/L (range 2.4 to 6.82); basal PRL of 4.6 ± 3.7 ng/ml (range 0.9 to 11.3) and peak of 7.0 ± 4.7 ng/ml (range 1.1 to 16.8). In patients with GHRH receptor mutations, basal (3.42 ± 0.40 U/L) and TRH-stimulated (17.4 ± 2.3 U/L) TSH levels were significantly higher when compared to the ones with *PROP1* mutations ($p < 0.05$). In patients with *PROP1* mutations, the mean+2SD was 2.45 U/L for basal TSH and 6.48 U/L for TSH peak and no overlap was found between TSH peak in both groups. The maximum PRL response to TRH in the patients with *PROP1* mutations was 16.8 ng/ml.

Conclusion: The range of TSH and PRL responses to the TRH test in patients with *PROP1* mutations presented here constitutes a model of exclusive pituitary hormone deficiencies and could be helpful to select patients for the genetic screening of mutations in *PROP1* gene.

Sources of Research Support: Grants from Conselho Nacional de desenvolvimento Cientifico e Tecnologico - CNPq 301339/2008-9 to B.M.; 300982/2009-7 to I.J.P.A.

Nothing to Disclose: FAC, LRC, VNB, MYN, IJPA, BBM

P1-253

Should Men with Isolated Secondary Hypogonadism with Low or Low Normal Gonadotrophins Have MRI Pituitary Scans? An Audit of 100 Patients.

E Rigby¹, TH Jones^{1,2} and v Muraleedharan¹.

¹Barnsley Hosp NHS Foundation Trust Barnsley, UK and ²Univ of Sheffield Sheffield, UK.

Background: Symptomatic testosterone deficiency in men with obesity, metabolic syndrome and/or type 2 diabetes is common. These patients often have low or low normal gonadotrophins. There is currently no published guidance on when to request an MRI pituitary scan in isolated hypogonadotrophic hypogonadal men. In our hospital we perform MRI pituitary scans in patients with testosterone deficiency and an LH of less than 4iu/l.

Method: 206 patients had an MRI pituitary scan between January 2005 and January 2010 and were identified using the hospital's radiology database. 188 of these patient's notes were available for review to identify those with testosterone deficiency, and low or low normal gonadotrophins, but otherwise normal pituitary function. The MRI reports of 100 patients were then examined to ascertain if there were any abnormalities of the pituitary gland.

Results: Mean age was 52 years (range 20-80). LH was less than 2iu/l in 37 patients, between 2 and 4iu/l in 52 patients and LH was greater than 4iu/l (Range 4.1-7.9) in 11 patients. 17 patients had an abnormal scan. Of these scans 10 were of patients with an LH less than 2iu/l, 10/37, (27.0%) (4 pituitary cysts, 2 Rathke's cysts, 3 empty or partially empty sella, 1 microadenoma); 7 were of patients with an LH 2-4iu/l, 7/52, (13.5%) (5 partially empty or empty sella, 2 microadenomas); 1 scan was from a patient with an LH >4iu/l, 1/11, (empty sella LH 4.7iu/l). The mean total testosterone for subjects with abnormal scans was 7.92 nmol/l (Range 0.7-9.9). An analysis of variance indicated no significant difference in levels of LH, FSH and age between patients with abnormal and normal scans.

Conclusion: A substantial number of the MRI pituitary scans analysed contained abnormalities. Some of the abnormalities may be incidental findings but some provide the patients with a diagnosis. No lesions identified by the MRI scan required interventional treatment of the structural abnormality. Pituitary MRI scans should be performed in men with testosterone deficiency and a low LH or low normal LH and remains a valuable diagnostic tool for patients with hypogonadotrophic hypogonadism. The lack of correlation with age, LH and FSH shows that these serve as poor indicators for pituitary disease in hypogonadotrophic hypogonadism.

Nothing to Disclose: ER, THJ, VM

P1-254

Rapid Changes in Thyroid Function after Transsphenoidal Adenectomy: Prediction of Recovery of Function.

JT Chaiban MD¹, RS Al-Aridi MD¹, KA Nyalakonda MD¹, DK Abdelmannan MD¹ and BM Arafah MD¹.

¹Univ Hosps Case Med Ctr and The Louis Stokes Veterans Administration Med Ctr Cleveland, OH.

Little is known about the changes in thyroid function in the immediate postop period. Earlier studies demonstrated a rapid rise in plasma ACTH and cortisol after adenectomy (1). In patients with pituitary adenoma and ACTH deficiency, recovery of HPA function is heralded by a rise in plasma ACTH immediately after surgery. With the availability of improved assays for TSH and freeT4, we decided to examine the changes in thyroid function in the immediate postop period in patients with pituitary adenomas who have NL thyroid function (n=68) and others with central hypothyroidism (n=15). Plasma/serum levels of ACTH, TSH, FreeT4 and Cortisol were repeatedly measured during the first 48 hours after adenectomy. Patients with hypercortisolism and others receiving thyroxine were excluded. Patients with NL thyroidal function preop had a significant rise in TSH and freeT4 levels shortly after surgery (table1).

Table1. Periop TSH and FT4 in 68 Patients with Normal HPT axis

| | 0 hr | 2-4 hrs | 6-8 hrs | 12-18 hrs | 48 hrs |
|--------------|-----------|------------|------------|------------|------------|
| TSH (uIU/ml) | 1.89±1.09 | 3.39±2.67* | 1.66±1.74 | 0.96±0.92* | 0.94±0.76* |
| FT4 (ng/dl) | 1.13±0.23 | 1.3±0.31* | 1.28±0.24* | 1.28±0.24* | 1.41±0.56* |

Of the 15 patients with preop central hypothyroidism (table2), 12 recovered function as documented clinically and biochemically 1-2 months postop.

Table2. Periop TSH and FT4 in 15 patients with central hypothyroidism

| | 0 hr | | 2-4 hrs | | 48 hrs | |
|-------------------------------|--------------|-------------|--------------|-------------|--------------|-------------|
| | TSH (uIU/ml) | FT4 (ng/dl) | TSH (uIU/ml) | FT4 (ng/dl) | TSH (uIU/ml) | FT4 (ng/dl) |
| Recovering Function (N=12) | 3.11±1.46* | 0.76±0.10 | 3.52±1.46* | 0.9±0.16 | 0.94±0.44 | 0.95±0.11 |
| Non Recovering Function (N=3) | 0.66±0.28 | 0.72±0.01 | 0.83±0.50 | 0.62±0.08 | 0.52±0.04 | 0.56±0.2 |

The data demonstrate that patients' recovery of thyroid function is noted as early as 4 hours after adenectomy. **Conclusion:** There was a rise in serum TSH and free T4 levels in patients with NL thyroidal function shortly after pituitary adenectomy. Similar findings were observed in patients with macroadenoma and hypothyroidism who recovered function after adenectomy. The data support the hypothesis that the hypopituitarism in this setting is largely due to compression of the portal vessels. Resumption of TRH release and improved TSH glycosylation after adenectomy result in improved TSH biologic activity and the subsequent rise in free T4.

(1) Arafah et al, J Clin Endocrinol Metab 1994;79(2):348

Nothing to Disclose: JTC, RSA-A, KAN, DKA, BMA

P1-255

Serum Cortisol as a Predictor of Exogenous Steroid Requirements for Patients Undergoing Pituitary Surgery.

A Goldberg B.Sc (Hon)¹ and J Goguen MD, MEd, FRCPC^{1,2}.

¹Univ of Toronto Toronto, Canada and ²St Michael's Hosp Toronto, Canada.

Background: Correct classification of pituitary patients' hypothalamus-pituitary-adrenal axis (HPA) function is essential to prevent life-threatening events in surgery while avoiding unnecessary steroid treatment. Although the insulin tolerance test (ITT) is the gold-standard in evaluating the HPA, it is often not used in pre-operative evaluation(1) and a screening cortisol value or ubiquitous coverage is used in place. There is currently literary incongruence as to the appropriate basal cortisol value for peri-operative steroid coverage, with recommendations for a cut-off as low as 9 mcg/dL(2). **Objective:** This study aims to clarify the use of screening basal cortisol in determining surgical steroid coverage, and to establish a safe value that would not risk adrenal crisis in times of stress, while obviating the need for universal steroid coverage. **Methods:** All diagnostic ITTs performed at our hospital over the past four years were reviewed for indication, basal and peak cortisol, minimum blood glucose achieved and clinical symptoms. Test diagnostic performances were compared at various cut off levels. **Results:** Of 101 ITTs performed, 95 were diagnostic and reached adequate hypoglycemia. Forty-four patients had suboptimal cortisol response to hypoglycemia (<18mcg/dL) indicating adrenal insufficiency (AI). Using a basal cortisol cutoff of <9 mcg/dL to govern treatment results in 17/44 (0.386 95%CI 0.244-0.545) with AI who would not receive steroids (figure 1A). A cutoff of 13 nmol/L will miss treating 4/44 (0.091 95% CI 0.025-0.217) who do have AI.

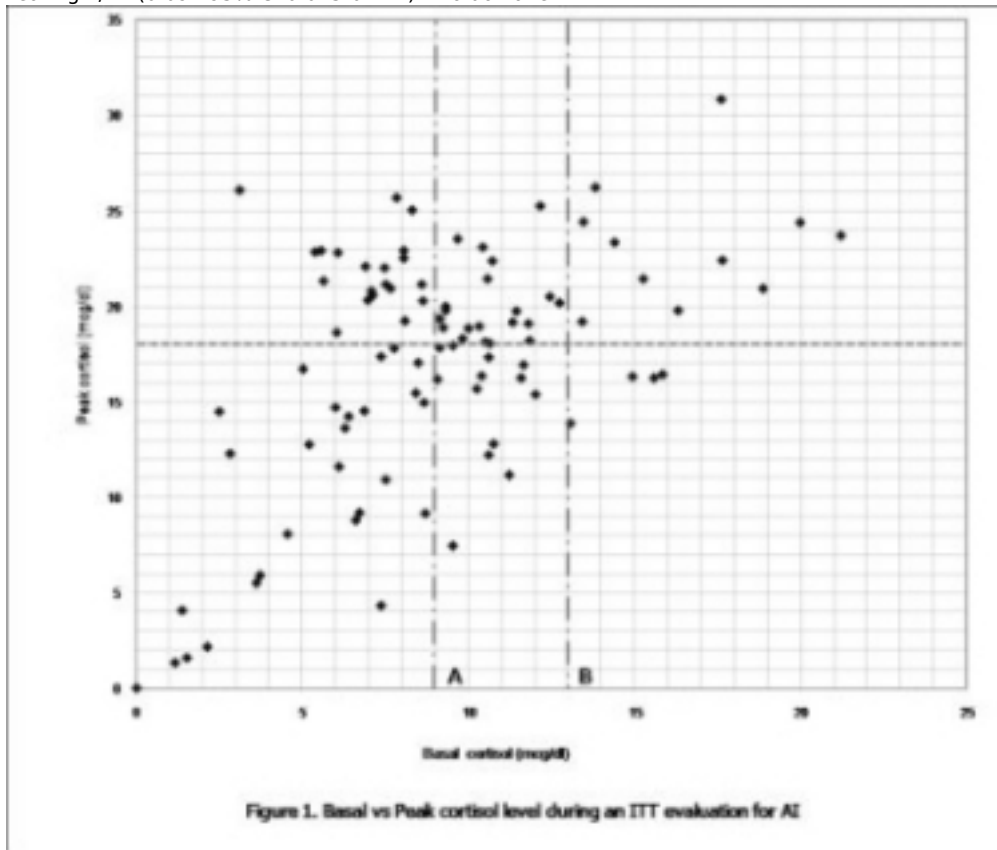


Table 1. Diagnostic performance of basal cortisol cutoff concentrations to detect adrenal insufficiency

| AI detection | 9 mcg/dL cutoff | 13 mcg/dL cutoff |
|-------------------------------------|------------------|------------------|
| Sensitivity (95% CI) | 61.4 (45.5-75.6) | 90.1 (78.3-97.5) |
| Specificity (95% CI) | 60.8 (46.1-74.2) | 23.5 (12.8-37.5) |
| Negative Predictive Value (95% CI) | 64.6 (49.5-77.8) | 75 (47.6-92.7) |
| False negative rate (1-sensitivity) | 35.4% | 9.9% |

Conclusion: Using a cutoff value of <13 mcg/dL will risk inadequately treating patients at risk for adrenal crisis that warrant peri-operative steroids. Further studies of peri-operative outcomes should be investigated.

(1) Pereira and Bevan, Pituitary 2008; 11: 347-351

(2) Wentworth et al., Clinical Endocrinology 2008; 68:29-35

Nothing to Disclose: AG, JG

P1-256

Past Compression of the Optic Chiasm Is Associated with Objective Shorter Sleep Duration in Patients with Pituitary Insufficiency.

AJ Borgers MD¹, N Romeijn MSc², E van Someren PhD², E Fliers MD PhD¹, A Alkemade PhD¹ and PH Bisschop MD PhD¹.

¹Academic Med Ctr Amsterdam, Netherlands and ²Netherlands Inst for Neuroscience Amsterdam, Netherlands.

Introduction

Patients treated for pituitary tumors show increased daytime sleepiness based on sleep questionnaires. Sleep is to a large extent regulated by the hypothalamic circadian pacemaker located in the suprachiasmatic nucleus (SCN). Because the SCN is located just superior to the optic chiasm, we hypothesized that a history of compression of the optic chiasm (CC) due to a (para)sellar tumor is associated with altered sleep patterns in patients with pituitary insufficiency.

Methods

Patients from the Endocrine Outpatient Clinic with pituitary insufficiency, and previously treated for a (para)sellar tumor, were invited to participate in this cross-sectional study. Four validated questionnaires (Epworth Sleepiness Scale, Pittsburgh Sleep Quality Index, Sleep Disorders Questionnaire and Athens Insomnia Scale) were used to evaluate the subjective sleepiness, subjective sleep quality and the presence of common sleep disorders. Objective measures of sleep patterns were assessed by wrist actigraphy for one week. We studied 36 patients (mean age 54.9±13.7yr; 69.4% men) with a history of CC and 17 patients (mean age 54.1±15.3yr, 41.2% men) without a history of CC. CC was defined as compression visualized by imaging and/or visual field defects in the presence of a (para)sellar tumor.

Results

No differences were found in subjective sleepiness scores and subjective sleep quality scores between both groups. Furthermore, no common sleep disorders were measured. However, patients with a history of CC had an objective shorter total sleep duration [median 453 (range 295-553) versus median 490 (range 432-740) minutes, p=0.016] compared to patients without a history of CC. Multiple linear regression analysis confirmed that a history of CC is a significant determinant of total sleep duration (P=0.003) after adjustment for age, sex, cranial radiotherapy and pituitary/hypothalamic surgery. A history of CC is responsible for 10.9% of this models' total goodness-of-fit (r²=41.5%).

Conclusion

The present study shows that a history of compression of the optic chiasm due to a (para)sellar tumor is associated with objective changes in total sleep duration in patients with pituitary insufficiency. Our data provides support for permanent impaired SCN function in patients treated for large pituitary tumors giving rise to optic chiasm compression.

Nothing to Disclose: AJB, NR, EvS, EF, AA, PHB

P1-257

Carotid Atheromatic Plaque Is Commonly Associated with Hypopituitary Men.

JW Hong¹, JY Kim¹, SY Rhee², CS Kim³, DJ Kim⁴ and EJ Lee^{1,5}.

¹Yonsei Univ Coll of Med Seoul, Republic of Korea ; ²Armed Forces Capital Hosp Gyeonggi, Republic of Korea ; ³Hallym Univ Sacred Heart Hosp Anyang, Republic of Korea ; ⁴Ajou Univ Sch of Med Gyeonggi, Republic of Korea and ⁵ Northwestern Univ Feinberg Sch of Med Chicago, IL.

Many cardiovascular risks are increased in hypopituitarism, and patients with this disease are more prone to cardiovascular disease. The aim of this study was to compare carotid artery plaque as well as major cardiovascular risk factors between male patients with hypopituitarism and control subjects. Furthermore, we analyzed associating factors to cause cardiovascular events in patients with hypopituitarism besides of growth hormone deficiency. Forty male patients aged 30-70 years with hypopituitarism were recruited at the Yonsei University Severance Hospital, Seoul, Korea between March 2008 and September 2008. All patients had appropriate replacement therapy with glucocorticoids, thyroxine and desmopressin, although growth hormone (GH) and sex steroids were not replaced. Plaque in the carotid arteries was observed more frequently in patients with hypopituitarism than age- and sex-matched control subjects (59.5% vs 2.5%, $p < 0.01$) without difference of carotid IMT. Patients with hypopituitarism had also higher waist circumference, waist to hip ratio, total cholesterol and LDL cholesterol than control subjects. In subgroup analysis in male patients with hypopituitarism including GH deficiency, lower testosterone levels were associated with higher waist circumference ($r = 0.424$, $P = 0.035$), not associated with BMI. In conclusion, hypopituitary males exhibit an increased incidence of carotid artery plaque, central obesity and higher total cholesterol level. Lower testosterone levels were associated with central obesity- a strong component of the metabolic syndrome, and unsubstituted testosterone deficiency might be an important cardiovascular contributing factor in patients with hypopituitarism.

Comparative analysis of male hypopituitary patients with age and sex-matched controls

| Variables | Control (n=40) | Hypopituitarism (n=40) | p-value |
|------------------------------|----------------|------------------------|---------|
| Age (yrs) | 54.63 ± 1.76 | 55.46 ± 2.00 | 0.773 |
| Waist circumference (cm) | 83.20 ± 0.67 | 87.56 ± 0.77 | <0.001 |
| WHR | 0.86 ± 0.01 | 0.91 ± 0.01 | <0.001 |
| Total cholesterol (mmol/L) | 4.86 ± 0.83 | 6.01 ± 1.08 | <0.001 |
| Mean c IMT (mm) | 0.69 ± 0.03 | 0.64 ± 0.03 | 0.310 |
| Plaque in carotid artery (%) | 2.5 | 59.5 | <0.001 |

by ANCOVA, adjusted by BMI, chi-square, mean ± S.E. WHR, waist hip ratio; cIMT, carotid intima-media thickness

JW Hong and JY Kim are equally contributed to this study.

Nothing to Disclose: JWH, JYK, SYR, CSK, DJK, EJJ

P1-258

Comparison of Lipid Profile, Blood Pressure, Fasting Blood Glucose and Waist Circumference in Patients with Metabolic Syndrome x Hypopituitarism.

J F S Pereira-Lima MD PhD¹, T M Figuera MD¹, C Lapnharski MD¹, C G S Leaes MD PhD¹, N P Ferreira MD PhD¹ and M C Oliveira MD PhD¹.

¹Neuroendocrinology Ctr of Santa Casa de Porto Alegre, UFCSA Porto Alegre, Brazil.

Introduction: Metabolic Syndrome (MS) is a complex disorder characterized by alterations in lipid profile, central obesity, hypertension and glucose intolerance, with a significant increased risk of cardiovascular events and death. Hypopituitarism (H) is the reduction or absence of one or more pituitary hormones. Recent studies show that patients with pituitary dysfunction have an increased incidence of obesity, insulin resistance and hyperlipidemia, resulting in increased cardiovascular mortality. **Objectives:** To evaluate the lipid profile, blood pressure, fasting plasma glucose (FPG) and abdominal circumference in patients with MS and in patients with H. Compare the data evaluated in patients with MS and patients with H and between patients with MS with and without H. **Study design:** Observational, retrospective chart review. **Patients and Methods:** 194 patients, 50 with MS and 144 with H. We excluded 2 with MS and 33 with H due to lack of data. The diagnosis of MS was the International Diabetes Federation and the H deficiency of one or more pituitary hormones. **Results:** 48 patients with MS, 66.7% women, mean age 61.3 years, weight 84.8 kg, body mass index (BMI) 33kg/m² and abdominal circumference (AC) 108.2 cm. The average cholesterol (C) 214.8 mg/dl, HDL 46.3 mg/dl, LDL cholesterol 126.5 mg/dl, triglycerides (Tr) 231.6 mg/dl, FPG 166.7 mg/dl. 111 patients with H, 65.8% women, average 54 years, weight 78.7 kg, BMI 29.8 kg/m² and CA 97.6 cm. The TC mean was 216.4 mg/dl, HDL 51.8 mg/dl, LDL 135mg/dl, Tr 151.4 mg/dl, FPG 95.2 mg/dl. CA was obtained in 66 patients, of which 27 met the criteria of MS (40.9%). Comparing the 111 with H and 48 with SM: H patients were younger (p=0.000), with lower weight (p=0.046), BMI (p=0.002) and CA (p=0.000). Patients with H showed higher values of HDL (p=0.012) and lower Tr (p=0.000). Showed lower values of FPG, incidence of DM and hypertension (p=0.000). Comparing the 27 with SM and H (SM/M) and 48 patients with MS: The SM/M were younger (p=0.000). Patients with MS have higher levels of FPG and incidence of DM (p=0.000). **Conclusion:** Patients with H showed lower values of FPG, incidence of central obesity, hypertension and a more favorable lipid profile compared to those with MS. Comparing patients with MS with and without H, no difference in the incidence of central obesity, LDL, HDL and Tr between the groups. Patients with SM without H showed higher levels of FPG and higher incidence of hypertension.

Nothing to Disclose: JFSP-L, TMF, CL, CGSL, NPF, MCO

P1-259

Results of French Collaborative Evaluation (DEPHY-TC) in Patients with Suspected Pituitary Abnormalities after Traumatic Brain Injury (TBI).

G Raverot Federation d'En¹, MF Malezet Service d'Endoc², ML Nunez Service d'Endoc³, N Morlet Barlat Departments of Endoc⁴, H Curalucci Etablissement de R[e⁵ and L di Nicola IPSEN Pharma SAS, B0⁶.

¹Hospices Civils de Lyon Lyon, France ; ²CHU Robert Debre Reims, France ; ³USN Haut Leveque, Pessac Pessac, France ; ⁴Assistance Publique-Hôpitaux de Marseille, Marseille, France ; ⁵Clinique St-Marti Marseille, France and ⁶Ipsen Pharma Boulogne, France.

Study aim: Pituitary function evaluation in patients with Traumatic Brain Injury (TBI).

Subjects: 146 patients (49 Female, 97 men, age: 31.9±16.2 y.) recovered from moderate to severe TBI. Prospective follow up has been performed 3/6 and 12 months from TBI for 79 subjects. Retrospective evaluation (from 0.3 to 30 y) included 67 subjects selected in front of overt clinical features (Obesity, Fatigue, Sexual disorders).

Methods: Clinical (neurological, endocrine, and general) and hormonal evaluations were performed using a standardized protocol including determination of FT4, TSH, PRL, IGF-I, Testosterone/E2, FSH and LH. Corticotrope and Somatotrope axes were evaluated using Glucagon-, Insulin Tolerance- or GHRH-Arginine tests.

Results :

Prospective group.

3-6 month after TBI (n = 79): 44.3% presented endocrine dysfunction : GH deficiency (GHD) (22%) Gonadotrope deficiency (19%), Corticotrope deficiency (13%), Thyrotrope deficiency (5%).

12 month after TBI (n=46) 34.8% presented endocrine dysfunction : GH deficiency (GHD) (15%) Gonadotrope deficiency (24%), Corticotrope deficiency (2%), Thyrotrope deficiency (2%).

Combined deficiency was observed in 12.6 % and 6.5% of cases initially and after 12 months respectively.

Six of 9 patients with GHD at first evaluation recovered normal somatotrope function after 12 month but 3 new patients with GHD were diagnosed at this time.

Retrospective group (n = 67) 45.3 % subjects presented endocrine dysfunction: GHD (32.8%), Gonadotrope deficiency (17.1%), Corticotrope deficiency (9.3%), Thyrotrope deficiency (4.7%). Combined deficiency was observed in 17.1 % of cases. Among patients with a follow-up after TBI > 1 y (n=99), GHD patients (n=25) presented significant increase of Body Mass Index (p<0.05) compare to GH sufficient patients (n=74).

Conclusion: our data in the prospective cohort confirm that pituitary dysfunction after TBI may evolve over time and requires multiple evaluations. Prospective interventions studies are needed to evaluate the benefits of hormonal supplementation in these patients.

Disclosures: LdN: Employee, Ipsen.

Nothing to Disclose: GR, MFM, MLN, NMB, HC

P1-260

Trauma Severity, but Not Hypopituitarism, Affects Cognitive Function after Traumatic Brain Injury: A Multi-Center Study in the Netherlands.

NE Kokshoorn MD¹, JWA Smit Prof¹, WA Nieuwlaat MD, PhD², NR Biermasz MD, PhD¹, PH Bisschop MD, PhD³, R Groote Veldman MD, PhD⁴, F Roelfsema MD, PhD¹, A Franken MD, PhD⁵, MJE Wassenaar MD¹, J Tiemensma MSc¹, JA Romijn Prof¹ and AM Pereira MD, PhD¹.

¹Leiden Univ Med Ctr Leiden, Netherlands ; ²Elisabeth Hosp Tilburg, Netherlands ; ³Amsterdam Med Ctr Amsterdam, Netherlands ; ⁴Med Spectrum Twente Enschede, Netherlands and ⁵Isala Clins Zwolle, Netherlands.

Background: Hypopituitarism after traumatic brain injury (TBI) is considered to be a prevalent condition, and may affect Quality of Life (QoL) and cognitive function. However, the prevalence of hypopituitarism differs considerably among reported studies due to differences in definitions, endocrine assessments of hypopituitarism, and confounding factors, like timing of evaluation and the severity of the trauma.

Aim: to evaluate the prevalence of hypopituitarism, QoL, and cognitive function in a large cohort of TBI patients after long term follow-up.

Patients and Methods: Ninety-four patients (64M, median age 45 yr) with follow-up duration after TBI > 1 yr and hospitalized for a minimum of 4 days were recruited from 5 centers in the Netherlands. Pituitary function was evaluated by fasting morning samples combined with insulin tolerance test (ITT, n=78) or, when contraindicated, by ACTH and GHRH-arginine test (n=16). QoL was assessed by 4 health related questionnaires (HADS, NHP, MFI-20, and SF-36); cognitive function by the minimal mental state examination (MMSE) for global cognitive functioning; verbal learning test of Rey (VLTR) and Rey's complex figure test (RCF) for memory; and trail making test (TMT), Stroop color-word test (SCWT) and letter digit substitution test (LDST) for executive functioning.

Results: Hypopituitarism was present in 6.4% (6/94) of cases (severe GHD 4.3% (n=4), hypogonadism 1.1% (n=1); hypocortisolism 2.1% (n=2)). Hypopituitarism affected QoL (several items in all questionnaires), but not cognitive function. However, trauma severity (moderate/severe vs mild injury) affected cognitive function: memory: VLTR (immediate repeats: p= 0.011) and executive functioning: SCWT (total interference p=0.009).

Conclusion: the prevalence of hypopituitarism after TBI was low after long-term follow-up. QoL was affected by hypopituitarism, but not by the severity of the trauma. In addition, trauma severity, but not hypopituitarism, significantly affected cognitive function.

Sources of Research Support: Pfizer Netherlands.

Nothing to Disclose: NEK, JWAS, WAN, NRB, PHB, RGV, FR, AF, MJEW, JT, JAR, AMP

P1-261

Pituitary Function in Patients after Aneurysmal SubArachnoid Hemorrhage: A Prospective 12-Months Study in 27 Patients.

S Lesven MD¹, E Sonnet MD¹, R Seizeur MD¹, N Roudaut MD¹, G Besson MD¹ and V Kerlan MD¹.

¹CHU Brest, France.

Introduction

As described after traumatic brain injury, aneurysmal SubArachnoid Hemorrhage (SAH) seems to be condition at high risk for the development of hypopituitarism. So the aim of this prospective study was to test the pituitary function in patients, 3 months (M3), 6 months (M6) and one year (M12) after SAH.

Methods: Dosage of TSH, FT4, total testosterone or estradiol, FSH, LH, IGF-1, basal morning cortisol and during a low dose corticotrophin stimulation test (abnormal when peak <19 microg/dl), were realized in patients with age under 70 (H/F:14/13, mean age: 49 ± 11 years) at M3, M6 and M12. The GH secretion was evaluated by at M6 and M12 by a glucagon test, When necessary, an eventual GH defect was confirmed by the use of an hypoglycaemia test (abnormal when peak <3 ng/ml).

Results

At M3, hypopituitarism was present in 11 patients (40.7 % of cases): Multiple (MD) and isolated (ID) deficiency in 10 (37 %) and 1 patients (3.7 %) respectively (no panhypopituitarism). ACTH, TSH, and gonadal deficiency was present in 2 (7.4 %), 4 (14.8 %) and 2 patients (7.4 %) respectively. IGF1 level was low in 12 patients (44.4 %).

At M6, hypopituitarism was present in 14 patients (51.9 % of cases): MD in 7 (25.9%) and ID in 7 patients (25.9%). (no panhypopituitarism). ACTH, TSH, gonadal and GH deficiency was present in 7 (25.9 %), 6 (22.2 %), 3 (11.1 %) and 3 patients (11.1%) respectively.

At M12, hypopituitarism was present in 15 patients (55.6% of cases): MD in 5 (18.5%) and ID in 10 patients (37.1%). (no panhypopituitarism). ACTH, TSH, gonadal and GH deficiency was present in 9 (33.3 %), 5 (18.5 %), 4 (14.8 %) and 7 patients (25.9 %) respectively.

Conclusion. Patients after SAH are at high risk to develop a pituitary deficiency. In our study, ACTH deficiency was the most frequent. Because of eventual pejorative consequences, it seems to be important in SAH patients to detect and treat an eventual pituitary deficiency

Nothing to Disclose: SL, ES, RS, NR, GB, VK

P1-262

Pituitary Insufficiency in the Acute Phase of Traumatic Brain Injury or Subarachnoid Hemorrhage: A Prospective Study.

P Sigurjonsson MD¹, ÁD Jonasdottir MD¹, IH Olafsson MD¹, S Karason MD, Phd, Assoc.prof^{1,2}, G Karlsdottir MD¹, G Sigthorsson MD, Phd¹, R Benediktsson MD, Phd, Prof^{1,2} and HÁ Sigurjonsdottir MD, Phd, Assoc.prof^{1,2}.

¹Landspítali Univ Hosp Reykjavik, Iceland and ²Univ of Iceland Reykjavik, Iceland.

Background: Traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) can in addition to death cause significant long term morbidity. Recent studies have shown that this may in part be due to transient or chronic hypopituitarism (HP) which occurs in up to 50% of patients. In Iceland all patients with moderate and severe TBI, Glasgow coma score 9-12 and <8 respectively, as well as all SAH are referred to The Dept. of Neurosurgery, at the Landspítali University Hospital in Reykjavik. The aim of this study was to assess the prevalence of HP immediately after TBI and SAH in Iceland.

Subjects and methods: During 11-months, 21 TBI patients were prospectively included, 6 moderate TBI and 15 severe TBI, 17 males and 4 females, mean age 34 ± 13 years (range 18-65 years). Seventeen patients with SAH were included, 11 males and 6 females, mean age 55 ± 14 years (range 30-85 years). Baseline hormone levels were measured on admission and 6 days later. Daily morning cortisol levels were measured on days 1-6 plus a synacthen test on day 6 or earlier if clinically justified. By our knowledge HP has not been reported in the acute phase of SAH before.

Results: On day 6, 3 of the 6 moderate TBI, 6 of the 15 severe TBI and 3 of the 17 SAH patients had developed central hypogonadism, 1 severe TBI patient central hypothyroidism, 1 SAH patient had somatotropin deficiency with low levels of IGF-1 and growth hormone and 1 severe TBI patient developed corticotropin deficiency and failed the synacthen test, he was treated with corticosteroids and retested 19 days after trauma when clinically stable and then had regained normal cortisol levels and a normal response on synacthen test. Two severe TBI and 1 SAH patient had multiple HP. Four moderate TBI, 4 severe TBI and 2 SAH patients had isolated hypogonadism. Lost to follow up on day 6 were 7 patients, 4 died and 3 did not attend.

Conclusion: The prevalence of HP in the acute phase following moderate and severe TBI and SAH is 42,9% and 17,6% respectively. Central hypogonadism was relatively frequent among moderate and severe TBI patients as well as SAH patients in the acute phase. Other pituitary insufficiencies seem more likely to occur in severe TBI and SAH patients as opposed to moderate TBI. This study indicates that patients with SAH can suffer from HP as well as patients with TBI, that is an important clinical finding.

Nothing to Disclose: PS, ADJ, IHO, SK, GK, GS, RB, HAS

P1-263

Salivary Free Cortisol in the Chronic Phase after Aneurysmal Subarachnoid Hemorrhage - A Different Story from What We Know about Serum Cortisol.

EM Poll Dipl.-Psych.¹, D Hellhammer Prof.², JM Gilsbach Prof.¹ and I Kreitschmann-Andermahr PD Dr. med.¹.

¹Univ Hosp RWTH Aachen Aachen, Germany and ²Univ of Trier Trier, Germany.

Spontaneous aneurysmal subarachnoid hemorrhage (SAH) is a form of stroke which constitutes a severe trauma to the brain and often leads to serious long-term medical and psychosocial sequelae which persist for years after the acute event. Recently, adrenocorticotrophic hormone deficiency has been identified as one possible consequence of the bleeding which is assumed to occur in around 20% of all survivors. Preliminary data suggest that a poor psychosocial outcome in SAH survivors is linked to alterations of cortisol secretion. Up to now, cortisol levels in SAH patients have been investigated with respect to basal values and in response to challenge tests, but not with regard to diurnal dynamics and feedback-regulation. In this study, basal serum cortisol and diurnal salivary cortisol profiles were investigated in 31 patients in the chronic phase following SAH as well as in 25 healthy controls. Additionally, low-dose dexamethasone suppression tests were performed and sensitivity to stress and stress resilience were measured with psychometric questionnaires (Neuropattern Diagnostic Kit).

As a result, SAH patients in general showed significantly more symptoms of stress-related physical complaints than controls. Patients were divided via cluster analysis in two separate groups: one with high (n=12) and one with low (n=19) stress load. Highly stressed SAH patients had significantly lower serum cortisol values than controls, while patients with a low stress load did not. On the other hand, free salivary cortisol levels in the morning (cortisol awakening response, CAR) were higher in the SAH low stress group when compared to controls, whilst the CAR of high-stress patients was normal. SAH patients in general exhibited a higher salivary CAR, but on the other hand slightly lower serum cortisol levels than controls. Administration of dexamethasone did not lead to any significant group differences. Cortisol levels in SAH patients were not associated with any clinical variables of the acute hemorrhage, such as severity of the bleeding or location of the aneurysm. The results of this study indicate that total and free serum cortisol levels give different information about the HPA-axis in patients after aneurysmal SAH and give rise to the suspicion that total serum cortisol levels alone may not be adequate for diagnosing adrenal function in this patient group. Additionally, stress may be an important mediator of HPA dysregulation in SAH patients.

Sources of Research Support: Scholarship Konrad Adenauer Stiftung for EM Poll; START Programme of the RWTH Aachen.

Nothing to Disclose: EMP, DH, JMG, IK-A

P1-264

Analysis of Body Weight and Nutritional Behaviour of Patients with Hypopituitarism Following Traumatic Brain Injury or Subarachnoidal Haemorrhage.

A Mueller-Oeffner Dr¹, B Gutt Dr¹, T Siegmund Dr¹, I Opherk Dr¹, J Zormaier Dr¹, M Hufnagl Dr¹ and P-M Schumm-Draeger Prof Dr¹.

¹Academic Teaching Hosp Munich Bogenhausen Munich, Germany.

Objective: The prevalence of hypopituitarism following traumatic brain injury (TBI) or subarachnoidal haemorrhage (SAH) is reported between 15-55%. Higher body mass index (BMI) is associated with cerebral outcome and hypopituitarism following TBI. We analysed the body weight and the eating habits of patients following TBI or SAH in relation to the development of hypopituitarism.

Method: Eighty-six consecutive patients (34 female, 52 men) of our centre (age 18-67y; mean 43y; BMI 28-46kg/m², mean 26kg/m²) underwent basal pituitary testing at least 6 months following TBI (n=47) or SAH (n=39). Pathological basal values were verified by dynamic testing (GHRH+ARG, CRH/ACTH, TRH or GnRH- tests). The choice of food and eating habits was assessed with a questionnaire.

Results: Hypopituitarism was distinguished in 23 patients (20%). An isolated affection of the somatotrophic axis was found in 7, of the gonadotrophic axis in 6, of the thyreotropic axis in 4 and of the corticotrophic axis in 4 patients. Two patients showed a somatotrophic and thyreotropic insufficiency. Since the TBI or SAH all patients gained 1,5 kg body weight and ate 3 meals per day, patients with hypopituitarism gained 2,5 kg (p=0,05) in average with 4 meals per day (p=0,02). Patients with an affection of the somatotrophic axis increased body weight at 3,6 kg (p=0,05) and ate more frequently daily vegetables (p=0,05). Patients with gonadotropin deficiency used less frequently vegetable oils (p=0,02). Patients with TSH deficiency declared more often not to eat fish once a week (p=0,02) and to eat more sweets (p=0,02) with voracious appetite for sweets (p=0,01). Patients with affection of the corticotrophic axis predicted to eat more with stress.

Conclusion: Patients with pituitary dysfunction gained more body weight since the TBI or SAH. Their nutritional behaviour is different in relation to the affected pituitary axis. It should be analysed in a prospective study, if the substitution of the insufficient axis results in changes of body weight and choice of food.

Nothing to Disclose: AM-O, BG, TS, IO, JZ, MH, P-MS

P1-265

Chronic Subdural Hematoma as a Cause of Pituitary Dysfunction.

M Kosak¹, V Masopust¹, D Netuka Ph.D.¹, Z Lacinova¹, M Krsek prof., CSc.¹, J Marek prof., DrSc.¹ and V Hana prof., CSc.¹.

¹Fac of Med 1, Charles Univ, Gen Fac Hosp Prague, Czech Republic.

Recently great attention has been given to hypopituitarism developed after craniocerebral injury and in patients with subarachnoid hemorrhage. Microhemorrhages, necrosis, tissue infarcts and vasoconstriction are reported as mechanisms of hypothalamo-pituitary dysfunction. Assessment of hypothalamo - pituitary endocrine functions in patients with chronic subdural hematomas has not been published yet, although dysfunction of hypothalamo- pituitary unit can be expected (head trauma, compression and edema of brain with shift of the midline structures).

Aims: Evaluation of the pituitary functions in patients with chronic subdural hematoma immediately after surgical treatment and during one year follow-up.

Patients and methods: We have examined 25 patients (20 men, 5 women, 51-88 years old, mean 71,2 +/- SD 9,9 years). Maximal thickness of the subdural hematoma in our group was 33 mm. In all cases except of two the shift of the third ventricle was present - in 12 patients more than 10 mm (maximum was 20 mm).

Fifteen patients (12 men/3 women) have been examined prospectively (prospective group) and 10 patients (8 men/ 2 women) were tested only 12 months after the operation (retrospective group). In the prospective group the patients in the first days after evacuation of the hematoma and then 3 and 12 months after the operation were tested. Basal levels of pituitary hormones and hormones of dependent peripheral glands, plasma cortisol during 250 mcg tetrasoactrin (Synacthen) test, GH after GHRH+arginin stimulation and TSH and prolactin after TRH stimulation were assessed.

Results: In the acute phase, central hypogonadism was diagnosed in four male patients among the prospectively followed patients. In three of them gonadotrophic axis normalised within 3 months after the operation (one patient hasn't been retested yet). In one patient partial hypocorticalism and central hypogonadism have developed after 3 months. In the retrospective group central hypogonadism was diagnosed in one patient. Relation between extent of subdural hematoma and hypothalamo - pituitary dysfunction has been analysed.

Conclusions: Preliminary data from our group of patients support the idea that chronic subdural hematoma may be complicated with a partial pituitary dysfunction which can develop even with a time delay. Further evaluation of a larger group is necessary.

Sources of Research Support: Grant of Czech Ministry of Health NS 9794 - 4.

Nothing to Disclose: MK, VM, DN, ZL, MK, JM, VH

P1-266

Effect of Dehydroepiandrosterone Sulphate Replacement on Quality of Life and Insulin Action in Hypopituitary Females: A Double Blind Placebo Controlled Study.

CM McHenry MD¹, PM Bell MD¹, SJ Hunter MD¹, CJ Thompson MD², CH Courtney MD¹, CN Ennis BSc¹, B Sheridan BSc¹, DR McCance MD¹, KR Mullan MD¹ and AB Atkinson MD¹.

¹Royal Victoria Hosp Belfast, UK ; ²Beaumont Hosp Dublin, Ireland and ³Royal Victoria Hosp Belfast, UK.

It has been suggested that addition of dehydroepiandrosterone sulphate (DHEAS) to standard adrenal replacement therapy may have beneficial effects on quality of life and glucose metabolism. Previous studies have yielded conflicting results probably related to differences in both the populations studied and assessment techniques used. We assessed effects of DHEAS in female hypopituitary patients on quality of life and insulin action.

A randomised double-blind placebo controlled crossover design was used. Fourteen patients on stable replacement therapy were assigned to DHEAS 50mg daily or placebo for 12 weeks with four week washout between treatments. Quality of life, using seven validated questionnaires (Short Form-36, Nottingham Health Profile, Fatigue Related Cognition Scale, General Health Questionnaire, Hospital Anxiety Depression Score, Psychological General Wellbeing Being Score, Adult Growth Hormone Deficiency Assessment), and insulin action by euglycaemic hyperinsulinaemic clamp were assessed at the end of each treatment period.

Thirteen patients completed the study. Serum concentrations of DHEAS (DHEAS therapy 5.4 ± 0.8 vs placebo $< 0.8 \pm 0.0$ $\mu\text{mol/l}$; $p < 0.001$) and androstenedione (DHEAS therapy 4.1 ± 0.8 vs placebo 1.3 ± 0.2 nmol/L) rose to within the normal range after DHEAS. There were no differences between treatments in testosterone, SHBG or IGF-1 concentrations. Quality of life measures were unchanged after DHEAS. There were no differences between treatments in fasting glucose, serum insulin concentrations or HbA1c. Triglyceride concentrations were lower following DHEAS (DHEAS therapy 1.24 ± 0.18 vs placebo 1.41 ± 0.19 mmol/l ; $p < 0.05$) but other lipid parameters were the same. Following DHEAS, there was no difference in glucose infusion rates required to maintain euglycaemia (DHEAS therapy 21.9 ± 2.5 vs placebo 24.5 ± 2.1 $\mu\text{mol/kg/min}$; $p = 0.4$).

In summary, there were no differences in quality of life or insulin action after DHEAS replacement therapy for 12 weeks. These results do not provide evidence for any positive effect on quality of life or insulin action and, therefore, do not support the addition of DHEAS to hypopituitary replacement therapy for these purposes.

Sources of Research Support: Doctoral fellowship from the R and D Office of the Department of Health and Personal Social Services, Northern Ireland.

Nothing to Disclose: CMM, PMB, SJH, CJT, CHC, CNE, BS, DRM, KRM, ABA

P1-267

The Effects of 3 Year Growth Hormone Replacement on the Lipid Profile of Adults with Growth Hormone Deficiency.

V Oguntolu¹, J Prague¹, P Choudhary¹ and S Aylwin¹.

¹Kings Coll Univ Hosp London, UK.

Introduction

Adult growth hormone deficiency (AGHD) is a well recognized clinical syndrome associated with cardiovascular morbidity and mortality. Reduction in total and LDL-cholesterol has been previously demonstrated, but there are few data after 3-years treatment. We studied the effects of GH replacement on the lipid profile in adult patients with GHD using a fixed graded initiation, followed by an individualized dose titration phase, for patients on long term treatment.

Design

A retrospective analysis of adult patients at Kings College Hospital GH database. Patients who had completed 3 years of replacement were selected. Recombinant human growth hormone (rhGH) was initiated at a dose of 0.3mg/day and increased to 0.5mg/day over the first 3 months period. Titration was subsequently made with the aim of achieving a target IGF-I level between the age-related mean and the upper limit of reference range.

Methods

70 adults (32 male and 38 female) who have completed a 3-year period of treatment with rhGH were selected for analysis. Fasting lipids (TChol, LDL, HDL and Triglycerides), IGF-I(ng/ml), waist/hip circumference, and weight were measured at each visit

Results

Measurements were made at baseline and 6, 12, 24 and 36months. Significant improvement was observed in all lipid parameters after six months. Further reduction in Tchol, LDL and Triglycerides occurred between 12, and 36months.

table1

| | Baseline (0mths) | 6months | 12months | 24months | 36months | p-values(0 vs 36mths) |
|-------------------|------------------|-----------|------------|------------|-----------|-----------------------|
| Total cholesterol | 6.05 (1.0) | 5.8(1.0) | 5.5(0.9) | 5.2(0.8) | 5.07(0.9) | p<0.0001 |
| LDL-cholesterol | 3.6 (0.9) | 3.4(0.8) | 2.92(0.95) | 2.73(0.8) | 2.6(0.9) | p<0.001 |
| HDL-cholesterol | 1.16(0.5) | 1.3(0.4) | 1.4(0.4) | 1.41(0.04) | 1.44(0.4) | p<0.001 |
| Triglycerides | 2.6 (1.1) | 2.32(1.0) | 2.2(1.0) | 2.06(0.44) | 1.98(1.0) | p<0.001 |

table2

| | 12months | 36months | p-value |
|-------------------|------------|-----------|---------|
| Total cholesterol | 5.49(0.93) | 5.07(0.9) | p=0.002 |
| LDL | 2.92(0.95) | 2.6(0.9) | p=0.02 |
| HDL | 1.4(0.4) | 1.44(0.4) | p=0.473 |
| Triglycerides | 2.2(1.0) | 1.98(1.0) | p=0.07 |

Conclusion

Treatment with GH over 3 year period in adults with GH deficiency leads to reduction in total, LDL cholesterol, triglyceride and increase in HDL. Our data indicate that significant improvement in lipid parameters become evident after 6months of treatment.

Disclosures: SA: Consultant, Ipsen, Novo Nordisk, Pfizer, Inc.

Nothing to Disclose: VO, JP, PC

P1-268

Cardiac Function in Growth Hormone Deficient Patients before and after One Year with Replacement Therapy: A Magnetic Resonance Imaging Study.

M Andreassen MD¹, J Faber MD, DMSc¹, A Kjaer MD, DMSc², CL Petersen MD³ and LO Kristensen DMSc¹.

¹Herlev Hosp, Univ of Copenhagen Herlev, Denmark ; ²Rigshospet, Univ of Copenhagen Copenhagen, Denmark and ³Frederiksberg Hosp, Univ of Copenhagen Frederiksberg, Denmark.

Context Assessed by conventional echocardiography the influence of growth hormone deficiency (GHD) and effects of replacement therapy on left ventricle (LV) function and mass (LVM) have shown inconsistent results.

Objective To evaluate cardiac function before and during replacement therapy employing the gold standard method cardiac magnetic resonance imaging (CMRI) and measurements of circulating levels of B-type natriuretic peptides.

Subjects and methods Sixteen patients (8 males and 8 females, mean age 49 years (range 18-75)) with severe GHD and 16 matched control subjects were included. CMRI was performed at baseline and after one year of GH replacement therapy (mean dose 0.34 mg/day). IGF-I, B-type natriuretic peptide (BNP) and the N-terminal part of pro-BNP (NT-proBNP) were measured after 0, 1, 2, 3, 6 and 12 months of treatment.

Results IGF-I Z-score increased from (median (IQR)) -2.3 (-3.8 to -1.4) to 0.5 (-0.3 to 1.7). LVM index (LVMI), ejection fraction (range 63-80%), cardiac output index and levels of BNP and NT-proBNP were similar at baseline in patients compared to controls (P-values from 0.09 to 0.37). The patients had significantly smaller LV end-diastolic volume index (P=0.032) and end-systolic volume index (P=0.038). No significant change in LV systolic function or LVM occurred during one year of GH treatment. BNP levels were unchanged (P=0.88), whereas NT-proBNP tended to decrease (P=0.052).

Conclusions Assessed by the highly sensitive and precise CMRI method, untreated GHD was not associated with impaired systolic function or reduced LVMI and GH replacement using physiological doses did not influence cardiac mass or function.

Sources of Research Support: Unrestricted research grant from NovoNordisk Scandinavia.

Nothing to Disclose: MA, JF, AK, CLP, LOK

P1-269

Effect of Growth Hormone Substitution Immediately after Pituitary Surgery on Metabolism, Body Composition, and Quality of Life.

J Aberle PD Dr¹, V Dannheim Dr¹, I Doeing¹, FU Beil Prof.¹ and J Flitsch PD Dr¹.

¹Univ of Hamburg Hamburg, Germany.

The effect of growth hormone substitution on in pituitary insufficient patients with growth hormone (GH) deficiency after pituitary surgery on metabolism, cardiovascular risk, and body composition is well known. Substitution is generally suggested in patients with a proven deficiency of the somatotrope axis if active tumor disease can be ruled out. However in many cases GH therapy is initiated months after pituitary surgery. Therefore it was our intention to find out, if an instant onset of substitution therapy after pituitary surgery is beneficial for patients.

So far we have recruited 20 patients after surgery of hormonally inactive pituitary adenoma. GH deficiency was proven by insulin hypoglycemia testing 3-5 days after surgery. In 10 patients GH treatment was initiated whereas 10 patients did not receive GH therapy. Follow up visits were at week 4, 12, and 24. At any visit laboratory parameters were determined, body impedance analysis was performed and quality of life questionnaires were filled out. Here we present the preliminary data of 20 patients. We will show how early GH treatment influences restitution of patients after pituitary surgery, body composition, and laboratory parameters as compared to a wait-and-see procedure.

Disclosures: JA: Research Funding, Novo Nordisk. JF: Researcher, Novo Nordisk.

Nothing to Disclose: VD, ID, FUB

P1-270

Incidence of Diabetes Mellitus in Growth Hormone (GH) Deficient Patients during GH Replacement Therapy - An Analysis of KIMS (Pfizer International Metabolic Database).

A Luger MD¹, AF Mattsson PhD², M Koltowska-Haggstrom MD, PhD², M Thunander MD³, M Goth MD⁴, J Verhelst MD⁵ and R Abs MD⁶.

¹Med Univ of Vienna Vienna, Austria ; ²Pfizer Endocrine Care Sollentuna, Sweden ; ³Central Hosp Växjö, Sweden ; ⁴Natl Med Ctr Budapest, Hungary ; ⁵Middelheim Hosp Antwerp, Belgium and ⁶Univ of Antwerp Antwerp, Belgium.

GH deficiency (GHD) as well as GH excess is associated with impaired glucose tolerance and diabetes. The aim of the present study was to evaluate incidence of diabetes in adults with GHD followed in KIMS. Subjects with adult-onset GHD aged 20-79 years without previous GH treatment and no diagnosis of diabetes at baseline were included in the study. Patients with Cushing's disease or acromegaly as underlying diseases were excluded. Diabetes was diagnosed according to the criteria of the American Diabetes Association and/or if reported as adverse event and/or if antidiabetic drugs were prescribed. GH dose was adjusted according to age-specific IGF-I reference values. Ratios of observed over expected number of cases were calculated (O/E) stratified for age and gender. Expected number of cases was calculated using population-based reference rates from a recently published study on diabetes incidence in southern Sweden (Thunander M et al., *Diab Res Clin Pract* 2008; 82:247). Multiple Poisson regression analyses on O/Es were also performed.

The studied group consisted of 5143 patients at baseline (50% females), mean age 49 years and a mean body mass index (BMI) of 29.1±5.9 kg/m². 523 patients developed diabetes (263 males, 260 females) over a median observation period of 3.1 years (range 0.01 -13 years; 20106 patient years). Diabetes incidence was 2.6 per 100 patient-years, a rate which was 6 times higher than expected (O/E=6.0 95% CI=5.5 - 6.6). However, the O/E ratio varied statistically significantly over several of the studied variables. In the multiple Poisson regression analysis O/E increased with BMI (9.4% per unit BMI; 95% CI: 7.8 -11%; p<0.0001) but decreased with attained age (around 4%/year; p<0.0001) and time since first GH treatment (-15.3% per year 95% CI: -19 - -11.5%; p<0.0001). Females had 23% higher O/E than males, 95% CI: 2.6 - 47%; p=0.025). In contrast, family history of diabetes, number of additional pituitary deficiencies, age at entry into KIMS, age at diagnosis of pituitary disease, IGF-I SDS and GH dose at the one year visit were not related to diabetes occurrence.

In conclusion, during GH replacement therapy incidence of diabetes is higher in GH deficient patients compared to a general population. However, decreasing risk with duration of GH therapy and lack of association with IGF-I SDS and GH dose indicate that GH replacement may not be the cause for the increased O/E ratio.

Disclosures: AL: Medical Advisory Board Member, Pfizer, Inc.; Chairman, Pfizer, Inc., Ipsen; Speaker, Pfizer, Inc., Novo Nordisk; Research Funding, Novo Nordisk. AFM: Employee, Pfizer, Inc. MK-H: Employee, Pfizer, Inc.

Nothing to Disclose: MT, MG, JV, RA

P1-271

Usefulness of High Resolution MRI and IPSS in Tumor Localization in Cushing's Disease.

Jung Soo Lim MD¹, Seung Ku Lee PhD¹, Se Hun Kim PhD¹, Sun Ho Kim PhD¹ and Eun Jig Lee PhD¹.

¹Yonsei Univ Coll of Med Seoul, Republic of Korea.

Background Cushing's disease is a rare disease with significant mortality and morbidity unless it is treated appropriately. The correct localization is essential because surgical removal is the treatment of choice, however, most tumors are too small for the detection.

Objective We evaluated the usefulness of high resolution MRI and inferior petrosal sinus sampling (IPSS) for the localization of tumor in Cushing's disease.

Methods Fifteen patients, 14 females and 1 male (range, 18-52 years) with Cushing's disease, underwent transsphenoidal surgery (TSS) by a single pituitary surgeon (SH Kim) from 2004 to 2009. IPSS was performed before TSS. While TSS, multiple staged resection and tumor tissue identification by frozen section (surgical and histological identification, SHI) were performed to determine the resection margin. All patients were cured confirmed by 24 hr urine free cortisol excretion.

Results Visible micro-lesion was identified in 10 (66.7%) on initial MRI. Seven (70%) of them had an agreement with IPSS lateralization, and among them, 6 were confirmed by SHI. Positive finding (10) on initial MRI had an agreement with SHI in 9 (90%). In 5 patients who had no visible lesions on initial MRI, only 1 (20%) showed an agreement between IPSS and SHI. After re-evaluation of the MRI images according to SHI retrospectively, tumor lateralization was changed in 2 cases (right -> bilateral, and unidentified -> bilateral). We also performed MRI again using different protocol in 2 patients whose tumors were not identified on initial MRI, the identified micro-lesions were confirmed by SHI. Overall MRI had an agreement with SHI in 12 (80%) in tumor lateralization. One False-negative IPSS result was noted. Overall, tumor lateralization by IPSS had an agreement with SHI in 7 patients (46.7%).

Conclusion High resolution MRI may be better in the localization of corticotrope adenoma than IPSS. In addition, while TSS, SHI is an important approach to identify tumor in order to remove tumor completely. Moreover, in the case when no definite lesion is found on initial MRI, it would be helpful to re-perform MRI using different protocol.

Nothing to Disclose: JSL, SKL, SHK, SHK, EJL

P1-272

Post-Operative ACTH and Cortisol Values and Its Correlation with Long Term Clinical Features in Cushing's Disease.

C Fajardo-Montanana¹, PA Riesgo-Suarez¹, C Messeguer-Escriva¹, J Gomez-Vela¹, JL Llacer-Ortega¹, L Gonzalez-Bonet¹, G Alaman-Laguarda¹, E Molla-Olmos¹, J Jornet-Fayos¹, A Cremades-Mira¹, JM Tomas-Torrent¹ and JL Piquer-Belloch¹.

¹Hosp Univ de La Ribera Alzira, Spain.

Introduction: It has been described that early post-operative cortisol levels lower than 2 mcg/dL might predict long term remission after transsphenoidal surgery for Cushing's disease. **Objective:** To study the early post-operative (< 72 hours) ACTH and cortisol levels in Cushing disease patients as a predictor factor of long-term remission. **Patients:** Twenty one consecutive patients, 5 men and 16 women, mean age 43.1 years (range 17-63) underwent transsphenoidal surgery for Cushing's disease in our hospital from 2005 to 2009. Mean followed-up period was 22 months (5-45). No corticosteroid replacement treatment was given. Post-operative ACTH and cortisol levels were measured every 4-6 hours. We study the clinical features, hormone values, radiological images, histological and surgical findings in the early postoperative period and at the end of the follow-up. **Results:** A total selective adenomectomy was intended in 20 patients in which a pituitary adenoma was identified during surgery. A right hemihypophysectomy based on petrosal venous sampling was performed in a male with no image in the preoperative MRI and with no intraoperative identification of the tumor. Early post-operative serum cortisol level was undetectable (< 2 mcg/dL) in 16 patients (100% of microadenomas (n= 9/9), 66 % of no MRI adenoma image (n=2/3) and 55.5% of macroadenomas (n= 5/9)). No new anterior pituitary deficits were added. One patient recovered a preoperative TSH deficit and three women recovered menstrual cycles. During the follow up period 18 (85.7%) patients achieved Cushing's disease remission: 100% (9/9) of microadenomas, 77.7% (7/9) of macroadenomas (7/9) and 66% (2/3) of patients with no adenoma identification in the preoperative MRI. Two patients had disease recurrence 1 year after achieving postoperative disease remission. **Conclusions:** Intraoperative identification and selective removal of the pituitary adenoma, even if they were not identified by preoperative MRI, correlated with 100% initial clinical remission in our patients. Early post-operative cortisol and ACTH measurements (without hormonal therapy replacement) have a prognostic value. Cortisol levels < 2 mcg/dl had a predictive value for clinical remission in patients with Cushing disease with pituitary microadenomas and for patients with no image of a pituitary adenoma in the preoperative MRI scan. Higher early postoperative cortisol levels did not exclude long term disease remission for macroadenomas.

Nothing to Disclose: CF-M, PAR-S, CM-E, JG-V, JLL-O, LG-B, GA-L, EM-O, JJ-F, AC-M, JMT-T, JLP-B

P1-273

Baseline Characteristics and Urine Free Cortisol (UFC) Variability of 162 Patients Enrolled in a Randomized, Double-Blind Phase III Study Assessing the Efficacy and Safety of Pasireotide (SOM230) in Patients with Cushing's Disease.

S Petersenn MD¹, J Newell-Price MD², JW Findling MD³, F Gu MD⁴, M Maldonado MD⁵, K Sen PhD⁶, LR Salgado MD⁷, A Colao MD, PhD⁸ and BMK Biller MD⁹.

¹ENDOC Ctr for Endocrine Tumors Hamburg, Germany ; ²Univ of Sheffield Sheffield, UK ; ³Med Coll of Wisconsin Milwaukee, WI ; ⁴Peking Union Med Coll Hosp Beijing, China ; ⁵Novartis Pharma AG Basel, Switzerland ; ⁶Novartis Pharma Corporation Florham Park, NJ ; ⁷Univ of Sao Paulo Med Sch Sao Paulo, Brazil ; ⁸Univ of Naples "Federico II" Naples, Italy and ⁹Massachusetts Gen Hosp Boston, MA.

Background: Cushing's disease represents an unmet medical need, with no approved medical therapies. Pasireotide is a multi-receptor targeted somatostatin analogue with high affinity for sst_{1,2,3} and sst₅. Based on encouraging Phase II results (1), a randomized, double-blind Phase III trial of pasireotide in patients with Cushing's disease is ongoing. Baseline characteristics of the patients in this study are reported, with a focus on analyzing the variability of UFC levels.

Methods: Patients with *de novo* or persistent/recurrent Cushing's disease were randomized to receive pasireotide in a 6m study. The primary efficacy endpoint is proportion of patients with mean 24-hour UFC $\leq 1 \times$ ULN at 6m. Because UFC is the primary endpoint, we analyzed the degree of variability at baseline. Four 24h-UFC samples were collected over 2 weeks for each patient, and the intra-patient variability was calculated. UFC was measured in a central lab by HPLC (normal range 30-145 nmol/day).

Results: 162 patients have been randomized, with accrual completed. Mean (\pm SD) age was 40.2y (\pm 11.9y), range 18-71y. 135 patients (83.3%) had persistent/recurrent disease, 27 (16.7%) were *de novo*. 128 patients (79.0%) had prior surgery, 7 (4.3%) prior irradiation, and 78 (48.1%) prior medication. Mean time since diagnosis was 54m (median 34m). Predominant comorbidities included hypertension (64.2%), osteoporosis (22.8%), diabetes mellitus (22.8%), hypothyroidism (21.0%), hypercholesterolemia (17.9%), hyperlipidemia (16.7%), depression (16.0%). Mean \pm SD baseline 24h-UFC was 970 \pm 1979 nmol/24h (range 195-22944). Mean intra-patient coefficient of variation (CV) was 31.3% (range 3.6-124.2%). The widest variation in an individual patient ranged from 600 to 14020 nmol/24h. Calculating a mean of the 4 UFCs for each patient reduces the SD in baseline UFC by 50%. Further analyses regarding the effect of multiple samples (4 vs 3 vs 2) on the reduction in intra-patient UFC variability will be reported.

Conclusions: The baseline characteristics of patients with *de novo* or persistent/recurrent Cushing's disease enrolled in a large Phase III study highlight the known adverse effects of chronic hypercortisolism. This study illustrates the wide variability in UFC measurements over a short period of time within individual patients. Calculating mean of 4 UFCs improves the SD by 50%. Analysis of baseline characteristics in a large cohort of Cushing's disease patients contributes to our understanding of this rare disease.

(1) Boscaro M et al. J Clin Endocrinol Metab 2009;94:115-122

Disclosures: SP: Speaker, Novartis Pharmaceuticals, Ipsen; Medical Advisory Board Member, Novartis Pharmaceuticals, Ipsen. JN-P: Consultant, Novartis Pharmaceuticals, Ipsen, Otsuka, Pfizer, Inc. JWF: Consultant, Novartis Pharmaceuticals, Corcept Therapeutics; Investigator, Novartis Pharmaceuticals, Corcept Therapeutics. MM: Employee, Novartis Pharmaceuticals. KS: Employee, Novartis Pharmaceuticals. BMKB: Study Investigator, Novartis Pharmaceuticals, Corcept; Consultant, Novartis Pharmaceuticals.

Nothing to Disclose: FG, LRS, AC

P1-274

Long-Term Treatment of Cushing's Disease with Pasireotide (SOM230): Results from a Phase II Extension Study.

M Boscaro¹, Y Zhang², K Sen², M Maldonado³, U Schoenherr³ and JW Findling⁴.

¹Polytechnic Univ of Marche Ancona, Italy ; ²Novartis Pharma Corporation Florham Park, NJ ; ³Novartis Pharma AG Basel, Switzerland and ⁴Med Coll of Wisconsin Milwaukee, WI.

Introduction: Pasireotide reduced UFC levels in 22 of 29 patients with Cushing's disease after 15 days' treatment (1). Of these 22 patients, five achieved normalized UFC (UFC-responders) and 17 had a reduction from baseline in UFC but did not achieve normalized UFC (UFC-reducers). Results of an extension phase to this study are presented.

Methods: Patients with *de novo* or persistent/recurrent Cushing's disease received pasireotide 600 µg sc bid for 15 days in the core study. Patients with significant clinical benefit after 15 days could enroll in an open-ended extension study. Patients with normalized UFC at end-of-core were to receive pasireotide 600 µg bid, with dose titration to 900 µg bid if required. All other patients were to receive pasireotide 900 µg bid. The primary endpoint was UFC response at month 6.

Results: 19 patients entered the extension phase, three of whom were UFC-responders at end-of-core. Mean treatment duration was 16 months (2mo-4.8y). Of 18 evaluable patients at 6 months, 10 (56%) had a reduction in UFC, of whom four (22%) were UFC-responders, and six (33%) were UFC-reducers. Of the four UFC-responders at 6 months, one was a UFC-responder and two were UFC-reducers at the end of the core study. In all patients, there was a trend toward a reduction in mean serum cortisol and plasma ACTH. Mean change in systolic blood pressure and bodyweight was -8.87mmHg and -7.4kg at 6 months. The most frequently reported adverse events (AEs) were diarrhea (13 patients), nausea (12 patients), hyperglycemia (11 patients) and abdominal pain (nine patients). Most AEs were grade 1 or 2; six patients reported grade 3 hyperglycemia and one patient reported grade 4 diabetes.

Conclusion: Long-term pasireotide treatment resulted in >50% of patients achieving normalization or a reduction in UFC, and was generally well tolerated. These results suggest pasireotide may be an effective long-term, pituitary-targeted treatment for Cushing's disease.

(1) Boscaro M et al., J Clin Endocrinol Metab 2009;94:115-122

Disclosures: YZ: Employee, Novartis Pharmaceuticals. KS: Employee, Novartis Pharmaceuticals. MM: Employee, Novartis Pharmaceuticals. US: Employee, Novartis Pharmaceuticals. JWF: Consultant, Novartis Pharmaceuticals, Corcept Therapeutics; Investigator, Novartis Pharmaceuticals, Corcept Therapeutics.

Nothing to Disclose: MB

P1-275

Subtle Cognitive Impairments in Patients with Long-Term Cure of Cushing's Disease.

J Tiemensma MSc¹, NE Kokshoorn Dr.¹, NR Biermasz Dr.¹, BSA Keijser Drs.¹, MJE Wassenaar Dr.¹, HAM Middelkoop Prof. Dr.¹, AM Pereira Dr.¹ and JA Romijn Prof. Dr.¹.

¹LUMC Leiden, Netherlands.

Context and objective: Active Cushing's disease is associated with cognitive impairments. We hypothesized that previous hypercortisolism in patients with Cushing's disease results in irreversible impairments in cognitive functioning. Therefore, our aim was to assess cognitive functioning after long-term cure of Cushing's disease.

Design: Cognitive assessment consisted of 11 tests, which evaluated global cognitive functioning, memory, and executive functioning.

Patients and control subjects: We included 74 patients cured of Cushing's disease and 74 controls matched for age, gender, and education. Furthermore, we included 54 patients previously treated for non-functioning pituitary macroadenomas (NFMA), and 54 controls matched for age, gender, and education.

Results: Compared to NFMA patients, patients cured from Cushing's disease had lower scores on the Mini Mental State Examination ($P=0.001$), and on the memory quotient of the Wechsler Memory Scale ($P=0.050$). Furthermore, patients cured from Cushing's disease tended to recall less words on the imprinting ($P=0.013$), immediate recall ($P=0.012$), and delayed recall ($P=0.003$) trials of the Verbal Learning Test of Rey and also recalled more intrusions on all trials ($P=0.002$, $P=0.003$, and $P=0.003$ respectively). On the Rey Complex Figure Test, patients cured from Cushing's disease had lower scores on both trials ($P=0.002$ and $P=0.007$) compared to NFMA patients. Patients cured from Cushing's disease also made less correct substitutions on the Letter-digit substitution test ($P=0.039$), and came up with less correct patterns on the Figure Fluency Test ($P=0.003$) compared to treated NFMA patients.

Conclusions: Cognitive function, reflecting memory and executive functions, is impaired in patients despite long-term cure of Cushing's disease. These observations indicate irreversible effects of previous hypercortisolism on cognitive function, and, thus, on the central nervous system. These observations may also be of relevance for patients treated with high dose exogenous glucocorticoids.

Nothing to Disclose: JT, NEK, NRB, BSAK, MJEW, HAMM, AMP, JAR

P1-276

Long Term Outcome in a Cohort of Patients with Cushing's Disease Treated at a Single Center in the Netherlands.

G van den Berg MD PhD¹, EW Hoving MD PhD¹, MN Kerstens MD PhD¹, MM van der Klauw MD PhD¹, LC Meiners MD PhD¹ and ACM van den Bergh MD PhD¹.

¹UMCG Groningen, Netherlands.

We report our experience in Cushing's disease over a period of 12 years (1998-2009), also on behalf of the other members of the Pituitary Working Group in our hospital. Thirty-nine patients underwent pituitary surgery, primary in 38 cases, and repeated surgery in one case, followed by secondary surgery in 4 patients and tertiary surgery in 2 patients. All surgery except for one was along the transsphenoidal route, presently using the endoscopic technique. In total, 47 operations were performed. Remission was defined as the disappearance of clinical signs, presence of low levels of serum cortisol shortly after surgery, with the temporary need for (hydro)cortisone substitution, and afterwards a normalized 24 hrs urine free cortisol together with adequate suppression of serum cortisol after 1 mg dexamethasone overnight (<50 nmol/l). The group consisted of 8 male and 31 female patients, representing 24 microadenomas, 6 macroadenomas, and 9 cases with occult disease. Inferior petrosal sinus sampling was performed in 8 of those 9 cases, confirming a pituitary origin. One microadenoma and one macroadenoma showed invasion of the cavernous sinus on MRI. The mean follow-up was 67 months (1-136). Remission was achieved in 34 patients (87%). Recurrent disease developed in 4 patients, after a follow-up period of 8-38 months (11%). Repeated surgery was unsuccessful in recurrent disease. All 4 patients underwent stereotactic radiotherapy (45 Gy/25 fractions). Primary failure was present in 5 patients, with remission after secondary surgery in 2 patients. A third patient was treated by radiotherapy, one patient died, and in a last patient subclinical disease was accepted until now. Overall repeated surgery was only successful in 3 out of 9. Occult disease was cured in 5 out of 9 cases. Two patients died due to cerebrovascular disease, 9 years and 6 months after surgery, the latter with persistent Cushing's disease. A third patient died, while being in remission for 3 years, after a myocardial infarction. The overall mortality was 8%. Stable remission is still present in 30 patients (76%), with a mean follow-up of 67 months (1-136). Cushing's disease is a very rare disease, even in a center for tertiary care, and results in dramatic clinical consequences for the patients involved. Management of these patients should be centralized in a few highly specialized centers, with a documented proper success rate. The main focus should be on the improvement of the surgical cure rates.

Nothing to Disclose: GvdB, EWH, MNK, MMvdK, LCM, ACMvdB

P1-277

Pituitary Tumors in MEN1 Syndrome.

RM Gomez MD¹, AG Diaz MD¹, JM Roganovich MD¹, S Lucas MD¹, MP Manavela MD¹, K Danilowicz MD¹, OD Bruno MD¹ and P Fainstein Day MD².

¹Hosp de Clins Buenos Aires, Argentina and ²Hosp Italiano Buenos Aires, Argentina.

Prevalence of pituitary tumors in Multiple Endocrine Neoplasm type 1 syndrome (MEN 1) varies from 10 to 60%. They are the first clinical manifestation in less than 25% of the patients. All types of anterior pituitary adenomas have been reported but prolactinomas, GH-secreting tumors and non-functioning tumors are the most frequent, whereas TSH and ACTH-secreting tumors are very rare. The aim of this communication is to report our experience in pituitary tumors in MEN 1 patients.

We analyzed 22 patients with typical clinical manifestations of MEN 1 (at least 2 or more major components of the syndrome: clinical and/or biochemical evidence of hyperparathyroidism, pituitary disease or duodenal-pancreatic tumors, or a family history including MEN 1 syndrome). A MEN 1 gene mutation was confirmed in 20 patients. Eleven out of 22 patients (50%) had anterior pituitary involvement: 5 corticotropinomas, 2 prolactinomas, 2 GH-secreting tumors and 2 non-functioning tumors. They were 8 women and 3 men, aged 14 to 51 years. In 8 of the 11 cases a pituitary adenoma was the first clinical presentation, 3 of them were corticotropinomas. Five patients showed macroadenomas. Only 3 patients remained without evidence of persistence or recurrence after treatment. In conclusion, pituitary involvement was frequent in MEN1 patients. The apparently high frequency of ACTH-producing pituitary tumors in this small series could be explained by a bias introduced by the high rate of derivation of Cushing's patients to our center. Nevertheless, despite the reported low incidence of ACTH-secreting tumors, systematic screening of MEN 1 syndrome should be recommended in cases of corticotropinoma. Management of pituitary adenomas in MEN 1 is similar to that in sporadic cases, although the persistence or recurrence of the disease is high.

Nothing to Disclose: RMG, AGD, JMR, SL, MPM, KD, ODB, PFD

P1-278

Absence of MEN1, AIP and P27Kip1 Mutation in Two Families with Familial Isolated Pituitary Adenoma.

T Sekiya PhD¹, RA Toledo PhD¹ and SP Toledo MD¹.

¹Univ of Sao Paulo Sao Paulo, Brazil.

Context: In addition to the MEN1 and PRKAR1A, two other genes, AIP and p27Kip1, have been recently associated to genetic predisposition to familial pituitary adenomas. Rare mutations in the p27Kip1 gene have been identified in MEN1-negative cases presenting MEN1 or MEN1-like phenotypes, while AIP mutations have been reported in approximately 15% of the kindreds with familial isolated pituitary adenoma (FIPA).

Objective: To analyze the MEN1, AIP and p27Kip1 genes in two not previously reported families with FIPA.

Patients: Two FIPA families were included in the study: a mother-daughter pair diagnosed with Cushing disease and two family members presenting with acromegaly. No MEN1 or CNC features were observed in all patients.

Methods: The entire MEN1, AIP and p27Kip1 genes were analyzed by PCR amplification and automatic sequencing.

Results: No mutation was identified in the MEN1, AIP and p27Kip1 genes. Heterozygous polymorphisms were observed in the patients indicating that full deletion of the analyzed genes is unlikely.

Discussion: Our results support the notion of genetic heterogeneity in FIPA. Further investigation will be necessary to identify new genes of FIPA.

Nothing to Disclose: TS, RAT, SPT

P1-279

Hyperplasia-Adenoma Sequence in Pituitary Tumorigenesis Related to AIP Mutation.

C Villa¹, MS Lagonigro², F Magri², R Brauner³, J Bouligand⁴, F Di Rocco⁵, C Sainte-Rose⁵, FX Roux⁶, A Daly⁷, A Beckers⁷ and L Chiovato².

¹Ctr Hosp Sainte Anne, INSERM U984, Univ Paris Descartes Paris, France ; ²Fondazione Salvatore Maugeri IRCCS, ISPEL Lab for Endocrine Disruptors, Dept of Endocrinology, Univ of Pavia Pavia, Italy ; ³Assistance Publique-Hôpitaux de Paris, Hosp Bicêtre, Univ Paris Descartes Paris, France ; ⁴Assistance Publique-Hôpitaux de Paris, Hosp Bicêtre, INSERM UMR-S693, Paris, France ; ⁵Hosp Necker Enfants-Malades, Univ Paris Descartes Paris, France ; ⁶Ctr Hosp Sainte Anne, Univ Paris Descartes Paris, France and ⁷Univ of Liege Liege, Belgium.

Pituitary adenoma predisposition has been recently associated with germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene, most often seen in familial isolated pituitary adenoma (FIPA) kindreds (1, 2). The mechanisms by which AIP mutations promote pituitary adenoma development remain uncertain. To gain insight into the role of mutated AIP in pituitary tumorigenesis, we performed a comprehensive analysis of loss of heterozygosity (LOH) in the normal adenohipophysis and abnormal tissue, in the context of a novel germline AIP gene mutation in a FIPA family with two young heterozygous twins with non-secreting pituitary adenomas. DNA was extracted from blood and paraffin embedded pituitary tissues, amplified by PCR. The fragments corresponding to the six exons of the AIP gene were sequenced and matched with the wild type reference. Both female twins were heterozygous for a previously undescribed germline AIP gene mutation (E216X). This mutation was inherited from the clinically unaffected father. Tissue-specific analysis of the E216X AIP mutation revealed LOH in the pituitary adenoma, whereas normal tissues had the AIP wild-type allele. Interestingly, zones of hyperplasia seen surrounding the adenoma also retained wild-type AIP. For the first time, the role of AIP gene in pituitary hyperplasia-adenoma sequence was investigated. Demonstration of specific LOH in the tumor tissue strongly suggests that AIP plays a role of tumor suppressor gene which determines a late pathologic event in pituitary tumorigenesis.

1. Vierimaa O et al, Science 2006
2. Daly AF et al, J Clin Endocrinol Metab 2007

Nothing to Disclose: CV, MSL, FM, RB, JB, FDR, CS-R, FXR, AD, AB, LC

P1-280

Sellar Metastases. Case Series of Patients Presenting to a Tertiary Care Center in the past Two Decades.

GS Bleumink MD,PhD¹, MW Schoofs MD,PhD¹, HL Tanghe MD,PhD¹, AJ van der Lelij MD,PhD¹, WW de Herder MD,PhD¹ and RA Feelders MD,PhD¹.

¹Erasmus MC Rotterdam, Netherlands.

Sellar metastases may mimic pituitary adenomas and are often difficult to distinguish on the basis of clinical signs or radiographic studies. Earlier studies have implicated diabetes insipidus as the typical presenting symptom, while anterior hypopituitarism, visual field defects and oculomotor palsy were found less frequently. However, the reported prevalence of signs and symptoms, especially of anterior pituitary failure, may have changed over the years because of improvement of diagnostic techniques.

We collected data from medical records on signs and symptoms, therapy and outcome of all cases of pituitary metastasis that were encountered between May 1988 and June 2009 in the Erasmus Medical Center in Rotterdam. We encountered 16 cases of sellar metastasis. Breast- (6) and prostate cancer (5) were the two most frequent encountered primary malignancies, followed by lymphomas (3). There was one case of lung carcinoma and one of leukemia. The majority of patients was male (56%). Mean age at diagnosis was 62. Presenting signs of sellar metastases are shown in Table 1.

Presenting signs and symptoms of sellar metastasis

| Sign/symptom | Percentage |
|----------------------------|------------|
| Pituitary insufficiency | 81% |
| Deterioration of vision | 50% |
| Optical chiasm compression | 40% |
| Visual field defects | 40% |
| Cranial nerve palsy | 38% |
| Headache | 38% |
| Diabetes insipidus | 25% |

Most frequent we found anterior hypopituitarism, which occurred in 81% of patients. In 6 of 12 performed bone scans hot spots were found in the sellar region. This corresponded with the MRI findings of infrasellar expansion or parasellar bone destruction. Somatostatin scintigraphy was positive in 2/6 patients. Ten of 14 patients (71%) showed improvement of signs and symptoms after neurosurgery, radiotherapy and/or chemotherapy. Mean time between diagnosis of sellar metastasis and death was 1.3 years.

It has been noted that, due to the use of more sensitive imaging techniques and endocrinological tests, the prevalence of anterior pituitary failure as presenting symptom in patients with pituitary metastasis is increasing over time. Bone and somatostatin receptor scintigraphy may give additional information, but are not highly specific for sellar metastasis. Sellar metastasis should be suspected when patients over 50 years old, with a history of cancer, present with a rapidly progressive lesion and symptoms of pituitary insufficiency, deterioration of vision and/or diabetes insipidus.

Nothing to Disclose: GSB, MWS, HLT, AjvdL, WWdH, RAF

P1-281

Phospho-Histone H3 (PHH3) Immunoreactivity in Non-Functioning Pituitary Adenomas.

E Hightower MD¹, J Devin MD², G Fuller MD, PhD², L Corley MA², IE McCutcheon MD², SG Waguespack MD², K Shah MD², K Hess PhD, MS² and ME Cabanillas MD².

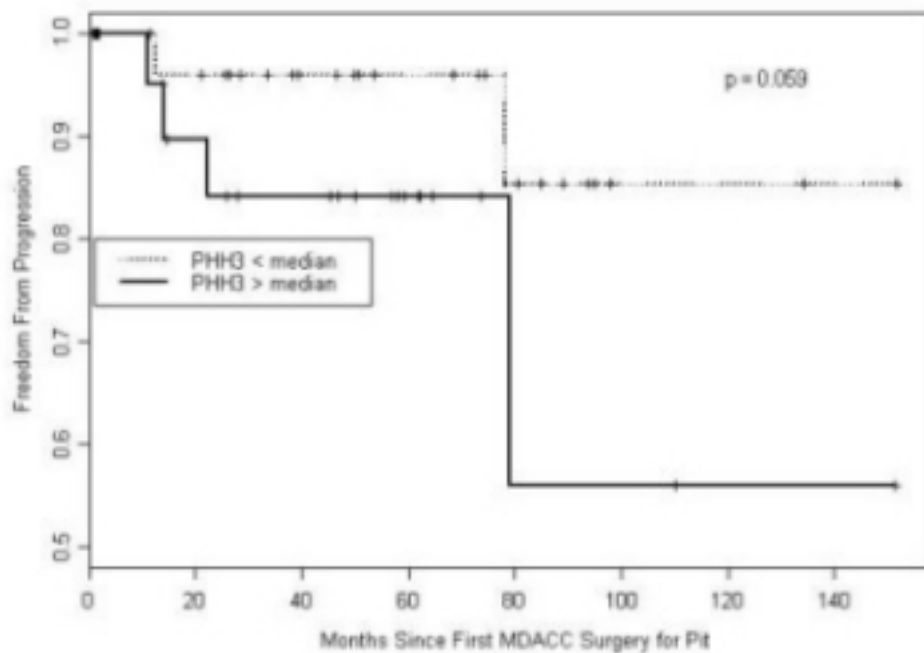
¹Univ of Texas Houston, TX and ²Univ of Texas MD Anderson Cancer Ctr Houston, TX.

Background: Nonfunctioning pituitary adenomas (NFPA) are typically benign neoplasms that can cause significant morbidity through local mass effect or invasion. Ki-67 reactivity and p53 expression are used to predict aggressive behavior, but no marker has been shown to reliably predict recurrence or progression. PHH3 is a protein phosphorylated during chromatin condensation in mitosis, and thus anti-PHH3 immunocytochemistry is uniquely able to reliably identify and quantitate mitotic activity. PHH3 is recognized as a prognostic indicator in patients with astrocytomas and assists in the grading of meningiomas(1,2). Its role in NFPA has yet to be evaluated.

Objectives: The study objectives were to determine the relationship among PHH3, Ki-67, and p53 in NFPA, and to evaluate the relationship between these indices and time to progression (TTP).

Methods: All NFPA patients from 1992-2006 operated on by a single neurosurgeon at MD Anderson Cancer Center (MDACC) were identified from a neurosurgical database (n=107). PHH3, Ki-67, and p53 were evaluated in each case with available tissue (n=76). Association strength between markers was assessed via Spearman's correlation coefficient. A rank correlation analysis was used to determine the association between the 3 proliferative markers and TTP.

Results: With 364 person-years of follow-up, 8 progression events were observed. 16 patients in whom the MDACC surgery did not represent the initial surgery were analyzed as a separate cohort (data not included). A modest correlation was found between Ki-67 and p53 (p=0.045). The PHH3 correlation with p53 approached significance (p=0.065). PHH3 did not correlate with Ki-67 (p=0.28). Of the 3 markers, only Ki-67 correlated with TTP (p=0.026). PHH3 reactivity suggested a shorter TTP, although this was not statistically significant (p=0.059).



Conclusion: Our results support previous data suggesting Ki-67 as a prognostic marker, but this assay is hindered by poor reproducibility and technical difficulties. PHH3 may provide a more reliable marker and a larger, prospective study of PHH3 reactivity in NFPA is warranted.

1. Colman H et al. "Assessment and Prognostic Significance of Mitotic Index Using the Mitosis Marker Phospho-histone H3 in Low and Intermediate-grade Infiltrating Astrocytomas." [2006] *Am J Surg Pathol* 30(5), 657-664.
2. Ribalta T et al. "The Mitosis-Specific Antibody Anti-Phosphohistone-H3 Facilitates Rapid Reliable Grading of Meningiomas According to WHO 2000 Criteria." [2004] *Am J Surg Pathol* 28(11), 1532-1536.

Nothing to Disclose: EH, JD, GF, LC, IEM, SGW, KS, KH, MEC

P1-282

Endoscopy Improves the Quality of Removal and the Endocrinological Outcome for Non Functioning Pituitary Adenomas Compared to Microsurgery.

E Jouanneau MD, PhD¹, M Messerer MD¹, G Raverot MD, PhD², M Rabilloud MD³, F Borson-Chazot MD, PhD², J Trouillas MD⁴ and G Perrin MD¹.

¹Neurological Hosp, Hospices Civils de Lyon, Claude Bernard Univ Lyon I Lyon, France ; ²Groupement Hosp Est, Hospices Civils de Lyon, Claude Bernard Univ Lyon I Lyon, France ; ³Claude Bernard Univ Lyon I Lyon, France and ⁴UFR Lyon RTH Laeannec, Claude Bernard Univ Lyon I Lyon, France.

Background: In absence of endocrinological symptoms, Non Functioning Pituitary Adenomas (NFPA) are usually diagnosed on visual symptoms induced by large macroadenomas. Thus, NFPA still challenge neurosurgeons with frequent post operative tumor remnants. Compared to microsurgery, endoscopy improves the surgical view. However, its superiority remains to be demonstrated. Our purpose was to compare the clinical results and the morbidity of endoscopy with those of microsurgery in 164 patients bearing a NFPA.

Methods: We compared the tumoral, endocrinological, ophthalmological post operative results, and morbidity in 2 consecutive series of patients with NFPA: 82 operated on by a sublabial approach with the microscope, between 2002 and 2005, and 82 patients operated on by a fully endonasal endoscopic technique, between 2005 and June 2009. Each patient was explored preoperatively and postoperatively annually by static and dynamic tests for pituitary functions, ophthalmologic exams and by 3D-contrast MRI.

Results: The two groups (endoscopic and microsurgical) did not differ regarding clinical features, tumor size and invasion of cavernous sinus ($p>0.05$). Postoperatively, the quality of resection was significantly improved ($p=0.002$) in the endoscopic group compared to the microsurgical one (Complete Removal, CR: 74/ 50%) and in the subgroup of tumors without cavernous sinus invasion, (CR: 86.9/72.5%; $p=0.04$). Regarding the anterior pituitary functions, in patients with a preoperative partial anterior deficit, the results were significantly improved ($p=0.01$) in the endoscopic group compared to microsurgical group (respectively, improvement: 56/25%; stabilization: 22/46% and aggravation: 22/29%). In patients with preoperative normal endocrinological status or total anterior deficit, no significant difference was found. In both groups, none of the patient with preoperative normal ophthalmologic exams worsened after surgery. In the endoscopic group, 100% of the symptomatic patients improved whereas 93% improved, 5% remained stable and 2% worsened in the microsurgical group ($p=0.35$). Lastly, no significant difference was found regarding the morbidity.

Conclusions: In this large series of patients with NFPA, surgery by endoscopy proves to be more efficient than microsurgery regarding the quality of resection and the endocrinological outcome. Thus, endoscopy may be recommended as the treatment of choice for such pituitary tumors.

Nothing to Disclose: EJ, MM, GR, MR, FB-C, JT, GP

P1-283

Endoscopic Endonasal Surgical Results of Pituitary Tumors with Cavernous Sinus Invasion.

Marvin Bergsneider MD¹, Marilene Wang MD¹ and Anthony Heaney MD, PhD¹.

¹David Geffen Sch of Med at UCLA Los Angeles, CA.

Introduction: Pituitary tumors with cavernous sinus invasion (CSI) demonstrate up to a 5-fold chance of residual tumor with an image-complete resection rate less than 10%. The objective of this study was to assess if an expanded endoscopic approach provides improved results.

Methods: This cohort was derived from a retrospective, consecutive series of 100 pituitary tumor patients undergoing endoscopic, endonasal resection in which no patient was excluded based on degree of tumor invasiveness. Confidence levels for CSI were based on positive-predictive values (PPV) established from objective MRI-defined criteria (Cottier et al, Radiology 2000; 215:463-469).

Results: Using PPV thresholds for CSI of 100 and 85%, a total of 12 and 31 patients, respectively, exhibited CSI. The mean tumor size was 27 ± 10 mm. Suprasellar extension > 10 mm was seen in 22/31 patients, with 6 patients with extra-diaphragmatic extension. An image-complete resection, based on MRI, was documented in 19/31 (61%) patients, but in only 5/12 (42%) of patients with 100% PPV CSI. Amongst this latter group, residual tumor was located in areas other than the cavernous sinus in 2/7 patients, therefore achieving a cavernous sinus clearance success rate of 58%.

Two patients (both in the 100% PPV group) experienced temporary 3rd nerve palsies. There were no carotid artery injuries.

Conclusion: The expanded, endoscopic endonasal approach facilitates exploration of the cavernous sinus under direct visualization, allowing image-complete resection rates greater than 50% in patients with CSI with minimal morbidity. Long-term follow-up will be required to assess clinical efficacy.

Nothing to Disclose: MB, MW, AH

P1-284

Effects of Pituitary Radiotherapy on Neurocognitive Functioning in Patients with Non Functioning Pituitary Adenoma.

P. Brummelman¹, M.F. Elderson¹, R.P.F. Dullaart MD PhD¹, G.J. Izaks MD PhD¹, C.A. Timmer¹, G. van den Berg MD PhD¹, A.C.M. van den Bergh MD PhD¹, B.H.R. Wolffenbuttel MD PhD¹ and A.P. van Beek MD PhD¹.

¹Univ Med Ctr Groningen, Univ of Groningen Groningen, Netherlands.

BACKGROUND: Neuropsychological deterioration is reported after pituitary radiotherapy (RT). However, reported results are inconsistent and previous studies are small or performed in cohorts with different pituitary pathology.

METHODS: We compared patients with non functioning pituitary adenomas (NFPA) who underwent surgery and adjuvant radiotherapy (RT+) with patients who had surgery only (RT-). Patients were only included when RT was given at least 6 months prior to the tests. Verbal memory was tested with a variant of Rey's Verbal Learning Test (RVLT). Executive functioning was measured with the Ruff Figural Fluency Test (RFFT). Anxiety and depression were measured with the Hospitality Anxiety and Depression Scale (HADS). All neuropsychological scores were adjusted for age, sex and education.

RESULTS: A total of 96 patients with NFPA participated in this study. Forty nine underwent surgery and adjuvant RT (32 men, age 60±11 y) and 47 underwent surgery only (30 men, age 62±9 y). Patients in the RT+ group were younger at surgery, more often underwent craniotomy and more frequently received hormonal substitution therapy. Education levels and HADS scores did not differ between the two groups. Median duration since RT was 11.6 y (range 0.6 - 29 y). On RVLT, the total patient group scored -0.95 SDS (standard deviation score) below the population mean for the immediate recall score (P=0.001) and -0.90 SDS for the delayed recall (P=0.001). RT+ patients scored significantly lower on the immediate recall (RT+:-1.19 SDS vs RT-: -0.69 SDS, P=0.037), but there was no significant difference on the delayed recall. When adjusted for immediate recall, RT+ patients had a significantly better delayed recall score (RT+: 0.05 SDS, RT-: -0.51 SDS, p=0.007).

On the RFFT, 71% of the RT+ group scored below the median of the reference population for the number of unique patterns (U) compared to 51% in the RT- group (P=0.048). The RT+ patients made less perseverative errors (P) when compared to the RT- patients (RT+: 32.7% below the median vs RT-: 53.2%, P=0.042). But, when adjusted for number of unique patterns (P/U) this difference disappeared.

CONCLUSION: Patients with NFPA who received postoperative radiotherapy scored different on measures of verbal memory and executive functioning than those who underwent surgery only. This association may be confounded by differences between groups in e.g. disease severity, type of surgery and hormone substitution.

Nothing to Disclose: PB, MFE, RPF, GJI, CAT, Gvdb, ACMvdb, BHRW, APvB

P1-285

Prognostic Indicators in Non-Functioning Pituitary Macroadenomas.

E Morin MD¹, J Lesage MD¹, J Ah-Lan MD¹, F Berthelet MD¹, C Beaugard MD¹, N Aris-Jilwan MD¹, H Beaugard MD¹, R Comtois MD¹, O Serri MD PhD¹ and S Vallette MD PhD¹.

¹CHUM Hosp Notre-Dame Montreal, Canada.

Objective: To determine clinical, radiological and pathological prognostic factors of non-functioning pituitary macroadenomas.

Design: Retrospective study of clinical data and treatment outcome of 120 patients with a non-functioning pituitary macroadenoma followed in our center for a minimal duration of 2 years (mean±SD, 8.2±5.8 yr; range, 2-27).

Patients: From 1982 to 2009, 259 patients (158 male, 111 female) were diagnosed in our center with a non-functioning pituitary macroadenoma. Of these, we analyzed 120 (70 male, 50 female) with a minimal follow-up of 2 year. Remission was defined as the absence of tumor remnant on imaging (CT-scan or MRI).

Results: Age at the time of diagnosis was 54±13 years (mean ± SD, range 21-83). At initial presentation, 51% of patients had headaches, 60% had visual field defects, 8% had opthalmoplegia and 62% had hypopituitarism. Mean adenoma largest diameter was 28±10mm (range,10-54), with invasion of surrounding structures in 48% of cases. First-line treatment was transsphenoidal surgery in 105 cases (87%), medical treatment in 5 cases and watchful waiting in 10 cases. Histopathological examination revealed no pituitary hormone immunoreactivity in 52%, gonadotroph adenoma in 31%, thyrotroph adenoma in 5% and multisecreting adenoma in 12% of cases. Remission was obtained in 26/105 (25%) patients after the first surgery and recurrences occurred in 4/26 cases. Remission was obtained in 3/22 patients following a second surgery. Tumor remnants were observed in 75% of patients 3 to 6 months following the first surgery. Tumor progression was observed in 58% of patients after surgery without additional treatment and in 39% of untreated patients. Radiotherapy, administered in 19 patients prevented tumor progression in 89% of cases. Considering patients untreated or treated by surgery alone, predictors for tumor progression were female gender (p=0.03), age at diagnosis below 40 yr (p=0.03), invasive adenomas (p=0.04). Conversely, male gender (p=0.03) and gonadotroph adenomas (p=0.09) seemed predictors of a better outcome. **Conclusion:** Since non-functioning macroadenomas are most often not cured by surgery, early identification of outcome predictors could improve long-term management of this disease.

Nothing to Disclose: EM, JL, JA-L, FB, CB, NA-J, HB, RC, OS, SV

P1-286

Healthcare Costs of Cushing's Disease from the US Commercially-Insured Perspective.

B Swearingen MD¹, L Boulanger MA, MBA², N Wu PhD², S Chen PhD², S Pulgar MPH³ and BMK Biller MD¹.

¹Massachusetts Gen Hosp Boston, MA ; ²Abt Bio-Pharma Solutions, Inc Lexington, MA and ³Novartis Pharm Florham Park, NJ.

Background: Cushing's disease (CD), a rare condition of cortisol excess resulting from an ACTH-producing pituitary adenoma, bears a significant morbidity and mortality burden. However, knowledge regarding the economic impact of CD is limited. **Methods:** This study assessed average annual costs for commercially-insured CD patients in the US 2004-2008 with a retrospective cohort design using a health insurance claims database of 29 million enrollees. CD patients were identified through diagnoses of Cushing's syndrome (ICD-9-CM: 255.0) and either benign pituitary adenoma (227.3) or hypophysectomy (07.6) and represented prevalent cases at different stages of treatment. Each CD patient was matched to 4 patients with non-functioning pituitary adenoma (NFPA) and 10 healthy controls (HC) by age and gender. NFPA patients were identified as those with a pituitary adenoma without Cushing's syndrome, acromegaly (253.0) or hyperprolactinemia (253.1). Comorbid conditions and annual direct healthcare costs were assessed through medical and pharmacy claims and costs were compared within each calendar year (CY). The analytic sample for each CY comprised patients and controls continuously enrolled for the entire CY. **Results:** Of the 877 identified CD patients, 79% were female and had an average age of 43 years (standard deviation [SD]: 14). Comorbidities were more prevalent among CD patients than NFPA or HC, including hypertension (43% vs. 24% vs. 17%, $p < 0.01$), diabetes (33% vs. 15% vs. 8%, $p < 0.01$) and hyperlipidemia (27% vs. 21% vs. 15%, $p < 0.01$). In CY 2008, CD patients had significantly higher total healthcare costs (mean: \$26,440, Standard Error [SE]: \$3,754) than NFPA controls (mean: \$13,708, SE: \$605, $p < 0.01$) or healthy controls (mean: \$5,954, SE: \$221, $p < 0.01$). Approximately one-third of total costs of CD patients were directly attributable to services related to CD. Cost differences were mainly due to an increased proportion of CD patients with inpatient admissions (20.7% [CD] vs. 15.8% [NFPA] vs. 7.1% [HC], $p < 0.01$) and more frequent outpatient hospital visits (6.5 vs. 3.8 vs. 1.8, $p < 0.01$). CD patients received more unique medications (mean: 10.0, SD: 7.6) than NFPA controls (mean: 7.4, SD: 6.6, $p < 0.01$) and HC (mean: 4.7, SD: 5.1, $p < 0.01$). Findings were similar for CY 2004-2007, and cost differences between cohorts increased over time. **Conclusions:** Patients with CD had more comorbidities than NFPA and HC, and incurred significantly higher annual healthcare costs.

Sources of Research Support: Research grant provided by Novartis Pharmaceuticals.

Nothing to Disclose: BS, LB, NW, SC, SP, BMKB

P1-287

Fibroblast Growth Factor Receptor 4 (FGFR4) in Cushing Disease Outcome: Influence of Expression and of the G388R Functional Polymorphism.

LP Brito MD¹, AM Lerario MD¹, MD Bronstein MD¹, BB Mendonca MD¹ and MCBV Fragoso MD¹.

¹Hosp das Clins da Fac de Med da USP Sao Paulo, Brazil.

Introduction: The normal human adenohypophysis expresses mRNAs for *FGFR1*, *FGFR2*, and *FGFR3*¹. Abnormal *FGFR4* expression has been detected in pituitary tumors, especially in larger and invasive adenomas^{2,3}. In addition, a single nucleotide polymorphism at codon 388 of the *FGFR4* gene, the substitution of glycine by arginine (G388R) has been identified³. Some studies demonstrated association of the arginine allele with adverse outcomes of human cancer^{4,5}. We hypothesized that *FGFR4* expression and genotype could be markers of adverse outcome of Cushing's disease (CD) after transsphenoidal surgery (TS).

Objectives: To analyze if there is association between the postoperative outcome of CD (remission / recurrence) and the *FGFR4* G388R genotype or the *FGFR4* expression in corticotropinomas. In parallel, we also evaluated clinical, hormonal and pathological findings known to be related to postoperative evolution.

Patients and Methods: Clinical, hormonal and pathological data of 76 patients who underwent the first TS were retrospectively reviewed. All patients were genotyped for G388R polymorphism. Genotyping of *FGFR4* G388R was determined by PCR-RFLP (*BstNI* restriction enzyme) in genomic DNA from peripheral blood or oral swab. *FGFR4* expression was assessed in 18 corticotropinomas. RNA was extracted according to Trizol reagent method. *FGFR4* mRNA expression was determined by real-time quantitative PCR using the Taqman system. It was used an assay that detects an amplicon in exons 8-9 boundary. A commercial pool of human pituitary glands was used as the reference sample.

Relative quantification was determined according to the $2^{-\Delta\Delta CT}$ method. A twofold change in mRNA levels was considered as significant.

Results: Homozygosis for *FGFR4* Gly388 alleles was associated with reduced disease-free survival (hazard ratio 6.91, CI 95%, 1.14 to 11.26; $p = 0.028$). *FGFR4* overexpression was found in 44% of the corticotropinomas and it was associated with lower postoperative remission rate ($p = 0.009$). Other variables associated with recurrence were Hardy tumor grade ($p = 0.017$), male gender ($p = 0.033$), lack of histological confirmation ($p = 0.026$) and cortisol levels $> 2 \mu\text{g/dL}$ in the early postoperative period ($p = 0.01$).

Conclusions: Our data suggests that homozygosis for *FGFR4* Gly388 alleles and *FGFR4* overexpression are associated with higher frequency of postoperative recurrence and persistence of Cushing's disease, respectively.

- (1) Abass SA et al., J Clin Endocrinol Metab, 1997; 1160-6.
- (2) Quian ZR et al., J Clin Endocrinol Metab, 2004; 1904-11.
- (3) Morita K et al., Clin Endocrinol 2008; 435-41.
- (4) Bange J et al., Cancer Res, 2002; 840-7.
- (5) Wang J et al., Clin Canc Res, 2004; 6169-78.

Sources of Research Support: FAPESP:06/52492-0.

Nothing to Disclose: LPB, AML, MDB, BBM, MCBVF

P1-288

Estrogen as a Presumed Risk Factor for Prolactinoma in a Male-to-Female Transsexual Patient.

VL Camara MD¹, UV Zanardi MD¹, A Glezer¹, DB Paraiba¹, MD Bronstein¹, BB Mendonca¹ and EMF Costa¹.

¹Univ of São Paulo São Paulo, Brazil.

Background: Estrogens significantly induce prolactin (PRL) synthesis and release from the pituitary gland in a dose-dependent and duration dependent manner. Estrogen administration is known to induce hyperprolactinemia, lactotroph hyperplasia and prolactinoma in rats. In humans, however, no definitive conclusions can be drawn about estrogen role on prolactinoma tumorigenesis.

Clinical case: A 42 year-old male to female (M2F) transgender patient referred to our institution for cross gender hormone treatment. The patient initiated estrogen treatment at the age of 17 and was receiving estradiol 17-enanthate 10 mg IM every 15 days. On physical examination, he had normal male sexual differentiation and development (penis length 7.5 x 2.0 cm, right testis 3.5 x 2.0 cm, left testis 3.0 x 2.1 cm) and breast development at Tunner V stage with mild bilateral galactorrhea. Visual fields were normal. Hormonal analysis was performed only after estrogen treatment and revealed an elevated serum PRL: 346 ng/mL (n < 10ng/mL), associated with suppressed LH and FSH (LH < 0.1 IU/L, FSH < 0.1 IU/L). Sellar MRI depicted a 1.5 x 1.3 cm lesion.

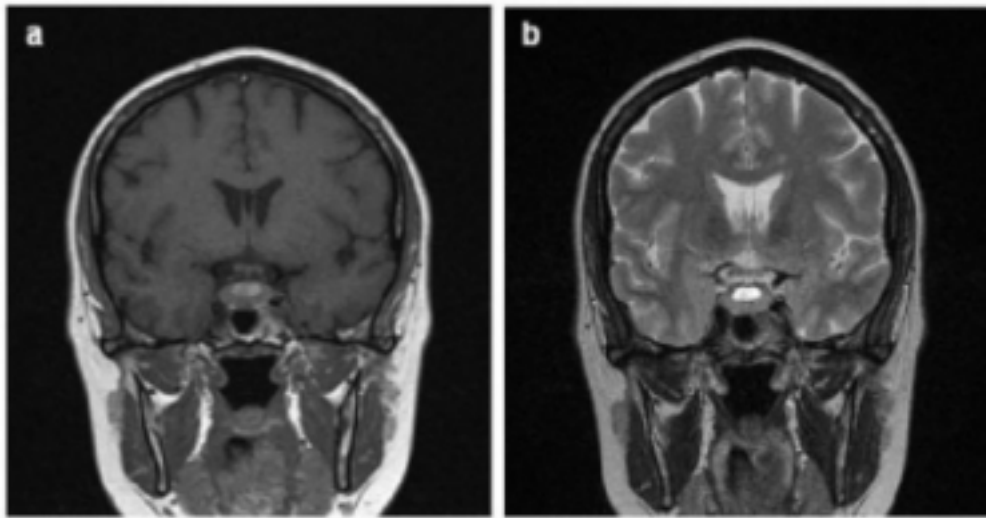


Fig. 1: Sellar lesion with hyperintense signal on T1 (a) and T2-weighted (b) images, suggesting a pituitary adenoma with a central cystic area.

Prolactinoma diagnosis was made and cabergoline was started. After 5 months, PRL level was 0.3 ng/mL and hypogonadotropic hypogonadism was solved (LH 5.6 IU/L, FSH 5.5 IU/L), despite regular estrogen intake.

Conclusions: This is the sixth reported case of prolactinoma in M2F transsexuals, suggesting the possible role of estrogen on prolactinoma tumorigenesis. Lactotroph adenoma have been reported in those patients on high or normal dose of estrogen treatment who presented previous normal PRL levels. Many potential mechanisms have been studied and proposed, including loss of inhibitory dopaminergic tone or direct action of estrogen in regulating pituitary proliferation through genomic and nongenomic mechanisms (1). Although causality has not been established, it is recommended to monitor serum PRL levels in the long-term estrogen treated M2F (2).

(1)Ben-Jonathan N et al. *Endocr Rev* 2001; 22(6):725-763

(2)Gooren LJ et al. *J Clin Endocrinol Metab* 2008; 93:19-25

Nothing to Disclose: VLC, UVZ, AG, DBP, MDB, BBM, EMFC

P1-289

Resolution of Hyperprolactinemia with Bromocriptine in Two Patients with Cabergoline Resistant Prolactinoma.

P Iyer M.D.¹ and M.E. Molitch M.D.².

¹Univ of South Florida Tampa, FL and ²Northwestern Univ Feinberg Sch of Med Chicago, IL.

Background: Dopamine agonists are usually effective in treatment of prolactinomas; however, resistance to dopaminergic agents has been described. Cabergoline is generally preferred to bromocriptine because of greater efficacy and lower adverse effects. We present two patients refractory to cabergoline therapy that responded to bromocriptine therapy.

Clinical Cases: Patient 1 is a 45 yr old female diagnosed with a pituitary microadenoma at age 30, upon presenting with galactorrhea and amenorrhea. She was treated with transvaginal bromocriptine for 9 years but then received no treatment for 2 yrs. When seen by us, her prolactin (PRL) was 102 ng/mL (2.3-26.7) and cabergoline 0.5 mg twice weekly was initiated. PRL remained elevated at one (90 ng/mL) and two years (80 ng/mL) despite an increase in the dose of cabergoline to 0.5 mg daily 3 mos prior to the last PRL level. Because of reports of potential cardiac valvulopathy with high dose cabergoline and cost, cabergoline was changed to bromocriptine, resulting in a normalization of PRL to 13 ng/mL in one year.

Patient 2 is a 15 yr old female who presented with headaches and secondary amenorrhea and was found to have a 17 mm macroadenoma with a PRL of 295 ng/mL. Cabergoline was started at 0.5 mg twice weekly and increased to 1 mg thrice weekly because the PRL level continued to rise to 340 ng/mL. After visual field defects developed, transphenoidal surgery was performed. Post surgery, the patient's PRL was 5 ng/mL but then rose to 212 ng/mL with increasing tumor size over 3 years despite escalation of cabergoline from 0.5 mg twice weekly to 1.0 mg thrice weekly. At that point, she was switched to bromocriptine at 10 mg daily and after 4.5 months the PRL fell to 133 ng/mL. However, after 2 mos, the macroadenoma continued to increase and a visual field defect developed so that she underwent another transsphenoidal surgery.

Conclusion: Some cabergoline resistant prolactinomas respond to bromocriptine, so that in such patients this change in treatment should be considered.

Nothing to Disclose: PI, MEM

P1-290

Successful Assisted Ventouse Delivery in a Pregnant Woman with Macroprolactinoma Compromising Optic Chiasm.

K Gunganah MBBS MRCP¹, S Chakravarti¹, S Chawda¹, J Pollock¹ and N Stojanovic FRCP¹.

¹Queens Hosp Romford, UK.

Background:

Over 30% of macroprolactinomas will cause symptomatic enlargement in pregnancy. The management and delivery of these patients can be challenging. We report the case of a pregnant woman with enlarging macroprolactinoma compromising the optic chiasm and visual fields, who was safely delivered by assisted Ventouse delivery.

Clinical Case:

A 27-year-old pregnant woman, with known macroprolactinoma, transferred her care to our centre at 23 weeks gestation. At the time, she was on a maintenance dose of Cabergoline 1mg twice weekly.

Her initial visual field peimetry was normal and pituitary MRI showed a 1.2 cm x 1.6 cm x1.8 cm macroadenoma abutting the optic chiasm with no evidence of compression. She was maintained on the same dose of Cabergoline and was having monthly visual field tests as well as regular endocrine, neurosurgical and obstetric follow ups.

Throughout pregnancy she complained of mild headaches. At 31 weeks gestation, a visual field defect was noted. Her Cabergoline was increased to 2 mg twice weekly. A subsequent pituitary MRI at 34 weeks gestation showed significant tumour enlargement, measuring 2.0 cm x 2.2 cm x 1.8 cm. The optic chiasm was elevated, as were the posterior aspects of both optic nerves suggesting a degree of nerve compression.

She had a planned delivery at 35 weeks gestation. Labor was induced and she had a successful and uneventful assisted Ventouse delivery. Subsequent dynamic pituitary tests were normal.

Two years after delivery her visual fields have returned to normal and the macroadenoma has significantly reduced in size on Cabergoline 1 mg daily.

Conclusion:

The literature on the mode of delivery in patients with large pituitary tumours compromising the optic chiasm is sparse. The choice of mode of delivery can be difficult. Caesarean section carries a risk of bleeding, hypotension and subsequent postpartum hypopituitarism (Sheehan syndrome). Assisted Ventouse delivery in comparison with normal delivery had the added benefit of limiting the duration of raised intracranial pressure. To the best of our knowledge, this is the first case reported of a pregnant woman, with symptomatic enlarging macroadenoma, safely delivered by assisted Ventouse delivery.

Nothing to Disclose: KG, SC, SC, JP, NS

P1-291

Cardiac Valvulopathy in a Prolactinoma Patient Treated with Cabergoline.

AT Siram MD¹, M Skamagas MD¹ and E Geer MD¹.

¹Mount Sinai Sch of Med New York, NY.

Background: Cabergoline, a dopamine agonist used to treat patients with prolactinomas, is associated with cardiac valvulopathy in patients with Parkinson's Disease (PD) (1). Cabergoline is a strong agonist of 5HT_{2B} receptors that are abundant on the cardiac valve myofibroblasts. Chronic stimulation of these receptors results in mitogenic stimulation of normally quiescent cells that may lead to overgrowth valvulopathy (2). Studies are still not conclusive on whether the dose and duration of cabergoline in prolactinoma patients poses a significant risk for valvulotoxicity as the cumulative doses tend to be much lower than those used in PD patients.

Clinical Case: A 53-year old gentleman with no significant past medical history was diagnosed with a pituitary macroprolactinoma (5cm) in 2005 when he presented with decreased libido and fatigue. Laboratory tests revealed a prolactin of 7360 ng/mL (nl 2.6-13.1ng/mL). The patient was started on cabergoline 1mg twice a week. The patient presented to our center in 2008 reporting improved symptoms on cabergoline and testosterone replacement. MRI of the pituitary revealed marked shrinkage of the pituitary tumor with the pituitary appearing normal in size. The patient's labs showed a prolactin 107 ng/mL and testosterone of 584 ng/dL (nl 280-800ng/dL) on replacement. He reported difficulty losing weight despite diet and exercise. Cabergoline was increased to 1.5 mg twice a week and repeat prolactin was 48 ng/mL. In 2009, MRI revealed no tumor seen and a shrunken concave pituitary. He was counseled on the potential risk of cardiac valve disease with cabergoline. Echocardiogram revealed trileaflet valve thickening, mild aortic regurgitation, and minimal mitral regurgitation. These aortic valve findings were new when compared to prior echocardiograms in 2005 and 2007. Repeat echocardiogram 7 months later revealed no change from the prior echocardiogram in 2009, and the patient remained asymptomatic. The endocrinologist has decided to closely monitor the patient with a repeat echocardiogram in 6 months. A switch to bromocriptine may be considered if there are clinical symptoms or worsening of the patient's valvulopathy.

Conclusion: This case demonstrates the development of aortic valve leaflet thickening after 4 years of cabergoline treatment of a macroprolactinoma, and suggests the importance of routine clinical and echocardiogram monitoring in such patients until studies elucidate the risk of valvulotoxicity.

(1) Zanettini R, Antonini A, Gatto G, Gentile R, Tesi S, Pezzoli. Valvular Heart Disease and the use of dopamine agonists for Parkinson's Disease. *NEJM* 2007; 356: 49-46.

(2) Roth BL. Drugs and Valvular Disease. *NEJM* 2007; 356:6-9

Nothing to Disclose: ATS, MS, EG

P1-292

Shrinkage of a Giant Prolactinoma with Cabergoline Therapy.

JR Kothapally M.D^{1,2} and RJ Anderson M.D^{1,2}.

¹Veterans Affairs Omaha, NE and ²Creighton Univ Omaha, NE.

BACKGROUND: Cabergoline therapy shrinks macroprolactinomas to variable degrees. Patients with very large tumors may experience apoplexy-like symptoms with tumor involution.

CLINICAL CASE: This white male presented at age 39 years with loss of peripheral vision for three weeks. Visual field testing showed a left upper visual field defect. MRI revealed a massive 5.5 cm pituitary tumor with bilateral cavernous sinus invasion and superior extension. Baseline laboratory values were: Prolactin >4000 ng/mL (normal, 2.0 -14); GH <1.5 ng/mL (<5); LH 0.9 mIU/mL (1.5-9.2); FSH 1.6 mIU/mL (1-14); Cortisol 13 mcg/dL (5-25); Total Testosterone 220 ng/dL (360-990); TSH <0.3 mIU/mL (0.3-5.0); Total T4 5.7mcg/dL (4.4-12.5). An emergency decompression via transfronto-pterional craniotomy was attempted, but failed due to a highly vascular tumor and bleeding with subsequent permanent right eye blindness and right 3rd and 6th nerve palsies. Pathology evaluation noted a pituitary adenoma. Radiation therapy was not used because of the risk of total blindness. Bromocriptine was started and titrated up to 50 mg daily with inadequate prolactin decline. The patient also received hydrocortisone, L-thyroxine and testosterone replacement. Cabergoline was added and increased to 5 mg twice per week while the bromocriptine was gradually stopped. Prolactin continued to decline. On cabergoline alone he experienced sudden, intermittent severe headaches without visual loss. Serial MRI studies showed a secondary empty sella and residual cavernous sinus tumor. Cabergoline has been decreased slowly to the current dose 4 mg twice per week. Now 21 years after his original surgery, the prolactin was 46 ng /mL. An echocardiogram showed no valvular abnormalities. A recent report of ten men with giant prolactinomas treated with cabergoline indicated three of them experienced a tumor volume reduction of > 95% after 12 months (1). Volume reduction of 50% in four patients and 25% in two patients also occurred. None of them reported apoplexy-like symptoms.

CONCLUSION: Dopamine agonists serve as the first-line therapy for giant and invasive prolactinomas, and as adjunctive therapy for incomplete surgical resection. Cabergoline can significantly decrease the tumor size and serum prolactin levels by variable amounts. Awareness of potential apoplexy-like symptoms during tumor involution will enhance the follow-up of these patients.

(1)S.M.Corsello et al, Clinical Endocrinology 2003;58,662-670

Nothing to Disclose: JRK, RJA

P1-293

Sibutramine-Induced Hyperprolactinemia: Case Report.

CGS Leaes MD PhD¹, JFS Pereira-Lima MD PhD¹ and MC Oliveira MD PhD¹.

¹Neuroendocrinology Ctr of Santa Casa de Porto Alegre, UFCSA Porto Alegre, Brazil.

Introduction: Several drugs may cause hyperprolactinemia, especially antipsychotic drugs and prokinetic drugs. Serum prolactin concentrations increase within hours after acute administration of these drugs and return to normal within two to four days after cessation of chronic therapy. So far, sibutramine, a sympathomimetic drug used in the management of obesity, was not described to be associated with altered prolactin levels. **Objective:** The purpose of this study is to present a case of sibutramine-induced hyperprolactinemia. **Case Report:** A 38-year-old white female patient seeks medical attention complaining of weight gain (Body mass index: 35) associated with anxiety. She started sibutramine treatment and presented with amenogalactorrhea. Hyperprolactinemia was diagnosed (prolactin of 46 and 89,6 ng/mL) with normal thyroid, renal and hepatic function, and a negative pregnancy test. A sella MRI was performed and sibutramine was suspended. Prolactin levels returned to normal within 15 days of sibutramine cessation and remained normal within 90 days of follow-up, with resolution of the amenogalactorrhea syndrome. **Conclusion:** sibutramine may be considered in differential diagnosis of drug-induced hyperprolactinemia.

Nothing to Disclose: CGSL, JFSP-L, MCO

P1-294

Rapid Pre-Operative Control of Hyperthyroidism with Lithium and Methimazole in a 17-year-Old Female with a TSH/GH-Secreting Pituitary Macroadenoma.

P Raghu MD¹, TK Hor MD¹ and L Munoz MD¹.

¹Rush Univ Med Ctr Chicago, IL.

Background: Pituitary adenomas that co-secrete TSH and GH are rare, comprising <0.5% of all pituitary adenomas. Pre-op control of thyroid hormone secretion is critical prior to imminent surgery and is usually achieved with antithyroid drugs or octreotide.

Clinical Case: 17-year-old female with no medical problems presented with primary amenorrhea and headaches. On physical exam she had tachycardia, edema in bilateral hands and feet, mildly enlarged hands, and a large goiter. MRI showed a 2.5 cm x 4.3 cm x 4.6 cm pituitary mass compressing the optic chiasm and the brain stem. Labs revealed central hypogonadism (LH 1.0 mIU/mL, FSH 2.81 mIU/mL, estradiol 27 pg/mL (0-433) and adrenal insufficiency (AI) (8 am cortisol 2.5 mcg/dL (6.7-22.6)). Prolactin (PRL) was 11.5 ng/mL (3.34-26.72) and PRL 1:100 was 9.95 ng/mL. Other labs include TSH 5.04 mIU/mL (0.3-4.2), FT4 4.77 ng/dL (0.58-1.64), FT3 15.79 pg/mL (2.5-3.9), alpha subunit (a-SU) 21 ng/mL (0.04-0.38), a-SU/TSH molar ratio 4.2, and SHBG 85 nmol/L (9-75), consistent with hyperthyroidism (HT) due to a TSHoma. IGF-1 was 627 ng/mL (193-731), GH was 6.5 ng/mL (0.0-6.0), and GH did not suppress with 75g of glucose (1 hr 8.2 ng/mL, 2 hrs 7.4 ng/mL), suggesting GH hypersecretion.

Patient was started on hydrocortisone for AI and beta-blockade and methimazole (MMI) 40 mg QD for pre-op control of HT. After 2 wks, FT4 (3.1 ng/dL) and FT3 (9.5 pg/mL) remained high, so MMI was titrated to 30 mg BID and lithium (Li) 300 mg BID was added. FT4 was 3.9 ng/dL and FT3 was 8.3 pg/mL 1 wk later, so Li was increased to 300 mg TID. TFTs markedly improved after 1 wk on this regimen: FT4 1.8 ng/dL and FT3 8.3 pg/mL. Repeat IGF-1 was 868 ng/mL.

At this time (4 wks after diagnosis), the patient underwent transcranial resection of the pituitary mass. The mass was partially resected and the post-op course was complicated by a left middle cerebral artery stroke. She remained on MMI post-op but Li was discontinued. TFTs initially improved, but 2 wks post-op were TSH 1.77 mIU/mL, FT4 2.6 ng/dL, FT3 9.9 pg/mL while on MMI 10 mg QD. MMI was titrated to 20 mg QD prior to discharge. GH and IGF-1 remained high at 11.6 ng/mL and 1251 ng/mL, respectively. Pathology revealed a pituitary adenoma which stained strongly for TSH, GH and PRL.

Conclusion: This is the first case report, that we are aware of, demonstrating the use of Li in conjunction with MMI to rapidly control HT in a patient with TSH/GH-secreting adenoma prior to surgery.

Nothing to Disclose: PR, TKH, LM

P1-295

Gonadotropin Secreting Pituitary Macroadenoma Causing Feminization.

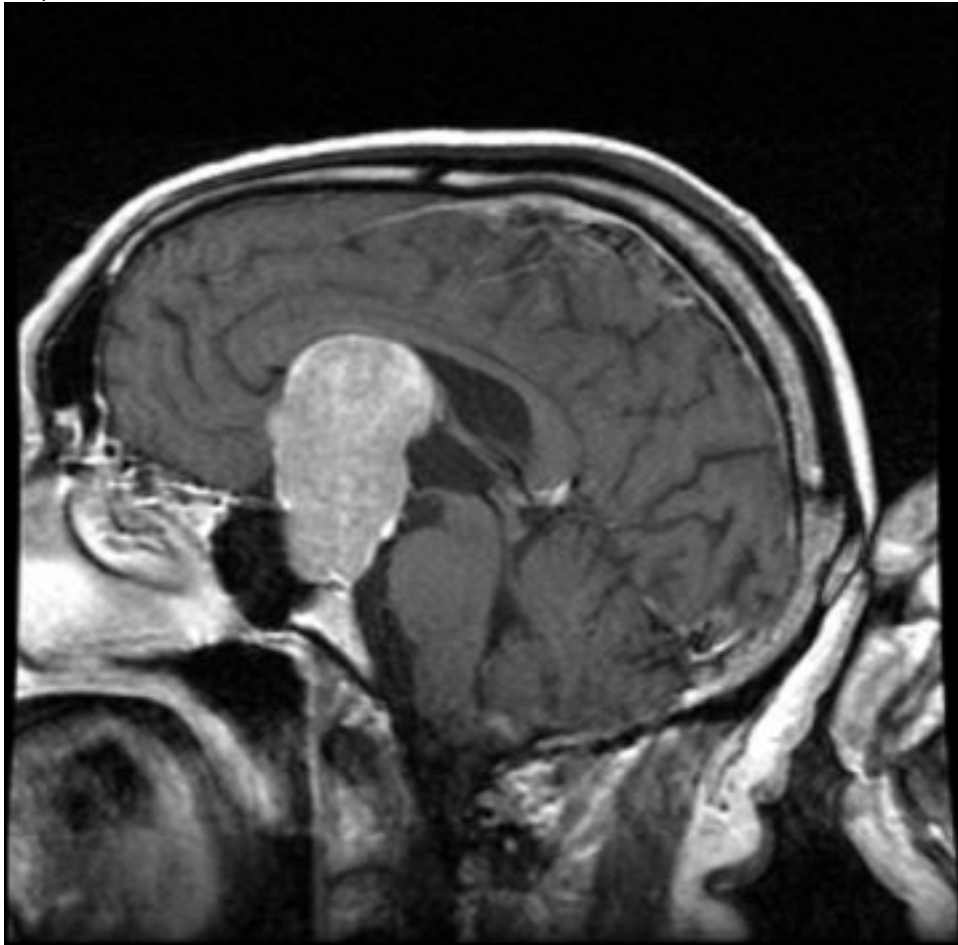
V Babu MD¹, C Blot MD¹, S Sukumaran Nair MD¹, P Cohen MD¹ and M Shimshi MD¹.

¹Bridgeport Hosp/Yale Univ Internal Med Residency Program Bridgeport, CT.

A Jamaican man aged 74 years[yr] presented with progressive visual loss for 4 yr. He also had frequent dizziness, decreased libido, gynecomastia, testicular pain for 1 yr. He denied headache, thyroid symptoms, muscle weakness, skin changes, or changes in weight.

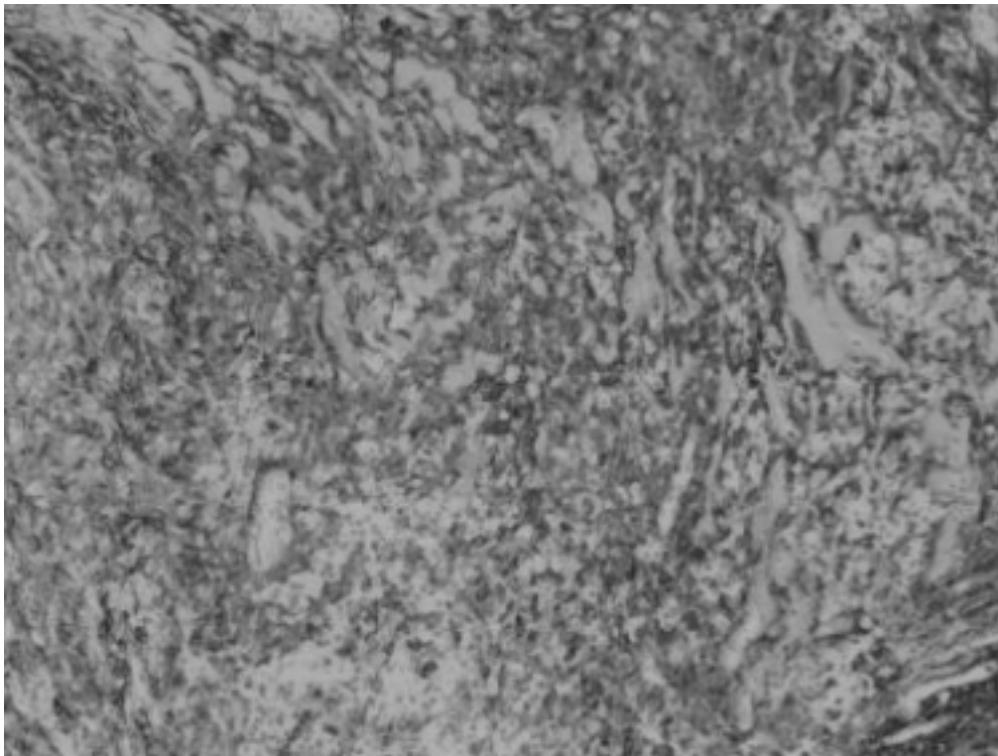
Positive findings on physical examination were: orthostasis [blood pressure 140/90 mmHg supine; 120/60 mmHg erect], complete visual loss in right eye, lateral field defects of left eye, bilateral gynecomastia, breast and testicular tenderness. Hair distribution and testicular size were normal.

MRI with contrast showed a 5X3 cm sellar mass with right frontal and inferior extension causing optic chiasm compression.



Laboratory evaluation showed elevated gonadotropins with FSH >200[1.5-12.4], LH 39.2[1.7-8.6], total testosterone 284[241-827], total estrogen 160.6[40-115] and remainder showed panhypopituitarism with TSH .02[.27-4.2], free T4 .77[.78-2.2], Am Cortisol 1.28, ACTH 15[7-50], GH<1 and prolactin 22.2[3.7-17.9]

He underwent frontal craniotomy and subtotal resection of pituitary macroadenoma. Histopathology showed cells diffusely positive for FSH, some compressed peripheral cells positive for prolactin and ACTH [likely normal pituitary cells], and negative TSH, GH and LH staining.



Ki67 was <1%.

Postoperatively he developed persistent DI and adrenal insufficiency and is currently on dexamethasone, l-thyroxine, DDAVP and testosterone. Postoperative labs were FSH 3.1, LH .872, testosterone <20, and estradiol 30.3[5.37-65.9].

Although gonadotroph adenomas are the most common pituitary macroadenomas, they rarely cause endocrine syndromes. FSH secreting gonadotroph adenomas with high total estrogen and normal testosterone causing feminization is previously unreported in men. The high FSH is caused by aromatization and conversion of testosterone to estrogen causing feminization.

Nothing to Disclose: VB, CB, SSN, PC, MS

P1-296

Somatostatin Analogues in Treatment of Clinically Non-Functioning Pituitary Adenomas - Does Pharmacotherapy Meet Expectations?.

J Kunert-Radek MD, PhD¹, N Zawada MD¹, H Pisarek PhD¹, M Gorska-Chrzastek MD, PhD¹ and M Pawlikowski MD, PhD¹.

¹Med Univ of Lodz Lodz, Poland.

Introduction: Somatostatin (SST) analogues have been used for more than 20 years in the treatment of acromegaly. Due to their antiproliferative and proapoptotic properties they are also proposed in the treatment of NFPA. The attempts of the treatment of NFPA with SST analogues have been relatively rare by now. The introduction of the treatment is possible on condition that there is presence of SSTR in the pituitary adenomas. The SSTR scintigraphy reveals the presence and the density of receptors in the adenoma, confirming the biological capability of these receptors to bind with ligands, especially type 2 of SSTR. Immunohistochemistry of SSTR in the surgically removed tumour's tissue allows the grading of all SSTR subtypes and give a possibility of choice of the SST analogue optimal for the treatment.

Aim of the presentation: The assessment of the effects of somatostatin analogues' treatment of non-functioning pituitary adenomas.

Analysis of clinical cases: The analysis has been carried out on 4 patients (3 women and 1 man, average age of 65 years) with clinically non-functioning macrotumours: 3 patients after several neurosurgical operations, including 1 patient also after radiotherapy and 1 patient with extraordinary hardness of the pituitary tumour, qualified for the next neurosurgery after pretreating with SST analogues, and 1 person refusing the surgical operation despite degeneration of the optic nerves. Scintigraphy revealed high density of SSTR in all four patients. Immunohistochemistry of surgically removed tumours' tissues of three operated patients confirmed the presence of 2 and 5 SSTR subtypes in the adenomas. All the patients received intramuscular injections of Sandostatin LAR 20mg every 4 weeks over 6 to 18 months.

Results: In all cases there was the good tolerance of the treatment with lack of undesirable effects. In none of the cases the progression of visual deterioration or further tumour expansion has been discovered.

Conclusions: Pharmacotherapy of NFPA with SST analogues is well tolerated treatment, providing the stabilisation of the tumour's expansion in both primary and recurrent adenomas at least in the analysed cases.

Nothing to Disclose: JK-R, NZ, HP, MG-C, MP

P1-297

A Sellar Mass in a Patient with Systemic Non Hodgkin Lymphoma: Is There a Role for PET Scan in Differential Diagnosis?.

Suzana San Juan Melo MD¹, Breno Balabem Alves MD¹, Antonio M Lerario MD, PhD¹, Maria Candida B V Fragoso MD, PhD¹, Valter A S Cescato MD, PhD¹, Andrea Glezer MD, PhD¹ and Marcello Delano Bronstein MD, PhD¹.

¹Univ of São Paulo Med Sch São Paulo, Brazil.

Background: Lymphomas originating in the sellar region are extremely rare, with 34 reports in the literature. Lymphoma metastases to pituitary are even rarer, with only 15 cases described. However, during the last decade there have been an increasing number of pituitary lymphomas diagnosed mainly due to immunosuppression and acquired immunodeficiency syndrome but also seen in immunocompetent individuals. Nevertheless, sellar mass in a patient with lymphoma can also be a pituitary adenoma. This association was described in 3 cases. MRI features may not differentiate among primary lymphoma, lymphoma metastasis to pituitary and pituitary adenomas.

Clinical Case: A 61 yrs- old man presented a sellar mass on a brain MRI performed for a systemic anaplastic non-Hodgkin lymphoma staging (Fig.1). He presented with hypopituitarism and visual impairment. All masses reduced after chemotherapy (cyclophosphamide, vincristine, doxorubicin and prednisolone), except the sellar one. F-18 FDG-PET imaging depicted a focal intense uptake only in the sellar region (Fig. 2). Surgery was performed and histology confirmed a non functioning pituitary adenoma.

Conclusion: Although the persistence of the sellar mass after chemotherapy was a clue pointing to pituitary adenoma, new techniques could help for sellar masses differential diagnosis. Despite lymphoma masses are known to have a high FDG-PET uptake, there are three previous cases of pituitary adenoma presenting, as our, a marked uptake. Therefore, more studies are needed to standardize pituitary adenoma profile in this imaging technique.

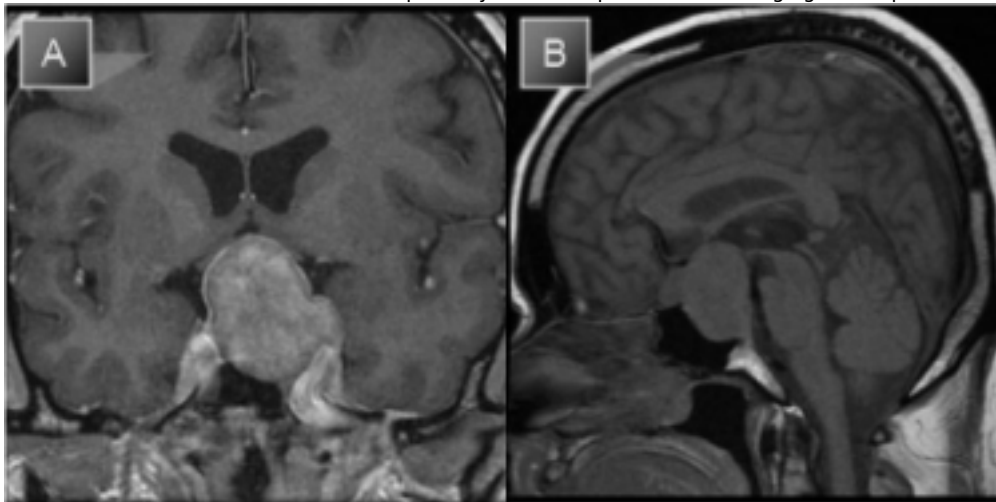


Fig. 1. (A) Coronal enhanced and sagittal unenhanced (B) T1-weighted MRI demonstrate a sellar mass with suprasellar expansion and chiasmatic compression.

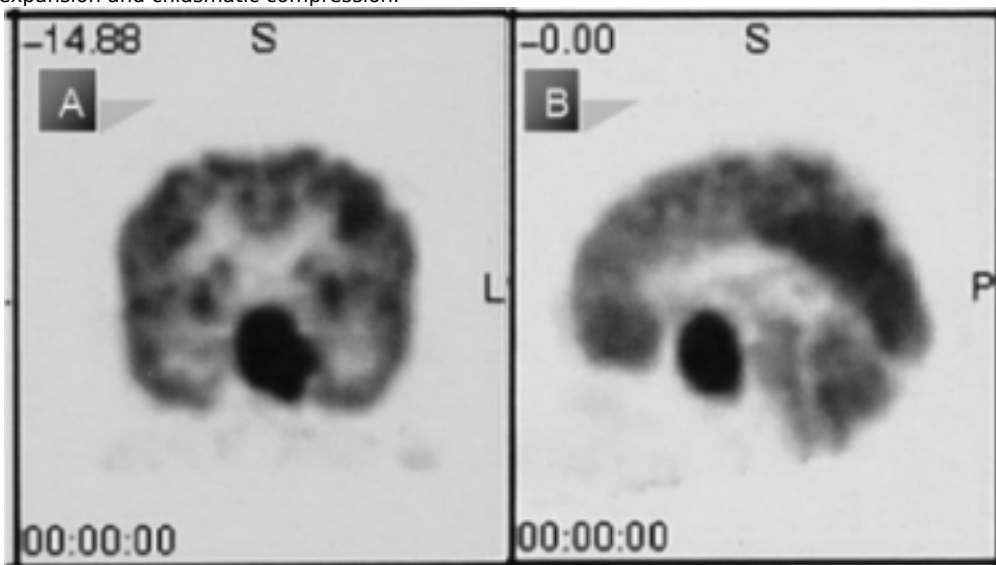


Fig. 2: FDG-PET images. (A) Coronal and (B) sagittal view revealing focal intense FDG uptake in the sellar region.

1. Mutsuko Yasuda et. al, Pituitary, 2009
2. Ogilvie C Megan, Pituitary 8:139-146, 2005
3. Giustina A, et.Al, J Clin Endocrinol Metab 86:4567-75
4. Clin Nucl Med. 2003;28(4):296-8

Nothing to Disclose: SSJM, BBA, AML, MCBVF, VASC, AG, MDB

P1-298

Pituitary Carcinoma.

AP Casarotto¹, TPB Silva¹, RM Junior PhD¹, MAZ Marino PhD¹, TZ Baldere¹, AN Almeida MDPHD¹, MKP Huayllas MS¹ and MA Costa¹.

¹Hosp Beneficencia Portuguesa Sao Paulo, Brazil.

This is an infrequent and unusual manifestation of neoplasm, characterized by cerebrospinal metastasis and/or extracranial. There are only 100 cases documented in the medical literature, thus representing 0.2% of pituitary tumors. Clinical Case: D.B.S., a 62 years old woman, with history of equilibrium dysfunction, diplopia, left ptosis, convergent strabismus, nausea, vomiting and vertigo. MR, showed a macro-adenoma (1.5 X 1.3 X 1 cm) involving the left cavernous sinus, and endocrine tests showed a non-functioning process. The patient was submitted to a transsphenoidal removal. After one month she showed contralateral ptosis and convergent strabismus, and a new MR demonstrated important growth of the sellar lesion (3.1 X 2.0 cm) with infra and supra-sellar invasion. A second transsphenoidal approach was performed followed by radiosurgery. A second pathological examination showed recurrent tumor with anaplasia and elevated mitotic index for atypical adenoma. Immunohistochemistry revealed a poorly differentiated pituitary carcinoma, with Ki67 positive in 70% of nuclei. In 3 months there was important worsening of the clinical picture, and a new MR demonstrated expansion of the primary lesion with meningeal and subependymal dissemination to the posterior fossa, fourth ventricle, cerebellum, medulla oblongata and cervical spinal cord, and ulterior death. Conclusion: Pituitary carcinomas are very aggressive tumors with a high mortality index. They usually are functioning adenomas, secreting PRL or ACTH. The non-functioning tumors, like this present case, are quite infrequent. Metastasis may occur rapidly, and systemic involvement is more common than cerebrospinal, rarely concomitant. There is no combination of histopathological data that make the diagnosis of this type of carcinoma. However, some studies have shown a correlation between the high mitotic activity (Ki67) with tumoral invasion, recurrences and metastatic potential. The differential diagnosis is made after disclosure of the metastatic lesions. After this confirmation, the treatment options are purely palliative, as chemotherapy and surgical removal. In summary, the present case represent and alert towards pituitary adenomas, with malignant behavior although they represent rarities.

Nothing to Disclose: APC, TPBS, RMJ, MAZM, TZB, ANA, MKPH, MAC

P1-299

Familial Pituitary Adenoma: A Report of Two Families.

NE Speil MD¹, P Jayaramaiah MD¹, R Trivedi MD¹, S Gupta MD¹ and R Khardori MD, Ph.D¹.

¹Southern Illinois Univ Sch of Med Springfield, IL.

Background: Pituitary adenomas are estimated to occur in familial setting in about 5% of cases. More than half are accounted for by MEN-1 and Carney Complex. Non-MEN/Carney Complex pituitary tumors also designated as familial isolated pituitary adenomas (FIPA) are being diagnosed with increased frequency.

Clinical cases: We report two cases that we have seen in our clinic in the past 10 years.

Our first case falls into the category of FIPA where the index case was diagnosed to have an aggressive tumor presenting with acromegaly while his mother had a non-functional pituitary tumor. He was 20 years old when diagnosed and underwent surgery (twice) and radiation therapy without remission. He was subsequently managed with Bromocriptine (30mg) daily after he was found to have adverse side effects from Sandostatin (nausea, diarrhea and abdominal cramps). His clinical, biochemical and radiological features were typical for textbook case of acromegaly. When he first presented to our clinic he had marked elevations in growth hormone and IGF-1. His IGF-1 and GH levels completely normalized with high dose Bromocriptine therapy.

Our second case is a 24 year old female who presented with headache and blurry vision during her 35th week of pregnancy. She was found to have a 17 mm pituitary adenoma without any hypersecretory disorder. Her pregnancy related hyperprolactinemia (PRL 260 ng/ml) resolved rapidly after she delivered. She was subsequently diagnosed to have Carney complex. Her mother has a non-functioning pituitary tumor without obvious hyper or hyposecretory disorder. She is currently undergoing more studies at a different medical center.

Conclusion: Recent analysis of Utah Population Data Base (UPDB) suggests a significantly elevated relative risk for first and third degree relatives of patients with pituitary tumors while the risk is not significantly different for second degree relatives. It is conceivable that we are missing many cases since tumors are often silent and therefore not detected. Had it not been for possible pregnancy related increase in tumor size, diagnosis in our second case would have been further delayed. Even though we now have a clinical and radiological diagnosis, the best treatment strategy still remains to be defined. Details on this cases will be provided and possible mechanisms of tumorigenesis discussed in further detail.

Nothing to Disclose: NES, PJ, RT, SG, RK

P1-300

Hypothalamic-Pituitary-Gonadal Axis Deterioration in Two Siblings with a Novel DAX 1 Gene Mutation.

L Iglesias M.D¹, M Castro-Magana M.D¹ and M Angulo M.D¹.

¹Winthrop Univ Hosp Mineola, NY.

Background: DAX 1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) is a nuclear receptor protein encoded by the *NROB1* gene (nuclear receptor subfamily 0, group B, member 1). It is an orphan receptor expressed in the adrenal cortex, gonads, ventromedial hypothalamus and pituitary gonadotropes. The roles of these nuclear receptors in the differentiation of the gonads and the adrenal cortex have been established through studies of the mutant phenotype in both mice and humans. However, the mechanisms underlying transcriptional regulation of these genes remain largely unknown. It has been postulated in addition that DAX 1 activity is essential in testicular development.

Clinical case: Two siblings, A and B developed severe adrenal crisis during their first two weeks of life with excellent response to glucocorticoids and mineralocorticoids replacement therapy. Both of them had normal penile length (4.5 and 5 cm) and testicular volume (1 and 2 ml) bilaterally, suggesting normal hypothalamic-pituitary-gonadal axis in utero. Both siblings were under good control while on glucocorticoid and mineralocorticoid therapy by age 15^{9/12} (A) and 14^{10/12} years(B), they had however, lack of puberty due to hypogonadotropic hypogonadism with testicular volume of 5.5 and 6 ml on A and B subject respectively. Genetic evaluation through DNA PCR amplification and sequencing of the *DAX1* nuclear receptor gene, revealed an 11 base pair duplication starting at nucleotide 321 (321dup11) They were placed on human chorionic gonadotropin (hCG) and recombinant FSH (rFSH) for a period of almost 2 years. Testicular volume increased to 11ml in A and 10 ml in B subject. Baseline serum levels of testosterone were low in both subjects (mean of 28 ng % +/- 11.3 SD) reaching a mean peak value of 1018 ng % +/- 217 SD while on hCG and rFSH treatment subsequently declining to levels close to those at baseline. In spite of the treatment, both siblings had progressive decrease in their testicular volume to 6 ml, which was associated with undetectable inhibin B levels and azoospermia by mean age 17.4 +/- 0.7 SD.

Conclusion: The lack of response to the administration of HCG and rFSH indicates primary testicular failure. Our cases suggests that not all developmental abnormalities caused by DAX 1 mutation will manifest at the same time and that gonadal development may be highly sensitive to the dose and pattern of DAX1 expression.

Nothing to Disclose: LI, MC-M, MA

P1-301

An 'Anti-Testis' Effect of DAX1 - Antagonism of the Testis-Specific Enhancer of SOX9.

LM Ludbrook PhD^{1,2}, P Bernard PhD¹, R Sekido PhD³, D Wilhelm PhD⁴, S Bagheri-Fam PhD¹, R Lovell-Badge PhD³ and VR Harley PhD^{1,2}.

¹Prince Henry's Inst of Med Res Melbourne, Australia ; ²Monash Univ Melbourne, Australia ; ³MRC Natl Inst of Med Res London, UK and ⁴Univ of Queensland Brisbane, Australia.

The role of the orphan nuclear hormone receptor *Dosage Sensitive Sex Reversal-Adrenal Hypoplasia Congenita on X chromosome, gene 1 (DAX1)* during gonad formation is contentious with pro-testis and anti-testis roles postulated. In humans, duplications of the *DAX1* gene at locus Xp21 cause Disorders of Sex Development (DSD), whereby XY individuals develop an ambiguous or female sex phenotype. Exactly how *DAX1* duplication causes the failure of typical testicular development is unclear. We hypothesized that, when present in excess, *DAX1* must repress the action of early testis-specific genes.

Using a mouse line transgenic for *Dax1* with gonad-specific expression, we investigated the expression of the critical testis-specific gene, *SOX9*. Immunostaining of *Dax1* transgenic XY gonads revealed reduced Sox9 protein levels when compared with wild type XY gonads. The low Sox9 protein levels may reflect a reduced transcriptional activity of the *Testis-Specific Enhancer of Sox9 (TES)*, which drives *Sox9* transcription in the developing XY gonad (1). Indeed, crossing *Dax1* transgenic mice with *TES-LacZ* reporter mice revealed that TES activity was repressed *in vivo* by high doses of *Dax1*. Moreover, in a heterozygous knockout mouse model for *Sox9* (50% dose of *Sox9* gene), the introduction of extra copies of *Dax1* starts to push the level of *Sox9* below a 'testis-determining' threshold, causing the formation of XY ovotestes expressing a female gonadal somatic cell marker, *FOXL2*, at both poles as shown by immunohistochemical analysis in developing XY embryos. These data support the hypothesis that when present at higher than typical doses *Dax1* functions in an 'anti-testis' manner by affecting *Sox9* expression. Using *in vitro* reporter activation assays and EMSA we show that *Dax1*-mediated repression of *Sox9* transcription is mediated through direct binding of *Dax1* to the nuclear hormone receptor Steroidogenic Factor-1 which in turn disrupts SF1 DNA binding to, and activation of, *TES*. With this work we have identified a potential mechanism for disruption of the male-specific sex determination pathway caused by *DAX1* duplication and leading to DSD in XY individuals.

(1) Sekido R and Lovell-Badge R., Nature 2008; 453:930

Nothing to Disclose: LML, PB, RS, DW, SB-F, RL-B, VRH

P1-302

Novel Intervening Sequence Mutation at the 5' Splice Site of the SOX9 Gene in an XY Infant Causing Campomelic Dysplasia Not Associated with Sex Reversal.

Cayce T. Jehaimi MD¹, Heather P. Crawford MD¹, Michael Z. Yafi MD¹ and Patrick G. Brosnan MD¹.

¹Univ of Texas Hlth Sci Ctr-Houston Houston, TX.

Background: Campomelic Dysplasia (CD) is an autosomal dominant disorder typically characterized by bowing of the long bones, Pierre-Robin sequence, cleft palate and absent corpus collosum among others (1,2). The majority of affected male infants (up to 75%) will also have sex reversal. The gene causing CD, SOX9, has been mapped to chromosome 17(17q24.3-q25.1). **Clinical case:** XY determined male infant born at 35wk gestation to healthy nonconsanguineous parents with birth weight of 2.3kg (25%) and length of 38cm (<10%). Soon after birth, absent corpus collosum and olfactory bulb hypoplasia were noted on brain MRI in addition to cleft palate, pretibial skin dimples, femora bowing and grade I hypospadias.





CD with SOX9 defect was suspected. All coding exons, 1-3, of the SOX9 gene on Ch 17 were amplified by PCR. DNA sequencing revealed an IVS1+1G>A transition. This mutation in an invariant conserved 5' splice site nucleotide caused aberrant mRNA processing and resulted in a phenotype consistent with CD. Patient was heterozygous. Further hormonal studies revealed robust testosterone and gonadotropin levels but with low anti-Müllerian hormone, suggesting possible Sertoli cell dysfunction. Additionally, TSH, GH & ACTH were all within normal limits.

| TSH (uU/mL) | Free T4 (ng/dL) | ACTH (pg/mL) | GH (ng/mL) | LH (mIU/mL) | FSH (mIU/mL) | AMH* (ng/mL) | Total Testosterone (ng/dL) |
|-------------|-----------------|--------------|------------|-------------|--------------|--------------|----------------------------|
| 2.8 | 1.43 | 35 | 43.8 | 11.7 | 3.8 | 26 | 168 |

*Normal range for males 14d-11months: 39-91

Conclusion: We describe a novel mutation of the SOX9 gene not associated with sex reversal. Skeletal anomalies at birth associated with any degree of genital ambiguity should warrant SOX9 gene analysis. Although our case report did not display sex reversal, the presence of hypospadias in association with abnormal long bone structures and Pierre-Robin sequence was enough evidence to suspect SOX9 gene defect.

- (1) Cameron FJ et al., Human Molecular Genetics 1996;10:1625
- (2) Kwok C et al., Am. J. Hum. Genet. 1995;57:1028
- (3) Schafer AJ et al., Phil. Trans. R. Soc. Lond 1995;350:271
- (4) Wagner T et al., Cell 1994;79:1111
- (5) Macpherson RI et al., Pediatr Radiol 1989;20:90

Nothing to Disclose: CTJ, HPC, MZY, PGB

P1-303

Mutant Steroidogenic Factor-1 from Patients with Disorders of Sex Development Show Reduced Activation of the Testis-Specific Enhancer of SOX9.

LM Ludbrook PhD^{1,2}, B Fisher BScHons^{1,2} and VR Harley PhD¹.

¹Prince Henry's Inst of Med Res Melbourne, Australia and ²Monash Univ Melbourne, Australia.

The orphan nuclear hormone receptor Steroidogenic Factor 1 (SF1; NR5A1) is expressed throughout hypothalamic, pituitary, gonadal and adrenal tissues. Naturally occurring human mutations combined with mouse knockout models have revealed a critical role for SF1 as a transcription factor at multiple stages during gonadal development and during development of the adrenal.

Missense mutation or truncation to SF1 in XY humans causes Disorders of Sex Development (DSD) with variable phenotypes. The precise mechanisms of SF1 action that fail in human DSD are not fully determined. This work aimed to utilise naturally occurring DSD-causing mutations in SF1 to increase our understanding of the sex determining function of SF1 in the developing male gonad. Recent work by others (1) identified SOX9 as a key target gene of SF1 during testis determination. SF1 activates Sox9 through a testis-specific enhancer element, termed TES. We tested the abilities of eleven clinical SF1 mutations to activate TES in reporter assays in HEK293T cells. Eight of the eleven SF1 mutants showed considerably reduced activation of TES compared to WT SF1. Furthermore, all mutations causing moderate to severe DSD phenotypes correlated with a more severe impairment of TES activation. In addition, all eleven of the mutants showed reduced synergistic activation of TES in co-transfection with the testis-determining co-factor SRY. Overall, this biochemical analysis of the function of mutant SF1 from DSD patients suggests that a failure of SOX9 upregulation, due to reduced activation of TES during testis development, could be the primary cause of the DSD in some patients with SF1 mutations.

(1) Sekido and Lovell-Badge, Nature 2008; 453(7197):930-934

Nothing to Disclose: LML, BF, VRH

P1-304

OCT4 Gonadal Gene Expression Related to the Presence of Y-Chromosome Sequences in Turner Syndrome.

B Bianco PhD¹, MVN Lipay PhD¹, KC Oliveira MSc¹, AD Guedes MD PhD¹ and ITN Verreschi MD PhD¹.

¹Fed Univ of Sao Paulo Sao Paulo, Brazil.

Background: Sex determination is genetically programmed in a critically timed and gene dosage-dependent manner. We hypothesized that the genes *DAX1*, *FOG2*, *GATA4*, *OCT4*, *SF1*, *SRY*, *TSPY*, *WT1* and *STRA8* that perform in the sexual differentiation and development, would have an altered expression in the dysgenetic gonads of Turner Syndrome (TS) patients possibly implying in the gonadal tumorigenesis. The present study aimed to investigate the presence of Y-chromosome mosaicism in TS patients and its association with gene expression *SRY*, *TSPY*, *SF1*, *WT1*, *DAX1*, *OCT4*, *GATA4*, *FOG2* and *STRA8* on gonadal tumorigenesis.

Material and Methods: One hundred and four TS patients and five fertile women as controls were studied. *SRY* and *TSPY* were screened by PCR in TS patients. Prophylactic gonadectomy was offered to all patients who tested positive for Y-chromosome sequences. Gonadal fragments of both TS and controls were studied by qRT-PCR for *SRY*, *TSPY*, *SF1*, *WT1*, *DAX1*, *OCT4*, *GATA4*, *FOG2* e *STRA8* genes.

Results: Y-mosaicism was found in 16.3% (n=17) of TS patients, all of them presented *SRY* and 3.8% also presented the *TSPY* gene. Twelve of the patients underwent the prophylactic surgery and the histopathological study disclosed bilateral gonadoblastoma in two cases, in another case, hilus cell hyperplasia and in a further case, hilus cell hyperplasia concurrently to stromal luteoma. In nine cases the pathologic examination did not reveal any tumoral process. No significant difference was found among the dysgenetic gonads and controls concerning the expression of the studied genes, except for *SRY*, *TSPY* e *OCT4* in both gonads of one patient whose chromosomal constitution was 45,X/45,X,add(15)(p11).

Discussion/Conclusion: *TSPY* genes are frequently expressed in tumors originated from germ cells and have been related to the development of gonadoblastoma. However, the most frequently used Y sequence in TS screening is *SRY* gene, due to its localization and important role in the signaling cascade of sex determining events. Furthermore, the *OCT4* gene is a pluripotent marker and the reactivation of this gene in the dysgenetic gonads occurs as part of the malignant transformation process typical of the gonadoblastoma. This way, the early detection of such events should be of great importance to preventing gonadal tumor development in TS patients.

Sources of Research Support: FAPESP (Grant No. 2007/01241-0).

Nothing to Disclose: BB, MVNL, KCO, ADG, ITNV

P1-305

Genetic Investigations in a 45,X Turner Patient with Spontaneous Puberty.

R Rossetti PhD^{1,2}, C Castronovo², C Cacciato^{1,2}, V Calcaterra³, D Rusconi², P Finelli^{1,2}, D Larizza³ and L Persani MD, PhD^{1,2}.

¹Univ degli Studi di Milano Milan, Italy ; ²IRCCS Inst Auxologico Italiano Milan, Italy and ³Univdi Pavia Pavia, Italy.

Turner syndrome (TS) is a disorder caused by numeric or structural abnormalities of the X chromosome. It is characterized by a variable spectrum of clinical features, the most common are short stature and ovarian dysgenesis. Spontaneous pubertal development in TS inversely correlates with the severity of karyotype abnormalities occurring in 5-10% of 45,X patients and in >30% of mosaics. These data support the concept that a double dose of certain X-linked genes is required for ovarian development. A critical locus for ovarian function has been defined at Xp11.2-q22.1. In a large array-CGH study on blood genomic DNA from 30 TS patients with 45,X on standard karyotyping, we identified an unique case with a duplication at chromosome Xp11.22 spanning 554 Kb and containing entirely only the BMP15 gene. This TS patient had spontaneous menarche at 14 years of age, followed by regular menses until 19, when she experienced secondary hypergonadotropic amenorrhea. As part of TS phenotype, she presents short stature. By Taqman CNV assay we investigated possible mosaicism in four tissues (blood, hair follicles, buccal and vaginal epithelium) and confirmed the duplication of the only BMP15 gene using two specific probes. In contrast, probes for GYG2 (Xp22.33) and SMARCA1 (Xq25) revealed just one copy for these genes. These results were further confirmed by i-FISH analysis using a BAC probe that covers the entire BMP15 gene and demonstrating a tandem duplication of this region on the X chromosome in 300 blood cells. Automatic sequencing of the entire coding region and intron-exon boundaries of the BMP15 gene revealed a wild-type sequence. In conclusion, a double dose of BMP15 gene is associated with spontaneous puberty and prolonged ovarian function in a 45,X TS patient. Since loss-of-function BMP15 mutations are associated with ovarian dysgenesis or premature ovarian failure in 46,XX women, these data support the concept that BMP15 gene dosage is critical for ovarian function in humans, and indicate BMP15 as the first ovarian determining gene on X chromosome.

Sources of Research Support: Telethon grant GGP09126; MIUR grant Prin 2006065999.

Nothing to Disclose: RR, CC, CC, VC, DR, PF, DL, LP

P1-306

Germ Cell Tumors (GCTs) in a Late-Treated Cohort of 46,XY Disorders of Sex Development (DSD) Patients.

DF Carvalho MD¹, FM Carvalho PhD², EMF Costa PhD¹, MAG Santos MSC¹, MY Nishi PhD¹, BB Mendonca PhD¹ and S Domenice PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil and ²Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

Introduction 46,XY patients with disorders of sex development (DSD) have a high risk factor for gonadal tumors' development. Type II germ cell tumors (GCTs) are the most frequent lesion found in dysgenetic gonads. A delayed maturation of germ cells in dysgenetic gonads predispose to malignant cell's transformation. Gonadoblastoma (GB) and intratubular germ cell neoplasia unclassified (ITGNU) are benign tumors that generally precede the invasive type II GCTs. Seminoma (S) and non-seminoma (NS) tumors are generally preceded by *in situ* neoplastic lesions. **Material** We analyzed retrospectively 15 late-treated 46,XY DSD patients (CA from 7 to 36 yrs-old) with previously diagnosed GCTs and its association with the diagnosis, age at surgery, location of the gonads and histological findings. A single expert pathologist reviewed all available tissue samples. **Results** In 7 patients with gonadal dysgenesis (one with Frasier syndrome) we found 2 S, 5 GB (3 of them associated with ITGNU and 1 with NS); in 3 patients with Turner syndrome with Y-chromosome lineage we found 3 GB (2 of them associated with ITGNU and one with S); in 1 patient with CAIS we found a ITGNU+S and in a patient with PAIS a ITGNU was found; in 3 patients with persistent Müllerian duct syndrome we found 2 S (one of them +ITGNU) and one NS. GCTs occurred before 15 yrs-old in two patients (CA 7 and 13 yrs-old) with 45,X/46,X,idic(Y)(pter→q11.2::q11.2→pter) and 45,X/46,XY karyotypes, respectively. All tumors were encountered in intra-abdominal gonads. **Discussion** GB affected mainly patients with gonadal dysgenesis, in accordance with the higher risk of GB in patients with a disruption in early stages of Sertoli cell differentiation. In our cohort, GB and ITGNU were equally found (53%) and a pathogenic link between both *in situ* tumors is possible considering the co-existence of these lesions in the same dysgenetic gonad. **Conclusion** Although a higher risk of ITGNU lesions has been associated with later-stage-gonadal development disorders, in our group we identified GB frequently associated with ITGNU in dysgenetic gonads, suggesting that a continuum spectrum of tumoral development might occur between these lesions.

(1) Cools M et al., Endocrine Reviews 2006; 27(5):468-484

(2) Mendonca BB et al., Clin Endocrinology 2009; 70:173-87

Nothing to Disclose: DFC, FMC, EMFC, MAGS, MYN, BBM, SD

P1-307

Frequency of Gly146Ala Polymorphism in Steroidogenic Factor 1 (*SF1/NR5A1*) Gene in Patients with Indeterminate 46,XY Disorder of Sexual Development (DSD).

PS Gadelha¹, CA Hohli¹, FO Machado¹, MP Brandao¹, AZ Machado¹, MG Santos¹, S Domenice¹, BB Mendonca¹ and EMF Costa¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

Steroidogenic factor-1 (*SF1/NR5A1*) regulates a variety of genes involved in steroidogenesis throughout the hypothalamic-pituitary-adrenal/gonadal axis. A large phenotypic spectrum has been associated with *SF1* mutations. *SF1* variants disrupt its function significantly enough to be a susceptibility factor for milder reproductive phenotypes. Studies have shown that the *SF1* polymorphism (pG146A) determines a mild alteration in *SF1* functional activity. It has been suggested that this variant might be a susceptibility factor for the development of micropenis or cryptorchidism.

Objective To analyze the frequency of the polymorphism pG146A in 46,XY DSD patients and to investigate if this variant constitutes a susceptibility factor for the development of this disorder. **Patients and Methods** Forty-four indeterminate 46,XY DSD patients (defects of androgen synthesis, action or metabolism were excluded) were evaluated to the presence of pG146A variant. Analysis of 13 patients with the variant showed that 4 were born small for gestational age, 7 presented micropenis and no Mullerian structures were identified. All patients presented normal testosterone response in hCG stimulation test (peak T >150ng/dl). The variant was searched in 109 Brazilian control males by the restriction enzyme digestion (*SphI*). **Results** The p.G146A variant was identified in heterozygous state in 13 out of 44 patients (29.5%) studied. Besides the association of pG146A with micropenis or cryptorchidism in previous study, in this DSD group it was not observed. The variant frequency was 19.3% (21/109) of control male (18 and 3 in heterozygous and homozygous state, respectively). The frequency of pG146A variant was slightly higher in 46,XY DSD patients compared to controls ($p=0,06$) and in controls it was similar to other studies. **Discussion** Some studies showed the diminished ability of p.G146A variant to transactivate *SF1* target genes. It suggests that the variant could also decrease the transcription of genes involved in androgen production, leading to genital abnormalities. The frequency of pG146A variant was higher in 46,XY DSD patients than in controls although it was not statistically significant in this cohort. **Conclusion** Even though no significant correlation had been determined between the presence of pG146A polymorphism and indeterminate 46, XY DSD, it does not exclude the potential contributions of decreased *SF-1* variants function in abnormal male genital development.

Nothing to Disclose: PSG, CAH, FOM, MPB, AZM, MGS, SD, BBM, EMFC

P1-308

Mapping a Causative Gene in Mayer-Rokitansky-Kuster-Hauser (MRKH) Syndrome in a Balanced 46, XX, T(3;16)(p21;p13.3).

HG Kim¹, R Ullmann², Y Shen³, LP Chorich¹, JF Gusella³, HH Ropers², V Kalscheuer², RH Reindollar⁴ and LC Layman¹.

¹Med Coll of Georgia Augusta, GA ; ²Max Planck Inst for Molecular Genetics Berlin, Germany ; ³Harvard Med Sch Boston, MA and ⁴Dartmouth Med Sch, Dartmouth-Hitchcock Med Ctr Lebanon, NH.

Mayer-Rokitansky-Kuster-Hauser syndrome (MRKH) is a severe reproductive developmental disorder resulting in the congenital absence of the uterus and vagina (CAUV). MRKH patients are 46,XX females with normal ovarian function. MRKH affects approximately 1/5,000 females and is the second most common cause of primary amenorrhea in girls. In familial cases, the syndrome appears to be transmitted as a female-limited autosomal dominant trait. Approximately 1/3 of patients have unilateral renal agenesis, and another 10-15% may have skeletal abnormalities, including the Klippel-Feil sequence with fusion of cervical vertebrae. The molecular basis of uterine and vaginal development is largely unknown. Although a number of genes have been implicated and studied, no causative gene has been identified to date. In humans only the *WNT4* gene has been associated in CAUV patients who also manifest mild hyperandrogenism. To date, only several chromosomal abnormalities have been identified with MRKH. These include an identical balanced chromosome translocation-46, XX, t(12;14)(q14;q31)- in two unrelated Indian girls with müllerian aplasia, maternally inherited terminal deletions of 4q and 22q11.21, as well as a recurrent 1.5 Mb microdeletion encompassing 16 known genes on 17q12. However, no disease gene has been identified in these chromosomal abnormalities. Because of the inherent reproductive impairment in MRKH, we hypothesize that a positional cloning strategy involving MRKH patients with balanced chromosomal rearrangements will lead to gene identification. We have ascertained a balanced 46,XX,t(3;16)(p21;p13.3) translocation in a female with MRKH. Comparative genomic hybridization (CGH) using the 244K Agilent CGH Array with an inter-probe distance of 7.8 Kb did not reveal any CNV changes. We therefore hypothesize that one of the breakpoints of this translocation is likely to harbor a gene responsible for this phenotype. We have already isolated derivative chromosomes 3 and 16 by FACS; and their genomic DNA was amplified for hybridization in array painting. This chromosome translocation affords the potential to define the first reported gene involved in MRKH, which will be instrumental toward understanding the embryology of this complex embryologic process.

Nothing to Disclose: HGK, RU, YS, LPC, JFG, HHR, VK, RHR, LCL

P1-309

Adrenocortical Insufficiency and 46, XY Disorders of Sex Development with Neonatal Onset Hereditary Coproporphyria.

K Hasegawa¹ and H Tanaka¹.

¹Okayama Univ Graduate Sch of Med, Dentistry & Pharma Scis Okayama, Japan.

Background: Genetic mutation of coproporphyrinogen oxidase, the sixth enzyme of the heme synthesis pathway, causes either hereditary coproporphyria (HCP) or harderoporphyria (1). HCP is a rare autosomal dominant disorder that chiefly exhibits a neurological attack. Its symptoms usually begin during late childhood or later: neonatal cases are rare. Harderoporphyria is an erythropoietic porphyria without acute neurological attacks by which harderoporphyrin is abnormally increased in patients' feces. Clinical symptoms of harderoporphyria include hemolytic anemia and jaundice; photosensitivity begins from the neonatal period. Homozygous or compound heterozygous K404E mutation in CPOX has been reported in patients with harderoporphyria. Recently, neonatal case with genetic mutation in CPOX that showed adrenocortical insufficiency and 46, XY disorders of sex development was reported by Takeuchi et al. (2).

Clinical case: A Japanese male neonate with jaundice and 46, XY disorders of sex development was found to have photosensitivity and adrenocortical insufficiency. Disorder of porphyrin metabolism was inferred to cause photosensitivity. Our patient showed severely impaired cortisol and testosterone production, but a urinary steroid profile analysis and serum steroid metabolites showed that known steroidogenic enzyme deficiencies including P450 oxidoreductase deficiency were not compatible to our patient. Clinical symptoms were compatible to harderoporphyria contrast to the pattern of increasing porphyrin metabolite that were compatible to those of typical hereditary coproporphyria. We found a heterozygous frame-shift mutation in exon 7 of the CPOX gene, but K404E in CPOX, which had been found in harderoporphyria, was not found. No other mutation was found in the coding region of any gene of protoporphyrinogen decarboxylase, uroporphyrinogen oxidase, or ferrochelatase.

Conclusion: Neonatal onset hereditary coproporphyria accompanies adrenocortical insufficiency and 46 XY, DSD.

(1) Schmitt, C. et al., Hum Mol Genet 2005;14: 3089

(2) Takeuchi H. et al., Blood 2001;98: 3871

Nothing to Disclose: KH, HT

P1-310

Absence of Inactivating Mutations in *FGF9* and *FGFR2* Genes in 46,XY Patients with Disorders of Sex Development (DSD) Due to Gonadal Dysgenesis.

AZ Machado Student¹, MG Santos Msc¹, EMF Costa PhD¹, BB Mendonca PhD¹, CR Gomes PhD¹, MP Brandao MD¹, MY Nishi PhD¹, RB Silva MD¹ and S Domenice PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

The expression of *Sox9* during testis' determination is critical for normal male gonadal development. *Fgf9/Fgfr2* signaling seems to be essential in the process of maintenance of *Sox9* expression after sex determination, an effect of auto regulative activity of *Sox9*, which is important to testis morphogenesis. *Fgf9* and *Fgfr2* knockout mouse present severe abnormalities in male gonadal development and causes male-to-female sex reversal. However, until now no association between these genes and testes development in humans could be confirmed. Our objective is to analyze if *FGF9* and *FGFR2* inactivating mutations are involved in the etiology of gonadal dysgenesis in 46,XY DSD patients.

Material and methods: We studied 39 patients with 46,XY gonadal dysgenesis (GD); 13 with the complete and 26 with the partial form. The entire coding region of *FGF9* (3 exons) and of the ligand binding domain of *FGFR2* (exons 7 to 10) were PCR amplified and sequenced directly using a BigDye Terminator in ABI PRISM 3100 DNA sequencer. **Results:** We did not find mutations in both genes in none of the patients. In *FGF9*, several previously described polymorphisms were identified (exon 1: rs35931146, rs10624265, rs35376466; exon 2: rs17070202, rs3818460, rs17070218, rs34748315, rs2274296; exon 3: rs9509841, rs9509842, rs17840929). In *FGFR2* a novel synonymous variant (c1035 T/C) on exon 9 was found in a partial GD patient. **Discussion:** *FGF9* was previously studied by Chen et al. in twenty-one patients with 46,XY DSD and no mutations was identified. Only a single nucleotide variant and a 3'-UTR microsatellite were found. The authors suggested that the 3'-UTR microsatellite of *FGF9* is a functional polymorphism that might play dual roles in regulation of *FGF9* expression. Deletions of 10q chromosome are classically associated with male genital abnormalities and *FGFR2* is one of the genes located at 10q26 region, suggesting its potential participation in the phenotype of 10q deletion syndrome. However, no inactivating mutations were found in our GD patients. **Conclusion:** Although these preliminary results did not identify inactivating mutation in *FGF9* and *FGFR2* genes in patients with 46,XY DSD due to GD, the role of this pathway in human testis embryogenesis may not be completely excluded. Further investigations, especially on the dosage effects of *FGF9* and *FGFR2* will be performed.

(1) Chen TM et al., 2007

Sources of Research Support: CNPq- 138091/2009-9; FAPESP- 08/55952-8.

Nothing to Disclose: AZM, MGS, EMFC, BBM, CRG, MPB, MYN, RBS, SD

P1-311

An Acceptor Site Mutation in Intron 1 of the Steroid 5 α -Reductase-2 (SRD5A2) Gene in Two Unrelated Cases of 46XY Male Pseudohermaphrodite.

Ali S. Alzahrani MD¹, Minjing Zou MD¹, Essa Baitei MD¹, Roua Al-Rijjal MD¹, Omalkhaire Al-Shaikh MD¹ and Yufei Shi MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Deficiency in steroid 5 α -reductase-2 is a rare cause of male pseudohermaphrodite. Mutations in the SRD5A2 gene encoding this enzyme have been described in most of the previous cases. In this report, we describe 2 unrelated cases of male pseudohermaphrodite, who did not seek medical advice until they reached pubertal age, in whom we have characterized a genetic mutation in the SRD5A2 gene. The cases were 18- and 15-year old who were raised as females (referred to subsequently as patient 1 and patient 2, respectively). They presented with primary amenorrhea and lack of development of female secondary sexual characteristics. Both patients had normal pubic and axillary hair, vulvae, clitoromegaly, masculinized body habitus, no breast development, and bilateral gonads in the inguinal regions. Both patients had 46XY karyotype. In patient 1, serum testosterone was elevated at 824 ng/dl (259-778) and dihydrotestosterone was on the low normal range for male of this age at 17.6 ng/dl (11.2-95.5) with testosterone:dihydrotestosterone ratio of 49 (normal <20). After 3 days of stimulation with beta-HCG, the levels increased to 1441 ng/dl and 39.1ng/dl, respectively. Serum 17-hydroxyprogesterone was normal at 2.1 nmol/l (0.3-3.3) and increased only to 16.8 nmol/l after 250 ug i.v synthetic ACTH stimulation (normal <30). In patient 2, serum testosterone and dihydrotestosterone were 717.6 ng/dl and 12.5 ng/dl, respectively (T:D ratio 57.4). After Beta-HCG stimulation, they increased to 1406 and 25.2 ng/dl, respectively. 17-hydroxyprogesterone was 7.2 nmol/l after synthetic ACTH stimulation. MRI of the pelvis of both patients showed absent female internal genitalia, rudimentary seminal vesicles and prostate glands and bilateral inguinal testes. After comprehensive counseling, both patients chose to be raised as males and surgery in the form of scrotoplasty and bilateral scrotal orchidopexy was performed for patient 1 while the patient 2 is scheduled for a similar procedure. The 5 coding exons and intron-exon boundaries of the SRD5A2 gene were amplified by PCR and subsequently sequenced from peripheral leukocyte DNA isolated from the patients and their parents. A biallelic acceptor site mutation in the SRD5A2 gene changing adenine to guanine ((IVS1 -2A>G) was found in both patients. A monoallelic (IVS1 -2A>G) mutation was present in their parents. We conclude that the steroid 5 α -reductase-2 deficiency in these 2 unrelated patients is caused by an acceptor site mutation in intron 1 ((IVS1 -2A>G) of the SRD5A2 gene, resulting in male pseudohermaphrodites.

Nothing to Disclose: ASA, MZ, EB, RA-R, OA-S, YS

P1-312

Absence of Mutations in *DMRT1* Gene in Patients with 46,XY Disorder of Sexual Development (DSD) Due to Gonadal Dysgenesis.

TE Silva Student¹, EMF Costa PhD¹, MG Santos MSc¹, MY Nishi PhD¹, AZ Machado Student¹, CR Gomes MD¹, RB Silva Student¹, MP Brandao PhD¹, BB Mendonca PhD¹ and S Domenice PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

The *Dmrt1* gene, a transcriptional factor, has an important role in testis development. *Dmrt1* knockout male mice showed severe testis hypoplasia and disorganization of testicular structures. In humans, *DMRT1* is located at 9p24.3 region. Classically, patients with deletions of 9p chromosome have urogenital abnormalities and also, in some cases, gonadal dysgenesis (GD). In patients with 9p deletion syndrome, usually others genes in addition to *DMRT1*, as *DMRT3* and *DMRT2* are deleted too, consequently the exact role of *DMRT1* in human genitourinary abnormalities has not been yet completely established. At moment, inactivating mutations in *DMRT1* have not been described in 46,XY patients with GD. **Objective** To investigate the presence of *DMRT1* inactivating mutations in 46,XY patients with DSD due to gonadal dysgenesis. **Material and Methods** We studied 38 patients with 46,XY GD; 25 patients with partial GD and 13 patients with complete GD. The entire coding region (exons 2-5) of *DMRT1* and dominate DM region (exon 1) were amplified by PCR. They were sequenced and analyzed by the ABI Prism 3100 Genetic Analyzer. **Results** A novel synonymous allele variant (c.923G>C) located at exon 3 of *DMRT1* was found in a partial GD patient and four polymorphisms previously described were also identified: rs3739583 in exon 1; rs16925431 and rs34946058 in exon 3; and rs35846503 in exon 5. **Discussion** Although in male mice the involvement of *Dmrt1* in the process of early male gonadogenesis had been demonstrated in experiments with knockout animals, in humans the role of *DMRT1* in testicular development has not yet been completely elucidated. It is not clear if haploinsufficiency of one or a combination of *DMRT* genes is responsible for this phenotype. Some 46,XY GD patients were analyzed to search for mutations in *DMRT1* and *DMRT2*, but no mutations were identified in these genes; only polymorphisms. **Conclusion** The absence of inactivating mutation in *DMRT1* confirms that this is not an usual mechanism of molecular etiology in 46,XY GD patients. The presence of microdeletions of *DMRT1* in these patients will be excluded.

- (1) Calvari V et al., Genomics 2000;65:203
- (2) Barbaro M et al., Eur J Hum Genet 2009;17:1439
- (3) Raymond CS et al., Genes Dev 2000;14:2587

Sources of Research Support: Fapesp # 2009/03872-3.

Nothing to Disclose: TES, EMFC, MGS, MYN, AZM, CRG, RBS, MPB, BBM, SD

P1-313

A Novel Heterozygous Nonsense Mutation (p.K248X) in *Wilms' Tumor Gene (WT1)* in a 46,XY Patient with Denys-Drash Syndrome.

TE Silva Student¹, RM Martin PhD¹, MY Nishi PhD¹, MG Santos MSc¹, EMF Costa PhD¹, BB Mendonca PhD¹ and S Domenice PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

The triad of Wilm's tumor (WT), nephropathy caused by diffuse or focal mesangial sclerosis and 46,XY disorder of sexual development (DSD) characterize the Denys-Drash syndrome (DDS). This syndrome is usually associated with a dominant-negative effect of *WT1*'s mutations. We described a novel heterozygous nonsense (p.K248X) *WT1* mutation in a 46,XY patient with DDS. **Patient** A 4-m-old child was referred with ambiguous genitalia. He had a bilateral intraabdominal testes and proximal hypospadias. Ultrasound revealed a left kidney tumor. Nephrectomy was performed (WT stage II) and chemotherapy. Bilateral gonadectomy had to be performed considering the difficulty to placed testes in the scrotum and no evidence of tumor was found. Mild proteinuria was detected at 17 yrs-old. **Methods** Genomic DNA was extracted from peripheral leukocytes by proteinase K-SDS-salting out methodology. The entire *WT1* coding region and the exon-intron boundaries regions were PCR amplified and sequenced by the ABI Prism 3100 Genetic Analyzer. **Results** A novel heterozygous mutation (742A>T) located at exon 4 of *WT1* was identified. The aminoacid lysine was changed to a stop codon at position 248 (p.K248X) resulting in a premature truncated *WT1* protein (ENSEMBLE 00000184937). **Discussion** More frequently the *WT1* mutations identified in DDS patients are missense mutations located at the zinc-finger region. In patients with truncated *WT1* protein WTs are usually diagnosed in the first year of life and bilateral tumors are frequently found. In our patient the WT was unilateral but was diagnosed precociously. The deleterious effect of the truncated *WT1* protein seems to be heterogeneous for kidney function. Some patients may develop a nephropathy later and with a benign evolution differing of the classical description of DDS. Genitourinary abnormalities have been associated with diverse *WT1* mutations making phenotype-genotype correlations very difficult. The (p.K248X) *WT1* mutation resulted in a *WT1* truncated protein before the nuclear localization signal (NLS) region, essential to *WT1* nuclear import. It has been hypothesized that the cytoplasmic mutant *WT1* might sequester some of the wild-type protein or partner proteins in the cytoplasm resulting in a reduced amount of nuclear *WT1*, preventing their normal function. **Conclusion** This case reinforces previous reports that *WT1* mutations may cause a large spectrum of disease, with clinical overlap of classical syndromes as DDS and Frasier.

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Nothing to Disclose: TES, RMM, MYN, MGS, EMFC, BBM, SD

P1-314

Characterization of CYP21A2 Gene Mutations in Dominican Subjects with Congenital Adrenal Hyperplasia.

C Tan PhD¹, JL Imperato-McGinley MD¹, I Labour MD², JJ Cordero MD¹, C Montero MD² and YS Zhu MD, PhD¹.

¹Weill Cornell Med Coll New York, NY and ²UNPHU Santo Domingo, Dominican Republic.

Congenital adrenal hyperplasia (CAH; MIM: 201910) is a group of autosomal recessive disorders and presents a wide range of clinical manifestations. About 90-95% of CAH are caused by steroid 21-hydroxylase deficiency due to mutations in 21-hydroxylase gene, CYP21A2. In the present study, we screened a total of 62 affected subjects and 161 family members from the Dominican Republic for CYP21A2 mutations. To identify the genetic defects, genomic DNA was isolated from the leukocytes of these subjects. The encoding region and the exon-intron junctions of CYP21A2 gene were amplified by 2-step PCR amplification with specific primers. The amplified products were purified and the DNA sequences were determined by automatic DNA sequencing. A CYP21A2 mutation(s) was detected in all 62 affected subjects. The most frequent mutation in this Dominican population is an A/C655G located on intron 2 (I2 splice) (19.4%). Other mutations detected include missense, nonsense and a small deletion in these patients (frequency): missense mutations P30L in exon 1 (9.7%), I172N in exon 4 (11.3%), S268T in exon 7 (16.1%), and V281L in exon 7 (4.8%); nonsense mutations Q318X in exon 8 (8.1%) and R483X in exon 10 (3.2%); and an 8-bp small deletion in exon 3 (6.5%). The frequency of single allele carrying two or more CYP21A2 mutations is approximately 11.3%. Furthermore, a new missense point mutation (R426C) was identified in exon 10 (8.1%), where a cytosine was substituted by thymidine, resulting in a replacement of arginine (CGC) by cysteine (TGC) at amino acid position 426. This mutation results in an 86% reduction in 21-hydroxylase enzymatic activity as determined by *in vitro* mutagenesis and transient transfection analysis in CV1 cells using 17-hydroxyprogesterone as a substrate. Heterozygosity for these mutations was detected in their parents and siblings. This study characterizes the CYP21A2 mutational spectrum of CAH patients with specific genetic features in this Dominican population, and includes a new mutation that has not been previously described.

Sources of Research Support: Grant from Argenbright Fund (JIM).

Nothing to Disclose: CT, JLI-M, IL, JJC, CM, YSZ

P1-315

Correlation of Genotype and Phenotype to Sex Assignment from the TCH Gender Medicine Clinic.

D Suresh¹, ME Axelrad¹, SK Gunn¹, L McCullough¹, O Smith¹, VR Sutton¹, LP Karaviti¹ and JE Dietrich¹.

¹Texas Children's Hosp, Baylor Coll of Med Houston, TX.

Background: 1 in 3000 infants is born with a disorder of sexual differentiation (DSD). Practice guidelines for sex assignment have not been established as there are few studies on outcome and decision making criteria that lead to satisfactory sex assignment. There are no studies exploring the concordance of gender assignment to genotype/phenotype

Objective: This study assesses the correlation of gender assignment with genotype and phenotype.

Methods: A retrospective chart review of all patients seen in Gender Medicine clinic at Texas Children's Hospital was conducted. We reviewed patient history, physical examination, hormonal studies, imaging studies, genotype and sex assignment.

Results: We assessed a total of 47 cases. In assessing the genotype, the presence of a Y component was labeled as male genotype. The concordance of male sex assignment to male genotype was 74% (54-86%). The concordance of female sex assignment to female genotype was 88% (63-98%). The total concordance of sex assignment to genotype was 77% (63-87%). In 8 cases where the sex assignment was discordant, complete androgen insensitivity was the most common diagnosis. The concordance of male sex assignment to male phenotype was 100% (87-100%) and female sex assignment to female phenotype was 95% (76-99%). The total concordance of sex assignment to phenotype was 97% (88-99%). In two patients where the phenotype was indeterminate, the sex assignment was based on genotype. There were 6 patients who had sex reassignment. The reassignment in 4 of these was based on genotype and in the other two was based on phenotype.

Conclusion: The correlation of sex assignment was related more to phenotype than genotype. However when the phenotype was indeterminate, the sex assignment was based on genotype. In cases of reassignment, the decision for reassignment by our gender medicine team was based on genotype more than phenotype, but factors such as biochemical markers, genetics, internal and external anatomy, psychosocial concerns were also addressed as part of the final decision-making process. These initial findings will be followed by prospective studies further evaluating the determinants of sex assignment and establishment of practice guidelines including educating the parents in the decision making process.

Nothing to Disclose: DS, MEA, SKG, LM, OS, VRS, LPK, JED

P1-316

Transsexuals' Response to Visual Stimuli - A fMRI Pilot Study.

PL Kapsner MD¹, M Bouchonville MD¹, M Garimella MD¹, T Teshiba¹ and A Mayer PhD¹.

¹Univ of New Mexico HSC Albuquerque, NM.

Purpose: Transsexuals desire to live as members of the opposite sex. The brain is sexually dimorphic. Transsexual's central part of the bed nucleus of the striae terminalis is more consistent in volume and somatostatin staining with their desired, rather than birth sex(1). Gender differences have been identified in fMRI response to emotional stimuli. Males lateralize to the right amygdala in response to happy stimuli; females don't lateralize(2). The study's aim was to develop a protocol and study transsexuals' fMRI response to visual stimuli.

Hypothesis: A protocol can be developed to assess transsexuals' response to visual stimuli using fMRI. No lateralization will occur in M-F transsexuals in the region of interest(ROI), the amygdala, with "happy" stimuli.

Methods: 8 M-F transsexuals were admitted overnight for 8am hormone levels (T, E2, E3, LH, FSH) followed by fMRI's. Using 25 female and 25 male NimStim images(3) (neutral, happy, angry, sad), a computer program was developed using a LCD screen that projected images, visualized by subjects in the scanner. A fixation point was used between images as "baseline". Images were identified using a touchpad. Response times and fMRI images were recorded. An asymmetry index(AI) was calculated using signals for the R and L amygdala as a percent of each subject's baseline as follows: (R-L)/(R+L). Analysis was performed using paired t-tests and 2x2 ANOVA.

Results: The desired protocol was successfully developed and implemented. One subject had high estrogen levels, another, post surgical, had low testosterone and estrogen levels. The emotion being displayed was correctly identified >80% of the time, consistent with the literature. Response times did not vary with the emotion or sex of the image. Based on the AI, there was no lateralization in fMRI signal in the ROI (all p values > 0.175). Standard deviations however were large.

Conclusion: Hormone evaluation showed subjects may not be taking medication regimens as prescribed. There was no lateralization in fMRI response in the amygdala consistent with biological females. It is not known if hormone therapy plays a role as only treated subjects were studied. in this pilot project. The standard deviation was large due to the small sample size also making data interpretation difficult. An fMRI protocol was developed and implemented to evaluate transsexual's response to visual stimuli and can be utilized to further study transsexualism, its etiology and diagnosis.

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(2)Kilgore WDS et al. NeuroReport 2001; 2543-2547

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Sources of Research Support: UNM SOM Research Allocation Committee Grant.

Nothing to Disclose: PLK, MB, MG, TT, AM

P1-317

Disorders of Sexual Differentiation: New Epigenetic Insights of Hypospadias.

A. Vottero MD¹, I. Viani PhD¹, R. Minari PhD¹, V. Gasco MD², S. Bernasconi MD¹ and L. Ghizzoni MD².

¹Univ of Parma Parma, Italy and ²Univ of Turin Turin, Italy.

The endocrine disruptors (EDs) have been involved in the pathogenesis of some disorders of sexual differentiation (DSD), in particular hypospadias, whose incidence has increased in well developed countries. Hypospadias is a malformation of the penis due to an incomplete development of the male urethra, whose exact etiology remains unknown in the majority of cases. We previously demonstrated that the androgen receptor (AR) gene in target tissues from patients with hypospadias is more methylated than in control children, resulting in a decreased expression of the AR. The aim of the present study was to evaluate the effects of some EDs on AR gene methylation. Fibroblasts obtained from foreskin tissue of children undergoing circumcision were cultured and, thereafter, stimulated with graded doses of different EDs, such as diethylstilbestrol (DES, 0.001, 0.01 µg/µl), O,P'-DDD (0.04, 0.4 µg/ml), and flutamide (0.001, 0.01 µg/µl). Genomic DNA was isolated and AR gene methylation analysis was performed by Real-Time PCR after sodium bisulfite treatment. Total RNA was also extracted and then reverse transcribed for the AR gene expression analysis by Real-Time PCR. The results were expressed as percentage of AR gene methylation vs baseline. Both DES and flutamide produced an increase in AR gene methylation (DES: from 40.33 ± 10.5 to 86.45 ± 13.2, P=0.05; flutamide: from 90.13 ± 11.3 to 146.41 ± 13.6, P=0.033) and consequently a decreased AR gene expression (DES: from 88.12 ± 8.4 to 25.52 ± 9, P=0.016; flutamide: from 72.21 ± 1.5 to 22.68 ± 5.7, P=0.007). O,P'-DDD showed an opposite effect, reducing the percentage of AR gene methylation and thus increasing the AR gene expression. In conclusion, these preliminary data suggest that EDs exerting anti-androgen effects such as DES and flutamide, increase the methylation rate of the AR gene and thus reduce the expression of the AR. Such a mechanism might be involved in the pathogenesis of hypospadias.

Nothing to Disclose: AV, IV, RM, VG, SB, LG

P1-318

Identification of Copy Number Variants and Characterization of Novel Key Genes Associated with Genitourinary Birth Defects.

Shuo Han BS¹, Mounia Tannour-Louet PhD¹, Sean T Corbett MD¹, Svetlana Yatsenko PhD¹, Lindsay Meyers BS¹, Sung-Hae Kang PhD¹, Sau Wai Cheung PhD, MBA¹ and Dolores Lamb PhD¹.

¹Baylor Coll of Med Houston, TX.

Congenital genitourinary(GU) abnormalities such as hypospadias, cryptorchidism and ambiguous genitalia are among the most common human birth defects. as patient care is complicated by surgical, psychological, and sexual concerns, these human reproductive disorders present difficult challenges for parents and physicians. Unfortunately, their etiology remains poorly understood. Since submicroscopic chromosomal anomalies underlie many genomic syndromes including mental retardation, developmental delays and heart defects, we hypothesize that inborn errors of urogenital tract development result from chromosomal aberrations that cannot be detected by a routine karyotype and may alter the function of key genes and regulatory pathways governing GU development. Using a clinically validated comparative genomic hybridization microarray, we identified copy number variants associated with inborn GU abnormalities. Anomalies were scattered across the genome but notably clustered in gene-enriched subtelomeric loci. Confirmed duplication and deletion events were significantly associated with GU defects($p=1.02 \times 10^{-26}$) when compared to 15,931 non-GU patient controls. De novo defects were observed at 1p36.33, 9p23p24 and 19q12-q13.11 for ambiguous genitalia and 10p14 and Xq28 for cryptorchidism and 12p13, 16p11.2 and 16q24.3 for hypospadias. Of interest, the gain on Xq28 was found in 2 unrelated patients and encompassed a single gene: vesicle-associated membrane protein 7(Vamp7). We found Vamp7 expressed in fetal and adult human and murine reproductive tissues. To unravel the precise function of VAMP7 in the development of the genital tract, Vamp7 knockdown was performed in NTERA-2 cells, which recapitulate the expression profile of endogenous markers of male sex determination and differentiation. Results indicate significant increase in gene expression of Hoxa13 and its downstream target Fgf8 which are two key players in male urogenital development. We also investigated whether testosterone had an effect on the expression of Vamp7 in NTERA-2 cells as these GU defects may result from defective androgen signaling. While Vamp7 gene expression was not affected, its protein levels increased in the presence of androgens. Taken together, the identification of these clinically significant copy number variants will aid in elucidating the molecular mechanisms underlying the pathogenesis of human genital development and define critical factors such as VAMP7 in male sexual development.

Sources of Research Support: 1R01DK078121 from the National Institute of Kidney and Digestive Diseases to DJL, the American Urological Association Foundation (SC and DJL), and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (P01HD36289) to DJL.

Nothing to Disclose: SH, MT-L, STC, SY, LM, S-HK, SWC, DL

P1-319

Identification of Submicroscopic Chromosome Defects Associated with Combined Hypospadias and Cryptorchidism.

SK Lewis PhD¹, M Tannour-Louet PhD¹, S Han BS¹, CJ Jorgez PhD¹, AP Bayne MD¹ and DJ Lamb PhD¹.

¹Baylor Coll of Med Houston, TX.

Developmental abnormalities of the male GU tract are the most common birth defects. These birth defects include cryptorchidism and hypospadias. Cryptorchidism is the failure of testis descent into the scrotum. It occurs in 2% of full-term newborn boys and results in impaired spermatogenesis. Hypospadias is a midline fusion defect of the male ventral urethra and occurs in nearly 1 in 125 live male births. The etiology of these congenital GU birth defects is poorly understood and likely involves genetic and environmental factors. Karyotype analysis is sometimes used to detect structural chromosomal defects in patients with congenital genitourinary defects, but a karyotype has limited resolution and cannot detect submicroscopic, clinically significant chromosomal rearrangements. We hypothesize that subtle chromosome aberrations are present in patients presenting with combined hypospadias and cryptorchidism and we used genome wide high-resolution comparative genomic hybridization arrays (aCGH) to detect these variants. It is likely that genes in these regions of copy number variation may be dosage sensitive and critical for GU tract development. Sex-matched genomic DNA from men of proven fertility served as a control. Genomic DNA was analyzed by aCGH using 720K NimbleGen arrays (Roche) and analyzed using Nexus Copy Number software (BioDiscovery). FISH and/or qPCR were employed to validate putative regions of duplication or deletion that were distinct from common copy number variants (CNV) found throughout the genome. Patients with combined hypospadias and cryptorchidism displayed distinct chromosomal regions of submicroscopic chromosome duplications or deletions not detected in normal pregnancy proven fertile controls or in public copy number variation databases. These regions include: 5p13, 9p11, 22q11 (copy number losses), 7q32-33, 10q24, 11q12, 15q11, 16p13, and 22q13 (copy number gains), which encodes genes with putative roles in GU tract and male external genitalia development in addition to fertility. Gene expression analyses and functional studies of candidate genes in these and other regions are needed to identify the significance of these findings for patients with combined hypospadias and cryptorchidism. Array CGH represents an accurate high-resolution method to identify clinically significant chromosomal aberrations associated with congenital GU tract defects.

Sources of Research Support: In part by K12 DK0083014, the Multidisciplinary K12 Urologic Research (KURe) Career Development Program to DJL (SL and CJ), 1R01DK078121 from the National Institute of Kidney and Digestive Diseases to DJL, and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (P01HD36289) to DJL.

Nothing to Disclose: SKL, MT-L, SH, CJJ, APB, DJL

P1-320

Correlations between Pre- and Postnatal Measurements of Penile and Clitoral Size.

Naomi Weintrob MD, MHA^{1,2}, Yoram Bental MD^{3,4}, Meir Weisbrod MD³, Yacov Shiff MD³, Ori Eyal MD^{1,2} and Reuven Sharony MD^{5,2}.

¹Dana Children Hosp, Tel Aviv Sourasky Med Ctr Tel Aviv, Israel ; ²Tel Aviv Univ Tel Aviv, Israel ; ³Laniado Hosp Natanya, Israel ; ⁴Technion, Israel Inst of Technology Haifa, Israel and ⁵Meir Med Ctr Kfar Saba, Israel.

Context: Ultrasound examination, usually at mid-gestation, is routinely used in standard clinical obstetric practice for the prenatal detection of various syndromes, major malformations and fetal growth, as well as the determination of fetal sex and detection of external genitalia anomalies. A reference range for prenatal penile length in relation to gestation age has recently been established and it led to frequent incorporation of the prenatal diagnosis of micropenis or clitoromegaly. Findings outside the normal range often cause parental anxiety, lead to further evaluation and sometimes to pregnancy termination. Comparisons of pre- and postnatal penile and clitoral size are lacking.

Objective: To correlate pre- and postnatal measurement of penile width and length and clitoral height and length.

Methods: Fetal penile and clitoral width and length in singleton pregnancies were measured twice by high-resolution ultrasonography. Postnatal measurements were carried out twice during the first postnatal week. Correlation between pre- and postnatal measurements were calculated by the Pearson correlation test.

Results: Paired pre- and postnatal measurements were performed in 45 males and 47 females. The correlations between measurements of fetal penile and clitoral length and width/height and week of gestation were highly significant ($p \leq 0.001$). Correlations between fetal and postnatal penile length and width were not significant. There was significant correlation between fetal clitoral length and height and post natal clitoral length ($r=0.34$, $p=0.019$, $r=0.36$, $p=0.012$, respectively), while correlation between fetal clitoral height and post natal clitoral width was not significant.

Conclusions: The lack of correlations between pre- and postnatal penile measurements, and the significant correlation between fetal and post natal clitoral length, suggests that while prenatal findings in females might be reliable indicators of postnatal measurements, this is not the case in males. This uncertainty in fetal penile measurements mandates exercising caution in parent counseling.

Nothing to Disclose: NW, YB, MW, YS, OE, RS

P1-321

G Protein Coupled Receptor 48 Up-Regulates Estrogen Receptor Alpha Expression Via cAMP/PKA Signaling in Male Reproductive Tract.

XY Li MD¹, Y Lu PhD¹, HY Sun PhD¹, MY Liu PhD², J Yang PhD¹ and G Ning MD¹.

¹Ruijin Hosp, Shanghai Jiao Tong Univ Sch of Med Shanghai, China and ²Texas A&M Univ Syst Hlth Sci Ctr Houston, TX.

The epididymis, derived from the anterior Wolffian or mesonephric duct, elongated, expanded and folded into a highly organized structure including the caput, the corpus and the cauda segments. Recent studies have demonstrated that estrogen participates in the development of male reproductive tracts. However, the expression regulation of estrogen receptors in male reproductive tracts is poorly understood.

G protein coupled receptor 48 (Gpr48), a newly identified orphan receptor, is dramatically expressed in human and mouse epididymis. Gpr48 null male mice demonstrated reproductive tract defects and infertility. In the present study, we found that estrogen receptor alpha(ERα) was dramatically reduced in epididymis and efferent ducts in Gpr48 null male mice.

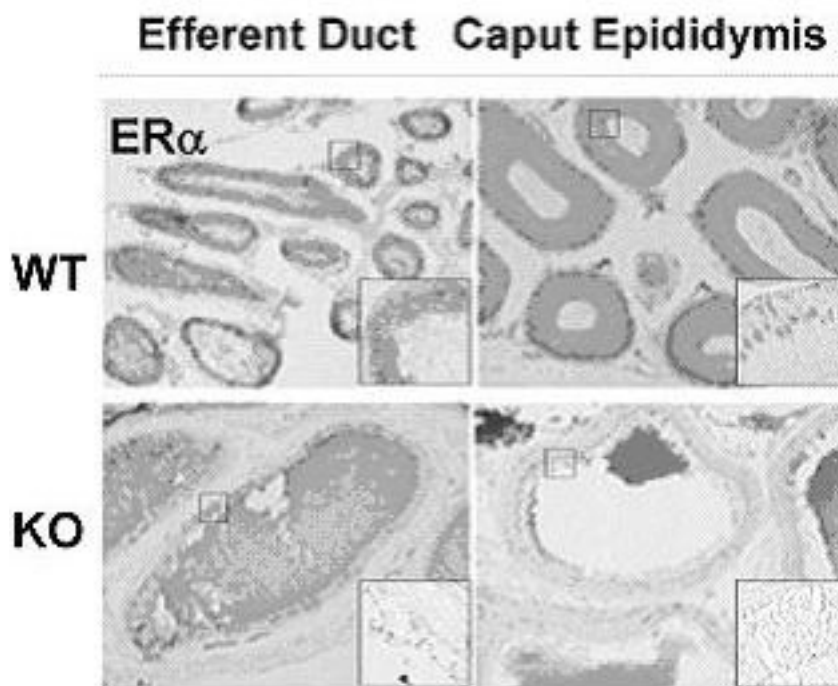
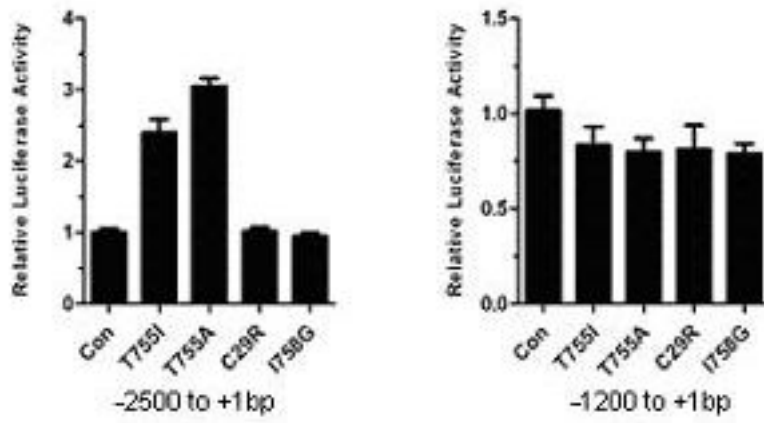


Fig. 1

We further revealed that ERα could be up-regulated by Gpr48 activation via cAMP/PKA signaling pathway. Moreover, we identified a cAMP responsive element (CRE) motif located at -1307 to -1300bp in the ERα promoter, which is able to interact with CREB.

The transcriptional activity assay



The functional Cre motif by ChIP assay

Fig.2

In conclusion, Gpr48 participates in the development of the male epididymis and efferent ducts through regulation of ERa expression via the cAMP/PKA signaling pathway.

Nothing to Disclose: XYL, YL, HYS, MYL, JY, GN

P1-322

Differences in Reproductive Phenotypes between C57BL/6 and Sv129 Mice Carrying Mutations in *Kiss1* and *Gpr54*: Evidence for Modifier Genes Affecting Reproduction.

JJ Yang PhD¹, AME DeGuillebon BS¹ and SB Seminara MD¹.

¹Massachusetts Gen Hosp Boston, MA.

Background: Different strain backgrounds can influence the phenotype of genetically engineered mice. Previous work on *Kiss1* and *Gpr54* mutant mice has been done in the Sv129 strain (129). We hypothesized that strain-specific genetic modifiers would produce differences in reproductive phenotypes created by *Kiss1* and *Gpr54* (*Kiss1r*) mutations in the C57BL/6 (B6) and 129 strains.

Methods: Reproductive phenotypes were examined in adult male and female mice carrying mutations in *Kiss1* or *Gpr54* on B6 and 129 background strains.

Results: B6 *Kiss1*^{-/-} and *Gpr54*^{-/-} males had severely shortened anogenital distance (AGD) compared to HET and WT littermates. In addition, both male and female B6 *Kiss1*^{-/-} and *Gpr54*^{-/-} mice had smaller gonads compared to HET and WT littermates.

Strikingly, the gonads of B6 *Kiss1*^{-/-} and *Gpr54*^{-/-} males were smaller than their 129 counterparts. In particular, B6 *Kiss1*^{-/-} and *Gpr54*^{-/-} males had disproportionately smaller testes than 129 *Kiss1*^{-/-} and *Gpr54*^{-/-} males. *Kiss1*^{-/-} and *Gpr54*^{-/-} B6 females also demonstrated smaller combined ovarian and uterine weights than their 129 counterparts.

Table 1. Reproductive Phenotypes of B6 and 129 *Kiss1* and *Gpr54* mice

| | | Testicles (mg) | Ovaries and Uterus (mg) | Male AGD (mm) |
|-----------------------------|-----|------------------|-------------------------|-----------------|
| <i>Kiss1</i> ^{-/-} | B6 | 5.4 ± 0.3 (10)* | 3.8 ± 0.8 (13)* | 11.7 ± 0.4 (9) |
| <i>Kiss1</i> ^{-/-} | 129 | 86.1 ± 9 (8) | 39.1 ± 7 (14) | 13.4 ± 0.4 (8) |
| <i>Kiss1</i> ^{+/-} | B6 | 118.5 ± 5 (18)* | 33.9 ± 4 (9)* | 15.5 ± 0.7 (16) |
| <i>Kiss1</i> ^{+/-} | 129 | 299.3 ± 6 (8) | 54.1 ± 6 (18) | 17.3 ± 0.5 (8) |
| WT | B6 | 176.2 ± 3 (26)* | 37.1 ± 3 (17)* | 15.5 ± 0.7 (27) |
| WT | 129 | 268 ± 11 (9) | 48.5 ± 4 (26) | 17.7 ± 0.4 (9) |
| <i>Gpr54</i> ^{+/-} | B6 | 175.8 ± 5 (10)* | 33.3 ± 4 (5)* | 19.0 ± 0.7 (12) |
| <i>Gpr54</i> ^{+/-} | 129 | 290 ± 5 (8) | 47.1 ± 4 (16) | 16.3 ± 0.5 (8) |
| <i>Gpr54</i> ^{-/-} | B6 | 5.06 ± 0.4 (10)* | 3.4 ± .6 (6)* | 12.9 ± 0.5 (11) |
| <i>Gpr54</i> ^{-/-} | 129 | 45.3 ± 5 (8) | 20.1 ± 6 (15) | 12.8 ± 0.4 (8) |

* p<0.05 compared to 129

Conclusion: These data suggest that one or more strain-specific genetic modifiers affect the expressivity of *Kiss1* and *Gpr54* mutations. Examination of *Kiss1* and *Gpr54* phenotypes in B6/129 hybrids will help characterize and identify these modifier gene(s) and further our understanding of the pathways that modulate GnRH secretion.

Nothing to Disclose: JJY, AMED, SBS

P1-323

5 Alpha Reductase Deficiency: Redefining the Diagnostic Process in the Adult Setting.

M Berra MD¹, SM Creighton FRCOG¹, G Rumbsby PhD FRCPath¹, JW Honour PhD FRCPath¹ and GS Conway MD FRCP¹.

¹UCL Hosps London, UK.

The presentation of 5 alpha reductase deficiency (5ARD), caused by SRD5A2 gene mutations can be very variable with a spectrum from males with hypospadias at birth to 46XY females with absent uterus and virilisation at puberty. Most of the known mutations in SRD5A2 are defined in cohorts of children with ambiguous genitalia, with an emphasis on the male end of the phenotypic spectrum. Presentation in adults may be expected to reveal a more female phenotype. In fact, partially virilised 46XY DSD is a poorly defined group in adults either because the original endocrine workup is not available or because an imprecise diagnostic label was previously applied – usually partial androgen insensitivity syndrome (PAIS). Moreover, a biochemical diagnosis is particularly difficult to achieve for subjects who have had gonadectomy although the ratio of 5 α /5 β metabolites of adrenal steroids in a urine steroid profile (USP) can indicate 5ARD. The diagnostic criteria for 5 α /5 β ratios are not clearly defined when used in this way. We therefore investigated the relationship between USP results and SRD5A2 genetic sequence in partially virilised 46 XY DSD adults. We aimed to determine the cut-off for USP 5 α /5 β steroid ratios compared to gene sequencing for identification of 5ARD. Methods: 35 46XY adults within the phenotypic spectrum of ambiguous genitalia at birth, absent uterus and histology compatible with testicular tissue were recruited. A urine steroid profile and SRD5A2 genetic screening was performed. 5 α reductase activity was assessed using the USP ratio of androsterone to aetiocholanolone (A/Ae) and 5 α tetrahydrocortisol to tetrahydrocortisol (aTHF /THF).

Results: DNA sequencing results were available for 16 subjects of whom 12 were found to have a mutation in SRD5A2 gene. Of these 5 were previously labelled as PAIS, 4 had no diagnosis and 3 had previously been diagnosed with 5ARD. All mutation positive subjects had A/Ae < 0.54 (normal range 0.67-2.20) and aTHF/THF < 0.50 (normal range 0.45-1.02). Using both biochemical and genetic data we estimated that 32% of our adult 46XY female DSD population have 5ARD. Conclusions: 5ARD is more prevalent than expected in the adult 46XY DSD population. A/Ae ratio appears to be more reliable than aTHF/THF ratio in discriminating the SRD5A2 mutation positive subjects with clear separation from the normal range and identifies those patients in whom sequencing will be beneficial for full diagnosis.

Nothing to Disclose: MB, SMC, GR, JWH, GSC

P1-324

Regulation of Spatial and Temporal Organization of Embryonic GnRH Neurons by IGF Signaling in Zebrafish.

TA Onuma PhD¹, Y Ding PhD¹, Y Zohar PhD², H Ando PhD³ and C Duan PhD¹.

¹Univ of Michigan Ann Arbor, MI ; ²Univ of Maryland Baltimore, MA and ³Kyushu Univ Fukuoka, Japan.

The initiation of puberty and function of the reproductive axis depend on the proper spatial and temporal organization of GnRH neurons. GnRH neurons emerge from the nasal compartment during embryogenesis and migrate into the forebrain hypothalamus. How their spatial and temporal organization is controlled during embryogenesis remains largely unknown. It is also known that somatic growth influences the timing of puberty, but the underlying molecular mechanisms are unclear. In this study, we used the transparent and free-living zebrafish embryos as an experimental model to test the hypothesis that insulin-like growth factor (IGF) signaling, a central growth-promoting signal in development, regulates GnRH neuronal development. To inhibit IGF-1R-mediated signaling in vivo in a temporally controlled manner, a transgenic zebrafish line was generated that expresses a dominant negative IGF-1R driven by the heat-inducible HSP70 promoter. In addition, a small molecule IGF-1R inhibitor was used to inhibit IGF signaling. Inhibition of IGF signaling delayed the emergence of GnRH2 neurons in the midbrain and that of GnRH3 neurons near the olfactory lobe. A GnRH3:EGFP transgenic line, in which GnRH3 neurons can be visualized by the expression of EGFP, was used to further analyze the effects of IGF1R inhibition. The in vivo imaging analysis revealed that some GnRH3 neurons appeared ectopically outside the olfactory bulb, and the organization of GnRH3 neuronal clusters was disrupted when IGF signaling was reduced. The effect of IGF signaling on GnRH3 neurons was dependent on the developmental stage. While inhibition of IGF1R-mediated signaling at 4 hpf triggered this abnormality, such treatment at 30 hpf had little effect. Likewise, inhibition of IGF signaling in larvae did not affect the migration of GnRH3 neurons to the hypothalamus. We further dissected the intracellular signaling pathways involved using pharmacological inhibitors specific for the MAPK and PI3K pathways. Our results show that inhibition of the PI3K but not MAPK pathway resulted in the abnormal organization of GnRH3 neurons. These results suggest that IGF signaling, mediated through the IGF-1R-PI3K signaling pathway, is crucial for the development of GnRH2 and GnRH3 neurons and for the proper spatial and temporal organization of GnRH3 neurons in the forebrain.

Sources of Research Support: NSF Grant IOB 0110864 and JSPS Fellowship 200609320.

Nothing to Disclose: TAO, YD, YZ, HA, CD

P1-325

TAC3/TACR3 Mutations Reveal Preferential Activation of GnRH Release by Neurokinin B in Neonatal Life Followed by Reversal in Adulthood.

E Gianetti MD¹, C Tusset PhD², S Noel PhD³, MG Au¹, AA Dwyer RN, FNP¹, VA Hughes¹, AP Abreu MD³, J Carroll³, E Trarbach PhD², LFG Silveira MD², EMF Costa MD², BB de Mendonca MD², M de Castro MD⁴, A Lofrano MD⁵, JE Hall MD¹, E Bolu MD⁶, M Ozata MD⁶, R Quinton MD⁷, JK Amory MD⁸, SE Stewart NP⁹, W Arlt MD¹⁰, TR Cole MD⁹, WF Crowley MD¹, UB Kaiser MD³, AC Latronico MD² and SB Seminara MD¹.

¹Massachusetts Gen Hosp Boston, MA ; ²HCFMUSP Sao Paulo, Brazil ; ³Brigham and Women's Hosp Boston, MA ; ⁴Univ de Sao Paulo - Fac de Med de Ribeirao Preto Ribeirao Preto, Brazil ; ⁵Hosp Univ de Brasilia Brasilia, Brazil ; ⁶Gulhane Sch of Med Ankara, Turkey ; ⁷Univ of Newcastle-upon-Tyne Newcastle-upon-Tyne, UK ; ⁸Univ of Washington Med Ctr Seattle, WA ; ⁹Birmingham Women's Hosp NHS Foundation Trust Edgbaston, UK and ¹⁰Univ of Birmingham Birmingham, UK.

Mutations in *TAC3* and *TACR3* (encoding neurokinin B and its receptor) have been identified in Turkish patients with hypogonadotropic hypogonadism (IHH), but broader populations have not yet been tested and detailed genotype-phenotype correlations have not been established.

345 probands and 292 controls with normosmic IHH were screened for sequence variants in the coding regions of *TAC3* and *TACR3*. The neuroendocrine phenotype of probands and family members carrying these rare sequence variants was examined throughout reproductive life and pre/post therapy.

Nineteen probands harbored 13 distinct coding sequence nucleotide variants in *TACR3* not observed in controls. Three variants were nonsense mutations and 6 led to non-synonymous amino acid changes. The remaining 4 did not change the amino acid sequence although a homozygous one of them is predicted to affect splicing.

Only one ligand mutation was identified in a female with a homozygous deletion of a single base pair, resulting in a complete loss of the neurokinin B decapeptide.

Of 16 males carrying coding sequence variants in *TACR3* and in whom information was available, 15 had microphallus. Seven of them were assessed after discontinuation of sex steroid therapy and six exhibited spontaneous activity of their hypothalamic-pituitary-gonadal (hpg) axis.

In the 7 females carrying coding sequence variants in either *TACR3* or *TAC3* and in whom information was available, none had spontaneous thelarche. Five of them were assessed after discontinuation of sex steroid therapy and 4 demonstrated evidence for reversibility of their hypogonadotropism.

In conclusion, mutations in the neurokinin B pathway occur in 5% of our patient population with normosmic idiopathic hypogonadotropic hypogonadism. While the neurokinin B pathway appears essential during early sexual development, its importance in sustaining the integrity of the hypothalamic-pituitary-gonadal axis appears attenuated over time. These studies imply that the neuroendocrine controls of GnRH secretion during the 'mini-puberty' of the neonatal period and puberty at adolescence may well differ.

Sources of Research Support: Eunice Kennedy Shriver NICHD/NIH through cooperative agreement [U54 5U54HD028138]; Grant Number M01-RR-01066 - Harvard Clinical and Translational Science Center - from the National Center for Research Resources and R01-HD-42708; Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP grant # 05/04726-0); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grant # 300209/2008-8); Medical Research Council UK (MRC Programme Grant G0900567); European Commission (FP7 Collaborative Project EuroDSD).

Nothing to Disclose: EG, CT, SN, MGA, AAD, VAH, APA, JC, ET, LFGS, EMFC, BBdM, MdC, AL, JEH, EB, MO, RQ, JKA, SES, WA, TRC, WFC, UBK, ACL, SBS

P1-326

Characterization of the Testis Specific Promoter Region in Human Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Gene.

A Miyata MD&PhD¹, A Tominaga MS¹, K Sasaki MD³, M Hatakeyama PhD², A Miura MS¹, K Inoue PhD¹, H Handa PhD² and N Minamino PhD³.

¹Kagoshima Univ Kagoshima, Japan ; ²Tokyo Inst of Technology Yokohama, Japan and ³Natl Cardiovascular Ctr Res Inst Suita, Japan.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide localized in testis at concentrations comparable to those found in brain. PACAP gene expression is precisely regulated during the spermatogenic cycle, suggesting involvement in spermatogenesis. The regulatory mechanism of PACAP gene expression in the testis was approached through investigation of the upstream region of the PACAP testis-specific exon (TSE). Although rat PACAP testis-specific exon (1) was reported, the detailed regulatory mechanism of PACAP gene expression in testis remains unknown. Therefore we attempted to characterize human testis specific promoter. In this study, we surveyed the human genome sequence-data base, and found a human homologue of TSE of the PACAP gene, which is localized 10.9 kb upstream from the transcriptional start site. RT-PCR analysis demonstrated mRNA derived from human PACAP TSE, and the testis-specific transcript of human PACAP gene was found to be spliced from the TSE into a region of intron 2 without a frame-shift. The resulting PACAP precursor has no signal peptide, suggesting that PACAP might function physiologically in an intracrine manner in the testis, as mentioned before elsewhere (2). It should be interesting that the luciferase reporter assay with a 1.2 kb-5'-flanking region of human PACAP TSE demonstrated potent promoter activity in F9 mouse testicular teratoma cells and NEC14 human testicular germinal cell tumor, but neither in Swiss-3T3 mouse fibroblast cells, nor PC12 rat pheochromocytoma cells. Then in the 1.2 kb-5'-flanking region of TSE, we identified an 80 bp-sequence as the region to exhibit the potent promoter activity in testicular F9 and NEC14 cells. Electrophoresis mobility shift assays showed that proteins from F9 nuclear extract interacted specifically with the 80 bp fragment. DNA affinity chromatography allowed isolation of specific nuclear proteins bound to the 80 bp fragment, one of which was identified as TIAR by mass spectrometry, suggesting that TIAR might be involved in the testis-specific expression of PACAP gene because of the previous report that TIAR knock out mice were sterile (3).

(1)Daniel PB et al., *Endocrinology* 2000; 141:1218

(2)Li M et al, *Endocrine* 2004; 23:59

(3)Beck AR et al., *Proc Natl Acad Sci.* 1998; 95:2331

Nothing to Disclose: AM, AT, KS, MH, AM, KI, HH, NM

P1-327

Molecular Characterisation of the Orphan Cyclin Dependent Kinase PCTAIRE-1/CDK16.

P Mikolcevic DI¹, R Sigl PhD¹, B Sohm PhD¹, K Pfaller Professor¹, MW Hess Professor¹ and S Geley Professor¹.

¹Med Univ of Innsbruck Innsbruck, Austria.

Cyclin-dependent kinases (CDKs) are well known for their function in cell cycle or transcriptional control, but the functions of several other members of this large serine/threonine kinase family are still elusive. CDK16, 17, and 18 (previously known as PCTAIRE kinases 1, 2 and 3) are highly conserved among metazoans but neither activator nor substrates of CDK16-18 are well established. CDK16 is expressed at high levels in the brain and in the testis but it is also found in several other tissues and cell lines. In the testis, CDK16 expression is highest in differentiated spermatids, suggesting a role in the differentiation of spermatozoa. Here we summarize our biochemical, cell biological as well as genetic analysis of CDK16. In gel filtration experiments using mouse testis cell lysate, Cdk16 eluted as a ~115 kDa complex and immunoprecipitated Cdk16 phosphorylated a ~116 kDa protein in an in vitro kinase assay using dephosphorylated testis cell lysate as substrate. These data suggested that active Cdk16 was associated with other proteins, which encouraged us to perform yeast two hybrid interaction screens. We used GAL4- and LexA-based systems in mating or conventional co-transformation approaches to identify potential proteins expressed in the testis for their ability to interact with Cdk16. Of 15 candidate proteins, only cyclin Y (CCNY) could be confirmed to interact with Cdk16 in co-immunoprecipitation and colocalisation experiments of epitope-tagged proteins expressed in human cells. In addition, when overexpressed Cdk16 is immunoprecipitated in the presence of overexpressed tagged CCNY, Cdk16 showed increased kinase activity towards myelin basic protein in an in vitro kinase assay. To determine the role of mouse Cdk16 in vivo, we created a mouse strain harbouring a conditional Cdk16 allele. Cdk16 deficient male but not female mice were found to be infertile. Histological examination of testis sections, however, demonstrated robust spermatogenesis, but spermatozoa from Cdk16 null mice were hypomotile and structurally abnormal. Transmission and scanning electron microscopy of epididymal sperm showed aberrantly formed sperm heads often contained in an excess of residual cytosol. In addition, the annulus was disconnected from the neck region, causing spermatozoa to bend. In summary, our data suggest that Cdk16 is activated by a cyclin and is essential for the differentiation of spermatozoa.

Nothing to Disclose: PM, RS, BS, KP, MWH, SG

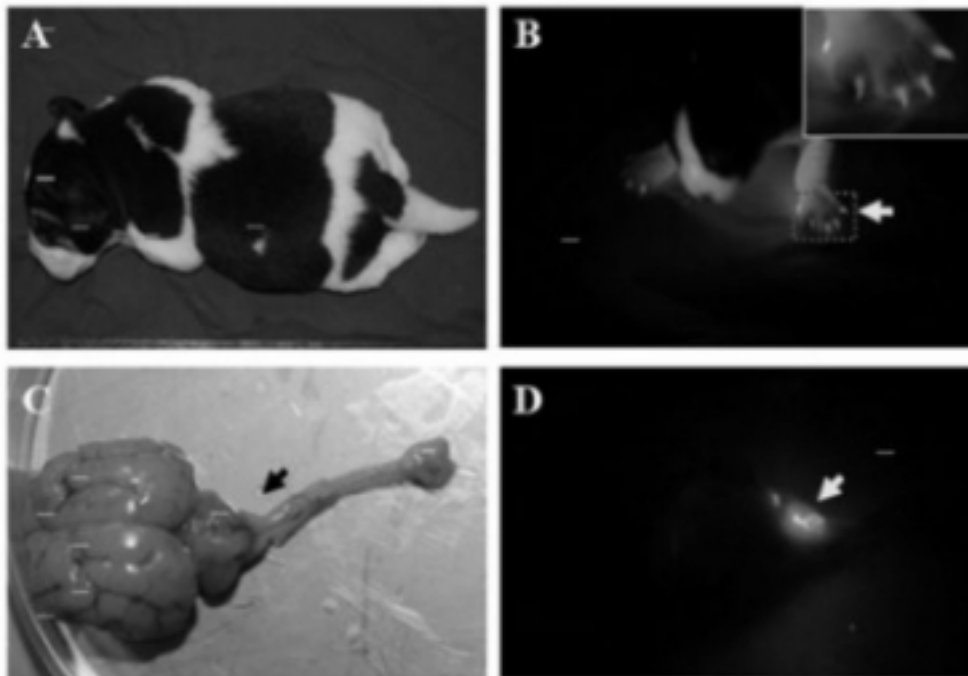
P1-328

Expression of Human Amyloid Precursor Protein (*hAPP*) in Transgenic Dogs Via Somatic Cell Nuclear Transfer for Alzheimer's Disease Model.

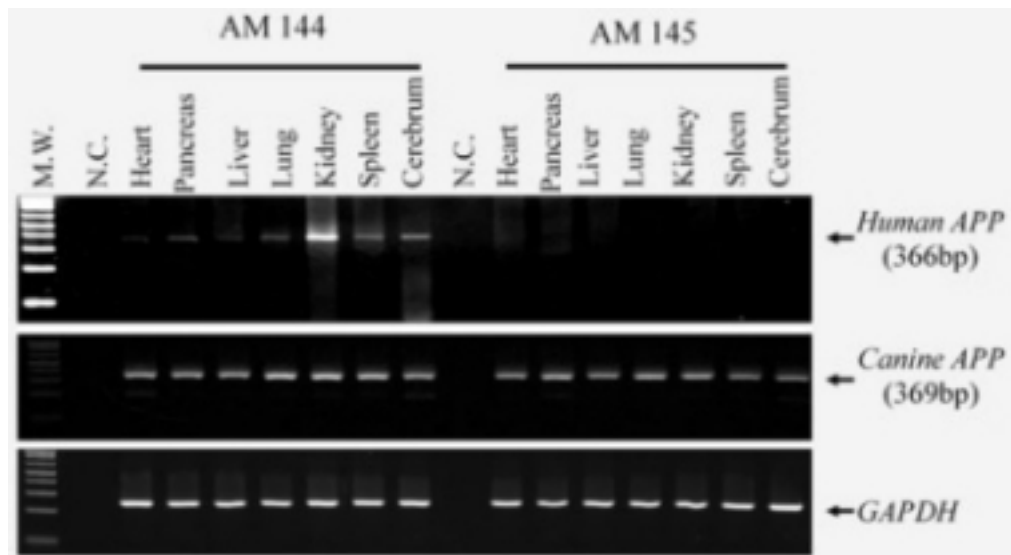
YW Jeong DVM¹, GS Lee DVM, PhD², JJ Kim¹, SW Park¹, KH Ko¹, M Kang¹, CJ Yang DVM¹, TK Jung DVM¹, EM Jung², YK Kim², SH Hyun DVM, PhD², T Shin DVM, PhD¹, EB Jeung DVM, PhD² and WS Hwang DVM, PhD¹.

¹SooAm Biotech Res Foundation Yongin-si, Republic of Korea and ²Chungbuk Natl Univ Cheongju-si, Republic of Korea.

Dogs are considered to be the most authentic animal model for studying multi-factorial human diseases as they share a common environment with mankind. It is very convenient to generate such models using somatic cell nuclear transfer in combination with transgenic technology, which provides a unique opportunity to study human genetic diseases through specific genetic manipulation. Alzheimer's disease is a common age-related neurodegenerative disorder, which is mainly caused by mutations in the amyloid precursor protein gene associated with beta amyloid plaque deposition as well as neurofibrillary tangles in brain tissue. The present study was carried out to produce transgenic dogs expressing the human APP gene with two well known mutations, London (V717F) and Swedish (K670M/N671L) types. The APP gene expression may restrictively express within neuronal cells by controlling Thy-1 promoter region. A total of 332 *in vivo* matured canine oocytes from 29 dogs, were then enucleated by aspiration and recombined with a transgenic donor cell. Out of the 17 surrogates, 4 dogs became pregnant and resulted in giving birth to 6 healthy live puppies. Five out of the six live puppies expressed green fluorescence.



To determine the expression of mutant human APP mRNA in canine tissue, we collected organ samples from a transgenic puppy and a non-transgenic puppy. Both animals expressed canine APP mRNA in various organs. The mutant human APP transcripts were only detected in the organs of the transgenic puppy, which was further confirmed by sequencing analysis.



This indicates that the transgenic puppies expressed the mutant hAPP gene in various vital organs without showing physiological abnormalities. The remaining transgenic puppies will be continuously monitored for any impaired behavior and for any age dependent neurodegenerative lesions.

Nothing to Disclose: YWJ, GSL, JJK, SWP, KHK, MK, CJY, TKJ, EMJ, YKK, SHH, TS, EBJ, WSH

P1-329

Significance of Mast Cells in Mammary Involution after Lactation: Production of Gonadotropin Releasing Hormone (GnRH) and Its Action in the Mammary Gland.

Mitsumori Kawaminami Ph.D.¹, Duangjai Rieanrakwong¹, Tomohiro Yonezawa Ph.D.¹ and Shiro Kurusu Ph.D.¹.

¹Kitasato Univ Towada City, Japan.

We have reported that mast cells are involved in mammary involution by producing GnRH (ENDO09). GnRH agonist increased both mammary epithelial expression of annexin A5, a marker protein of GnRH action, and TUNEL-positive cells. In the present study, we studied further the significance of mast cells in the accomplishment of involution with three experimental approaches.

1) The effect of ergocryptine that suppresses prolactin secretion on the number of mammary mast cells was examined. When ergocryptine was administered to lactating rats for 2 days from lactation day 10, mammary mast cells were increased even though pups were remained. When involution was induced by teat sealing with surgical adhesive in a lateral gland, mast cell number was not changed from another side gland despite of increased epithelial apoptosis. We have already observed that prolactin suppressed the post-lactational increase of mammary mast cells. Getting together, the cessation of high levels of prolactin secretion after lactation is the trigger of mast cell migration to the mammary gland.

2) Involution after weaning was significantly delayed in mast cell-deficient Wsh (C57BL/6-Wsh/Wsh) mice. In C57BL/6J control mice, glandular tissues were almost completely replaced with adipose tissues by five days after weaning. However, the mammary tissues of Wsh mouse still showed a distinct alveolar structure on the same day. Administration of GnRH agonist locally to the mammary glands facilitated involution in Wsh mouse. These results clearly demonstrate that mast cells and their production of GnRH are essential for the initiation of involution after weaning.

3) When GnRH agonist was added to a culture of HC11 mammary epithelial cells for 24 hr, the cells decreased in a dose-dependent manner. GnRH agonist as well as leukemia inhibitory factor (LIF), a cytokine involved in mammary involution, induced TUNEL-positive HC11 cells and augmented Caspase 3 activity. Western blotting showed that GnRH agonist as well as LIF suppressed the expression of anti-apoptotic factor Akt and Bcl-2, whereas pro-apoptotic factor Bax was upregulated.

Present results clearly demonstrate that the mast cell migration into the mammary tissues after lactation is initiated by the reduction of plasma prolactin and the mast cell is prerequisite for the initiation of normal involution. GnRH produced by mast cells directly acts on mammary epithelial cells and induces apoptosis.

Nothing to Disclose: MK, DR, TY, SK

P1-330

Endothelial Nitric Oxide Synthase Uncoupling in a Chemically Induced Model of Menopause Is Associated with the Development of Endothelial Dysfunction.

S Kumar PhD¹, S Sharma PhD¹, S Aggarwal MD¹, R Rafikov PhD¹, SK Noonepalle MS¹, DW Stepp PhD¹, PB Hoyer PhD² and SM Black PhD¹.

¹Med Coll of Georgia Augusta, GA and ²Univ of Arizona Tucson, AZ.

In this study, we have used the ovary intact mouse model analogous to menopause in humans that is induced by injecting 4-vinylcyclohexene diepoxide (VCD). VCD selectively accelerates the natural loss of small primordial and primary follicles without affecting other tissues, including the rest of the ovary producing ovarian failure and a transition into menopause. Our aim was to determine if the decreases in circulating estrogen levels in the VCD mouse could decrease endothelial nitric oxide synthase (eNOS) expression and influence NO production and endothelial function. Utilizing western blot analysis we found that the levels of estrogen receptors α and β were significantly decreased in the aorta of VCD mice compared to vehicle controls. Furthermore, we observed a significant decrease in eNOS protein levels in VCD mice. Recently our group has reported that estrogen plays a role in regulating the expression of GTP cyclohydrolase1 (GCH1) and cellular tetrahydrobiopterin levels (BH₄). GCH1 protein levels were significantly decreased in the VCD mice and this was associated with a decrease in the levels of cAMP levels and phospho-CREB. Further we found that aortic BH₄ levels were also significantly decreased in VCD mice. BH₄ is an important cofactor for NOS coupling and NO production. NO levels in the aorta of VCD mice were significantly decreased while NOS-derived superoxide was significantly increased indicative of uncoupled eNOS. Finally, we found that there was a significant attenuation in vasodilation to acetylcholine in the aorta of the VCD mouse. We conclude that during menopause, loss of estrogen not only results in reduced levels of eNOS and lower NO production but also causes increase oxidative stress through uncoupling of eNOS leading to the development of endothelial dysfunction.

Sources of Research Support: In part by a Seed Award from the Cardiovascular Discovery Institute of the Medical College of Georgia.

Nothing to Disclose: SK, SS, SA, RR, SKN, DWS, PBH, SMB

P1-331

Genetic Deletion of the Angiotensin-(1-7) Receptor Mas Leads to Marked Changes in Female Reproduction.

K Honorato-Sampaio MS¹, RF Andrade PhD¹, COD Horta¹, RAS Santos MD, PhD¹, M Bader² and AM Reis PhD¹.

¹Fed Univ of Minas Gerais Belo Horizonte, Brazil and ²Max-Delbrück-Ctr for Molecular Med Berlin, Germany.

Several studies have shown the presence of the renin-angiotensin system (RAS) components in mammalian ovaries and their involvement in ovarian physiology. We have shown the presence of Angiotensin-(1-7) [Ang-(1-7)], an important biologically active component of the RAS, and of its Mas-receptor in rat and rabbit ovaries, and the involvement of Ang-(1-7) in the rabbit ovulatory process. The aim of this study was to evaluate the effect of genetic deletion of the G protein-coupled receptor, Mas, in the female reproduction. Immature mice (26 days-old) were primed with 5 IU of pregnant mare's gonadotropin (PMSG) followed 48h later by 5IU of human chorionic gonadotropin (hCG). After 14-16h the oocytes were collected from the oviducts. Plasma estradiol level (E2) was measured by RIA. Histology of the ovaries was used to estimate total number of primordial, primary, secondary and antral follicles. Size of the litters was determined from breeding data of mice pool (2-6 month-old) housed at Federal University of Minas Gerais. The estrous cycle of adult mice (3 months-old) was determined throughout 18 days by daily vaginal smears. Spontaneous ovulation was determined by collecting oocytes from the mice oviducts on the morning of estrus. Histological analysis showed that Mas-knockout mice had a significant smaller pool of primordial follicles than wild type (WT) animals. However, Mas-knockout animals showed a higher number of antral follicles after PMSG stimulation. Estrus cycle analysis showed that knockout females had shorter proestrus and longer diestrus phase. Knockout mice presented a smaller litter size (5.95 ± 0.17 pups/litter) than wild type (7.14 ± 0.17 pups/litter). No difference was observed in the number of oocytes in oviducts between knockout and wild types (around 30 oocytes per oviducts) following stimulation of ovulation. However, Mas-knockout mice presented fewer oocytes in oviducts (4.5 ± 0.7) than wild animals (8.6 ± 0.8) in the spontaneous ovulation. These results indicate that Angiotensin-(1-7) receptor Mas plays an important role on female reproduction, involving events such as oocyte recruitment and ovulation, confirming previous studies from our laboratory showing the effects of Ang-(1-7) on ovulation.

Sources of Research Support: CNPQ, FAPEMIG and CAPES.

Nothing to Disclose: KH-S, RFA, CODH, RASS, MB, AMR

P1-332

Increased Fetal Cortisol Reduces Fetal but Not Maternal Plasma NTproCNP in Late Ovine Gestation.

TCR Prickett PhD¹, BA McNeill BSc(hons)², MH Oliver MD³, JE Harding MD³ and EA Espiner MD¹.

¹Univ of Otago, Christchurch Christchurch, New Zealand ; ²Lincoln Univ Canterbury, New Zealand and ³Univ of Auckland Auckland, New Zealand.

C-type natriuretic peptide (CNP) is an important growth factor expressed in a wide range of tissues during development and post natal life. CNP production, known to be essential to post natal linear growth in both rodents and humans, is strongly correlated with linear growth velocity in lambs, rodents and children and is rapidly and reversibly reduced by catabolic interventions such as caloric restriction and glucocorticoids which are well recognized inhibitors of skeletal growth. Similar decreases in plasma CNP forms occur in the fetus during maternal caloric restriction or glucocorticoid administration but the effect of physiological increments in fetal cortisol on CNP production is unknown.

Recent studies of ovine pregnancy [1, 2] show that both CNP and the bio inactive amino terminal fragment of proCNP 1-103 (NTproCNP) are secreted by the placenta and circulate at high concentrations in maternal plasma from the end of the first trimester [2]. In contrast, the ovine placenta does not appear to contribute to circulating fetal CNP forms which are lower than maternal concentrations [1, 2].

Since maternal plasma CNP forms decline suddenly within the last week of ovine pregnancy (138-145 days) [2], a phase of rapidly increasing fetal cortisol production, we hypothesized that contrived increases in fetal cortisol within the physiological range in late gestation would inhibit not only fetal but also maternal concentrations of NTproCNP.

Accordingly we have studied the fetal and maternal responses of NTproCNP to sustained low dose infusions of cortisol (1.25mg/day/kg for 11 days) delivered to either the fetus (n=12) or the mother (n=10) from day 117 gestation. Fetal plasma cortisol was increased 9 fold (p<0.001) to peak values of 38 ±14 ng/ml. Compared to control (maternal saline infusions, n=12), fetal plasma NTproCNP was progressively reduced (p<0.004) during fetal cortisol infusions which also reduced fetal girth growth (p<0.05). In contrast, maternal NTproCNP was unaffected by cortisol whether delivered via the fetus or maternal circulations. Neither fetal girth growth nor fetal plasma NTproCNP was affected by cortisol delivered to the mother. We conclude that fetal but not placental tissue production of CNP is reduced by physiological increments in fetal cortisol. Failure to reduce maternal NTproCNP may relate to the continuing presence of the placental barrier to cortisol at this stage of pregnancy.

[1] Prickett TC et al., Endocrinology 2007;148:4015

[2] McNeill BA et al., Endocrinology 2009;150:4777

Nothing to Disclose: TCRP, BAM, MHO, JEH, EAE

P1-333

Effects of Progesterone Receptor-B Expression Ablation on Prepartum Remodeling of the Cervix.

T.Y. Chhaya BS¹, E.A. Apostolakis PhD², T.J. Lechuga BS¹, R.M. Diaz MD¹, A.E. Burns BA¹, L. Force MD¹, B.T. Oshiro MD¹ and S.M. Yellon PhD¹.

¹Loma Linda Univ Sch of Med Loma Linda, CA and ²Baylor Coll of Med Houston, TX.

Trophic actions of progesterone in reproductive functions are, in part, mediated by interaction with the classical intracellular receptor isoform PR-B (1). The PR-A isoform serves as a dominant transcriptional repressor to antagonize actions of progesterone bound to PR-B. Withdrawal of progestational support for pregnancy is integral to the final common pathways for parturition (2, 3), but the role of progesterone receptor isoforms in the cervix with respect to degradation of extracellular matrix and immune cell immigration at term is not known (4, 5). To determine if PR-B participates in cervical remodeling, pregnant transgenic mice lacking expression of this isoform (PRBKO; 6) and controls were killed before or after birth. The cervix was processed, stained, and analyzed as previously described (7). In PRBKO mice, births occurred normally on day 19 post-breeding as in controls. By the day before birth, cervixes from PRBKO mice had reduced numbers of cell nuclei/area, diminished collagen content and structure, as well as increased numbers of resident macrophages compared to nonpregnant mice. These results were equivalent to that in controls and indicate that PR-B does not regulate growth, degradation of the extracellular matrix or proinflammatory processes that regulate recruitment of macrophages in the cervix before term. By contrast, neutrophil immigration did not occur in cervixes from PRBKO compared to that in prepartum controls. Although PR-B may facilitate recruitment of neutrophils, a reduced census of neutrophils does not appear to impact the prepartum remodeling process. These findings focus attention on the PR-A isoform or non-classical progesterone receptors as important for progestational actions that promote growth and reduce residency by immune cells in the cervix during pregnancy. To complete cervical remodeling, the findings raise the possibility that PR-A or other receptors may be important for neuroinflammatory processes that are associated with withdrawal of progesterone, ripening of the cervix, and the mechanism for parturition.

- (1) Mulac-Jericevic et al., Science 2000; 289:175.
- (2) Haluska et al., JSGI 2002; 9:125.
- (3) Merlini et al., JCEM 2007; 92:1927.
- (4) Mackler et al., JSGI 2003; 10:323.
- (5) Yellon et al., Biol Reprod 2008; 78:438.
- (6) Mulac-Jericevic & Conneely, Reprod 2004; 128:139.
- (7) Yellon et al., Biol Reprod 2009; 81:1.

Sources of Research Support: NIH HD054931.

Nothing to Disclose: TYC, EAA, TJL, RMD, AEB, LF, BTO, SMY

P1-334

Menopause Increases NOS Uncoupling and Reduces Heart Function after Myocardial Infarction.

S Kumar PhD¹, S Sharma PhD¹, SK Noonepalle MS¹, J Liu BS¹, T Hu PhD¹, PB Hoyer PhD² and SM Black PhD¹.

¹Med Coll of Georgia Augusta, GA and ²Univ of Arizona Tucson, AZ.

Women undergo many physiological changes after menopause becoming more prone to the development of cardiovascular disease including cardiac failure. To begin to determine the factors that contribute to the increased cardiovascular risk, we used the ovary intact mouse model of menopause induced by exposure to 4-vinylcyclohexene diepoxide (VCD), which selectively accelerates the natural loss of small primordial and primary follicles without affecting other tissues, including the rest of the ovary. This study tested the hypothesis that increased eNOS uncoupling, due to the loss of estrogen signaling in the VCD mouse, would lead to enhanced heart damage after surgically induced myocardial infarction (MI). VCD lead to a significant decrease in the protein levels of both estrogen receptor α and β as well as eNOS expression. The decrease in eNOS protein correlated with a decrease in NO metabolites but also an increase in eNOS-derived superoxide indicative of eNOS uncoupling. The increase in eNOS uncoupling was due to decreased expression of GTP cyclohydrolase1 (GCH1) and tetrahydrobiopterin (BH₄) levels. Next myocardial infarcts (MI) were produced by permanently ligating the LAD and magnetic resonance imaging (MRI) was utilized to measure changes in left ventricular Ejection Fraction (LVEF). Our data indicate that the menopausal phenotype induced by VCD significantly decreases the LVEF after MI compared to vehicle-treated control mice. Thus, we conclude that during menopause, loss of estrogen not only results in reduced levels of eNOS, lower NO production but also causes an increase in oxidative stress through uncoupling of eNOS which enhances the damage to the heart during an MI.

Sources of Research Support: In part by a Seed Award from the Cardiovascular Discovery Institute of the Medical College of Georgia.

Nothing to Disclose: SK, SS, SKN, JL, TH, PBH, SMB

P1-335

Maternal Serum Resistin and Adiponectin: Comparison of Singleton and Twin Pregnancies and the Effect of Serum Estradiol or Progesterone.

V Daniel Castracane PhD¹, Pamela DiMarino BS², Sarah O'Neal BS² and Robert P Kauffman MD².

¹Texas Tech Univ HSC at the Permian Basin Sch of Med Odessa, TX and ²Texas Tech Univ HSC at Amarillo Sch of Med Amarillo, TX.

In a recent report (Endocrine Society 2009), we compared maternal serum levels of leptin and the soluble leptin receptor from singleton and twin pregnancies. That study indicated trivial, if any, contribution from the placenta toward the maternal serum hormone levels of either analyte, but leptin levels in both groups increased in proportion with the BMI. In the present study, we examined maternal serum levels of resistin and adiponectin, adipokines with important roles in metabolism produced by the placenta, to determine whether a placental contribution exists for these hormones. Uncomplicated pregnant women between 15 and 20 weeks of gestation provided a single blood sample. A random sampling of singleton (n=42) and twin (n=39) pregnancies were included in this study. Serum resistin and total adiponectin were measured with ELISA assays (Millipore) and serum estradiol and progesterone with an automated chemiluminescent assay system (Immulate, Siemens). Independent samples t-tests were used to compare continuous variables. Resistin and adiponectin between singleton and twins were corrected (independently) for estradiol, progesterone, and BMI levels using ANCOVA.

Despite significant differences in circulating estradiol and progesterone levels between singletons and twins ($p < 0.0001$ for each hormone), resistin ($p = 0.76$) and adiponectin ($p = 0.85$) levels were similar. Circulating estradiol and progesterone concentrations had no effect on resistin or adiponectin in either group. Similarly, BMI did not influence resistin and adiponectin levels.

The increased placental mass associated with twins does not influence maternal serum resistin or adiponectin compared to singletons. Despite the placental expression of these hormones, our results implicate maternal adipose tissue as the primary sources of resistin and adiponectin in the serum of pregnant women. Placental expression of these adipokines seems to be designed for paracrine functions.

Nothing to Disclose: VDC, PD, SO, RPK

P1-336

Tacrolimus and Sirolimus Reduce Estradiol Action in the Normal Female Rat.

V Shivaswamy MBBS, MS^{1,2}, L Ochsner MSN¹, D Maroni MS¹, J Passer BS¹, FG Hamel PhD^{1,2}, JS Davis PhD^{1,2} and JL Larsen MD^{1,2}.

¹Univ of Nebraska Med Ctr Omaha, NE ; ²Univ of Nebraska Med Ctr Omaha, NE ; ³Univ of Nebraska Med Ctr Omaha, NE and ⁴Veterans Administration Med Ctr Omaha, NE.

Reproductive function is variably changed after organ transplantation. Immunosuppressants, tacrolimus (TAC) and sirolimus (SIR) contribute to post transplant diabetes mellitus (PTDM) and are associated with ovarian cysts. We have previously shown that treatment of female rats for 4 weeks with TAC, SIR and TAC-SIR altered estrus cycling. We examined the effects of TAC and SIR on reproductive organs in normal female rats. Sprague-Dawley female rats (150-200g) were grouped based on estrus stage (n=5/group), and received daily treatments for 4wk: TAC alone (4mg/kg/d), SIR alone (2mg/kg/d), TAC + SIR, or control (C: sunflower oil/10% ethanol). Body weights and vaginal smears were taken daily, and glucose measured twice weekly. On the last day of treatment glucose and insulin responses were measured, after oral glucose challenge (1g/kg), and blood and tissues were harvested. TAC, SIR, and TAC-SIR increased daily glucose concentrations (p<0.05) compared to C. TAC and SIR were hyperglycemic after oral glucose compared to C (p<0.05), accompanied by hyperinsulinemia in SIR only, compared to C (p<0.05). Estrus cycles were reduced in all groups compared to C (p<0.05). The uterine horns were smaller in all treatment groups compared to C (p<0.05) suggesting reduced responsiveness to estradiol. Western blot analysis of ovarian proteins showed lower levels of phosphorylated ribosomal protein S6, a target for mTOR/p70S6Kinase, indicating that SIR was effective at the level of the ovary. SIR inhibited aromatase expression in ovaries, compared to TAC, TAC-SIR and C (p <0.05), providing a mechanism for the reduction in uterine size. Estradiol, testosterone and progesterone levels were variable within groups and were not different among groups. In summary, in an animal model of immunosuppressant induced diabetes, all treatments disrupted estrus cycling. Reductions in uterine size following TAC and SIR treatment suggested reduced estrogen action; even though serum concentrations of steroid hormones at the time of sacrifice were not statistically different. In conclusion, TAC and SIR affect normal reproductive function in female rats possibly by reducing estrogenic actions which may have implications beyond the reproductive system in transplant patients.

Sources of Research Support: Grants from the Department of Veterans Affairs Biomedical Laboratory Research and Development Merit Review Research Program (JSD), the Olson Center for Women's Health at the University of Nebraska Medical Center (JSD) and a University of Nebraska Medical Center Assistantship (DM).

Nothing to Disclose: VS, LO, DM, JP, FGH, JSD, JLL

P1-337

Mechanisms of Stress-Induced Pregnancy Failure in Mice: Role of Maternal Neuro-Endocrine-Immune Responses.

VJ Parker BSc(HonS)¹ and AJ Douglas PhD¹.

¹Univ of Edinburgh Edinburgh, UK.

Prolonged stress exposure in early gestation often results in pregnancy failure associated with inhibition of progesterone secretion and the consequent cytokine imbalance. Prolactin modulates progesterone secretion, independently mediates implantation, has a role in immune regulation and is a mediator of neuro-endocrine-immune systems that underlie pregnancy establishment. Therefore, we hypothesised that stress would also decrease prolactin secretion. Furthermore, as dopamine is the main inhibitor of prolactin, we predicted that decreased prolactin was due to increased tuberoinfundibular dopamine (TIDA) neuron activation. Progesterone and prolactin suppress the expression of the pro-inflammatory cytokine interleukin (IL)-6, so we also expected that stress would elevate IL-6 release in early pregnancy vs. virgin, due to depleted progesterone/prolactin. To test the effect of stress on the prolactin system, virgin and d5.5 pregnant c57/Bl6 mice were either given lipopolysaccharide intraperitoneally (LPS i.p.: 12.5µg) or fasted for 24hrs; controls were given vehicle or fed, respectively. Blood was collected after decapitation and assayed for hormones and IL-6 by radioimmunoassay or ELISA. Alternatively, mice were perfused-fixed and TIDA neuron activation was analysed by double immunocytochemistry for tyrosine hydroxylase (enzyme in dopamine synthesis)/Fos expression. Stress significantly decreased plasma progesterone and prolactin and increased IL6 and increased activation of TIDA neurons in the arcuate nucleus in early pregnant vs. virgin mice. The elevated activation of TIDA neurons, correlated with decreased prolactin secretion, in pregnancy following stress shows that changes in control of TIDA neurons play an important role. To test if stress alters prolactin negative feedback to TIDA neurons to cause inhibited prolactin secretion we injected ovine prolactin (1 mg/g body weight in 100ml saline; i.p.) into stressed and non-stressed mice and measured plasma prolactin. Prolactin concentration was inhibited in non-stressed pregnant mice but not in stressed pregnant mice suggesting that stress attenuates negative feedback. This cannot explain the stress-induced increase in TIDA activation or decrease in prolactin secretion in early pregnancy so other stress-related hypothalamic mechanisms must be responsible.

Sources of Research Support: The Society for Endocrinology (UK). VJP was funded by the BBSRC.

Nothing to Disclose: VJP, AJD

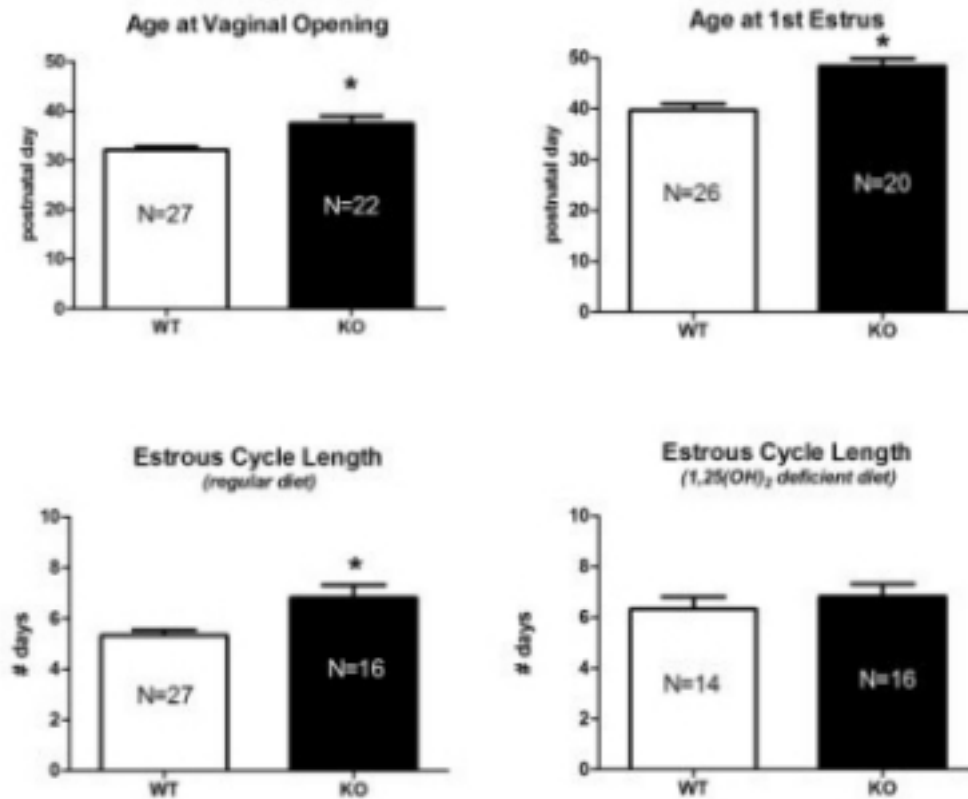
P1-338

1, 25-(OH)₂ Vitamin D₃ Deficiency Disrupts Female Hypothalamic-Pituitary Physiology.

CL Dicken MD¹, DD Israel PhD¹, J Shu MD¹ and GS Neal-Perry MD, PhD¹.

¹Albert Einstein Coll of Med Bronx, NY.

Vitamin D₃ (Vit D₃) deficiency has reached epidemic levels in the US. While the role of Vit D₃ in bone health is well known; several recent clinical studies suggest Vit D₃ is also essential for fertility. Rodent models for Vit D₃ deficiency using either transgenic vitamin D receptor (VDR) or *CYP27B1* enzyme knockout (KO) female mice demonstrate arrested folliculogenesis and hypogonadism. The mechanism(s) by which Vit D₃ deficiency produces this abnormal reproductive phenotype is unclear. Our preliminary data indicate that ovaries in wild-type (WT) and *CYP27B1* KO mice respond similarly to ovarian hyperstimulation, thereby suggesting that the primary defect is not ovarian. Moreover we have also demonstrated that GnRH neurons colocalize VDR, implying a role for Vit D₃ in GnRH neurophysiology. **We therefore hypothesized that Vit D₃ is essential for normal female hypothalamic-pituitary axis physiology and the pubertal transition.** WT (n=14-27) and *CYP27B1* KO (n=16-22) mice were used to study the effects of Vit D₃ deficiency on the pubertal transition and the estrous cycle. All dams received a regular diet throughout gestation and lactation. Pups were weaned at 21 d, genotyped, and maintained on either a Vit D₃ deficient or regular diet. Mice were monitored daily for vaginal opening (VO). Daily vaginal smears were performed for 8 wks to document 1st estrus, the time between VO and 1st estrus, and estrous cycle length. **Results:** *CYP27B1* KO mice had a significantly delayed VO ($p < 0.001$) and 1st estrus ($p < 0.0001$). *CYP27B1* KO mice exhibited prolonged estrous cycles ($p < 0.01$). WT mice fed a Vit D₃ deficient diet exhibited prolonged estrous cycle lengths equivalent to that of *CYP27B1* KO mice ($p > 0.05$).



* $p < 0.01$

Conclusion: Our findings strongly suggest that Vit D₃ deficiency adversely affects the pubertal transition and the establishment of normal estrous cycle length. These data are consistent with our hypothesis that Vit D₃ has a central role in female reproduction and is essential for normal hypothalamic-pituitary physiology.

- (1) Ozkan S et al., Fertil Steril 2009;
- (2) Panda DK et al., Proc Natl Acad Sci USA 2001; 98:7498
- (3) Yoshizawa T et al., Nat Genet 1997; 16:391

Sources of Research Support: Montefiore Medical Center, Department of Obstetrics & Gynecology; Albert Einstein College of Medicine; Zondek Award.

Nothing to Disclose: CLD, DDI, JS, GSN-P

P1-339

Replication of Association of Insulin Receptor Gene Polymorphisms with Polycystic Ovary Syndrome (PCOS).

MO Goodarzi¹, YV Lowuers², KD Taylor¹, MR Jones¹, J Cui¹, S Kwon¹, YDI Chen¹, L Stolk², AG Uitterlinden², JSE Laven² and R Azziz¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA and ²Erasmus MC Rotterdam, Netherlands.

Because insulin resistance is present in most women with PCOS, genetic association studies have focused on components of the insulin-signaling pathway, with inconclusive results. We previously reported (ENDO 2008) our genotyping of 41 genes related to insulin signaling as candidates for PCOS. We selected the insulin receptor (*INSR*) plus 40 genes in two limbs of the pathway: (a) the PI3-kinase, and (b) CAP/Cbl pathways (the MAPK pathway was not included). SNPs were chosen using Caucasian (CEU) HapMap linkage disequilibrium (LD) data to maximize coverage of each gene. In the discovery cohort, we genotyped 384 SNPs in 275 White women with PCOS and 173 White controls using the oligo-ligation assay on an Illumina BeadStation. SNPs per gene ranged from 3 (*IRS1*) to 35 (*INSR*). In this cohort, five SNPs (rs12459488, rs12971499, rs2252673, rs7258382, rs10401628) in the *INSR* gene were associated with PCOS (P values 0.009-0.02). Given the clear importance of this gene, in the current study we sought to replicate these associations in an independent cohort consisting of 526 Caucasian PCOS women and 3585 unselected controls from the general population (the Rotterdam study). Two of the five *INSR* SNPs replicated association with PCOS. Carriers of the minor G allele at rs2252673 had an increased odds of PCOS in both the discovery cohort (odds ratio (OR)=1.97, 95%CI: 1.10-3.36, P=0.02) and the replication cohort (OR=1.32, 95%CI: 1.08-1.60, P=0.006). On the other hand, minor allele carriers of rs7258382 were protected against PCOS in both the discovery (OR=0.48, 95%CI: 0.27-0.83, P=0.009) and the replication (OR=0.80, 95%CI: 0.64-0.99, P=0.04) cohorts. These two SNPs are not in LD with each other; they are also not in LD with any other SNPs in the CEU HapMap database. A study of Korean women found a trend towards increased PCOS susceptibility for the G allele of rs2252673 (1), consistent with our results. Most of the remaining studies of *INSR* in PCOS focused on rs1799817 (His1058 C/T in exon 17), for which a recent meta-analysis found no overall association with PCOS (2). No prior study has comprehensively tagged variation across the large (181 kb) *INSR* gene, as done in the discovery cohort. This approach allowed us to identify two variants, one predisposing to and one protective against PCOS, with associations that were replicated in an independent and larger cohort. These data should invigorate interest in *INSR* as a PCOS susceptibility gene.

(1) Lee EJ et al., Fertil Steril 2008;89:1213

(2) Ionnidis A et al., Mol Genet Metab 2009 Oct 22 Epub

Sources of Research Support: NIH grant R01-DK073632 (to RA); NIH grant R01-HD029364 (to RA); NIH grant R01-DK079888 (to MOG); NIH grant M01-RR000425 (General Clinical Research Center Grant at CSMC); Helping Hand of Los Angeles, Inc.

Nothing to Disclose: MOG, YVL, KDT, MRJ, JC, SK, YDIC, LS, AGU, JSEL, RA

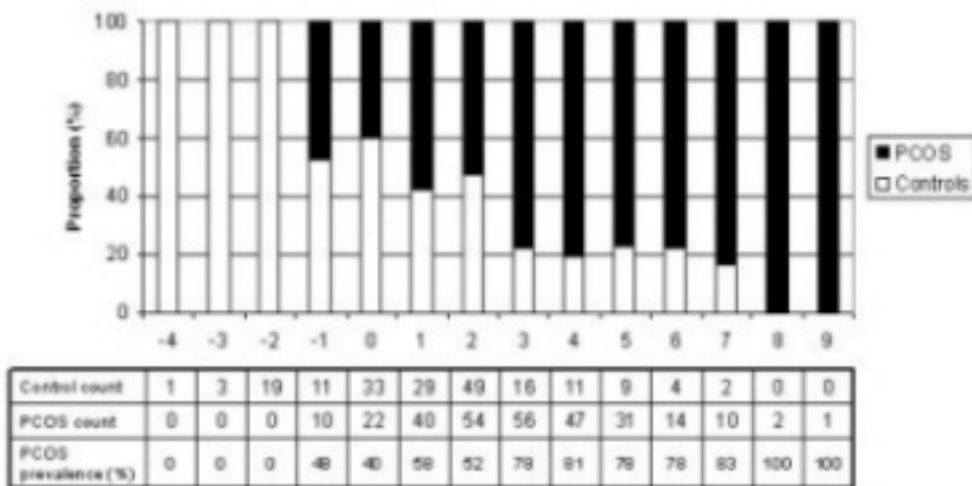
P1-340

Integration of Risk and Protective Alleles Predicts Susceptibility to Polycystic Ovary Syndrome.

MO Goodarzi MD, PhD¹, MR Jones BSc¹, YDI Chen PhD¹, J Cui MS¹, X Guo PhD¹ and R Azziz MD, MPH, MBA¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA.

PCOS is considered a common, complex genetic disorder. Susceptibility to such disorders is determined by the inheritance of multiple genetic factors, each with a moderate effect on disease risk. Few studies have jointly considered multiple genes as risk factors for PCOS. Our objective was to simultaneously assess the effect on PCOS susceptibility of 10 genes (*AKT2*, *AR*, *CYP3A7*, *FEM1A*, *FEM1B*, *GSK3B*, *PRKAG3*, *SGTA*, *SRD5A1*, *SRD5A2*) previously found by us to be associated with PCOS, providing the first analysis of multiple loci integrated as a genetic risk score. The study cohort consisted of 287 White women with PCOS and 187 White controls, recruited from the reproductive endocrinology clinic and surrounding community of the University of Alabama at Birmingham. Genotyping and analysis was carried out at Cedars-Sinai Medical Center. Considering 13 independent ($r^2 < 0.8$) loci from 10 genes, we constructed a genetic risk score (GRS) consisting of the number of PCOS-predisposing alleles minus the number of protective alleles. Association of GRS with PCOS was evaluated using logistic regression, adjusting for BMI and age. The GRS differed significantly between cases (median GRS=3) and controls (median GRS=1) ($P < 0.0001$). Each unit increment in the GRS was associated with a 1.56-fold increase in the odds of PCOS (95% CI 1.36-1.79, $P < 0.0001$). The **Figure** displays the prevalence and distribution of PCOS at each GRS level. No subject with a risk score of -4 to -2 had PCOS. At the other end of the spectrum, all subjects with risk scores of 8 or 9 had PCOS. The prevalence of PCOS steadily rose as the GRS increased. The regression model of GRS as a predictor of PCOS was robustly confirmed using internal cross-validation. This is the first demonstration that overall genetic burden is a predictor of PCOS status in a dose-related manner, similar to type 2 diabetes and coronary heart disease, supporting the concept that PCOS results from contributions from multiple susceptibility loci.



Sources of Research Support: NIH grant R01-DK073632 (to RA); NIH grant R01-HD029364 (to RA); NIH grant R01-DK079888 (to MOG); NIH grant M01-RR000425 (General Clinical Research Center Grant at CSMC); Helping Hand of Los Angeles, Inc.

Nothing to Disclose: MOG, MRJ, YDIC, JC, XG, RA

P1-341

The Impact of Proband Glucose Tolerance Status on Body Mass Index (BMI) and Adiposity in Adolescent Girls at High Risk for Polycystic Ovary Syndrome (PCOS).

DH Geller MD, PhD¹, S Ten MD², R Mathew MD³, SE Oberfield MD⁴, MI Hernandez MD⁵, F Cassorla MD⁵, F Orio MD, PhD⁶, JP Baillargeon MD⁷ and R Azziz MD¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA ; ²Infants and Children's Hosp of Brooklyn, Maimonides Med Ctr Brooklyn, NY ; ³ Vanderbilt Univ Med Sch Nashville, TN ; ⁴Columbia Univ Coll of Physicians and Surgs New York, NY ; ⁵IDIMI, Univ of Chile Santiago, Chile ; ⁶Univ "Federico II" Naples, Italy and ⁷Univ of Sherbrooke Sherbrooke, Canada.

INTRODUCTION: PCOS is a common endocrine-metabolic abnormality of women, ~70% demonstrating insulin resistance beyond that determined by their degree of obesity. Adolescent daughters and sisters of women with PCOS are at high risk to develop the disorder, and these girls demonstrate abnormal insulin dynamics as early as pubertal onset, as a function of the proband glucose tolerance. As the peri-pubertal development of obesity would alter insulin action, exacerbating the risk of developing PCOS, we studied the degree of adiposity and its relationship with metabolic parameters in girls at risk for PCOS across the pubertal spectrum.

METHODS: In 53 peri-pubertal daughters and siblings of women with PCOS we determined insulin dynamics indices: HOMA-IR and HOMA%B by homeostasis modeling, and insulin sensitivity (SI), acute insulin response (AIRg) and disposition index (DI) by IVGTT. We calculated body adiposity using bioelectrical impedance (BI) in 19 subjects. For analysis, subjects were segregated by the glucose tolerance of the proband (affected mother or older sister): glucose intolerant (PCOS-GI) or normal glucose tolerance (PCOS-NGT).

RESULTS: Cross-sectional BMI Z-score was greater during the first half of pubertal maturation in PCOS-GI girls after adjusting for proband relationship (mother or sister), both at Tanner 1 and across Tanner 1-3. Longitudinal modeling confirmed significantly greater BMI Z-scores in PCOS-GI girls ($p=0.049$) unsegregated by Tanner. Within-Tanner analysis revealed differential BMI Z-scores between cohorts across puberty: greater in PCOS-GI girls at T1 ($p=0.002$), with diverging BMI Z-score linear change, T1-2 ($p=0.019$). Proband glucose tolerance impacted body composition, with greater % fat in PCOS-GI girls ($p=0.047$) after adjusting for proband relationship and subject BMI; conversely, group impedance means were indistinguishable. After adjusting for subject BMI and proband relationship, partial correlations were observed between HOMA%B and % fat, and SI, AIRg & DI with impedance.

CONCLUSIONS: Girls with PCOS-GI probands exhibited greater BMI than those with PCOS-NGT probands across maturation, and significantly greater adiposity even after adjusting for their BMI. Proband glucose tolerance may impact both metabolic performance and body composition in girls predisposed to PCOS. These data suggest that emerging peri-pubertal adiposity independent of BMI may signal greater predisposition to metabolic derangements in this population.

Nothing to Disclose: DHG, ST, RM, SEO, MIH, FC, FO, JPB, RA

P1-342

Effects of Prenatal Exposure to Testosterone May Be Due to Its Conversion to Estradiol in Female Lambs.

Monica P Recabarren¹, Pedro P Rojas-Garcia¹, Maria C Vizcarra¹, Sergio Palma¹, Teresa Sir-Petermann² and Sergio E Recabarren¹.

¹Univ of Concepcion Chillan, Chile and ²Univ of Chile Santiago, Chile.

Adult female sheep prenatally exposed to an excess of testosterone (T) exhibit features that resemble those shown by women with the polycystic ovary syndrome among them, hypersecretion of LH, presence of cysts in the ovary and absence of the preovulatory LH peak. We have previously shown that during prepubertal development, in female lambs prenatally exposed to testosterone, the response of LH to a GnRH analog, is higher at 5 weeks of age (1), indicating that this alterations in the reproductive axis may start early in postnatal life. These alterations could be due to the testosterone imprinting or due to its aromatization to estradiol. To answer this hypothesis, we compared the LH response to a GnRH analoge in 5-weeks old female lamb born to mothers treated with DHT, which do not convert to estradiol (n=6) and T (n=4) (30 mg DHT or T between 30 and 90 days of gestation, followed by 40 mg DHT o T between 90 and 120 days of gestation (birth at term: 147 days). Treatment was given twice weekly by intramuscular (im) injections. A third group of sheep mothers were given the vehicle of the hormone during the same gestational days and used as control. All lambs were born at term. At 5 weeks of age, early prepubertal age, 10 mg of leuprolide/kg body weight (time 0) was given intravenously to all lambs. Blood samples were taken at time 0 and at 3, 6, 9, 12, 18, 24, 30, 36, 42 and 48 hours after the GnRH analoge and plasma LH was measured in each sample by RIA. LH secretion in response to GnRH was significantly higher at 3 and 6 hours in both T-female lambs and DHT-female lambs in comparison to Control female lambs. Mean concentration of LH in T-female lambs was twice the LH concentrations showed by DHT-female lambs at 3 hours post GnRH (One-way ANOVA). The 48-hours area under the curve (AUC) of the LH secretion was significantly higher in DHT and T-female lambs compared to Control female lambs, but the DHT-female lambs showed lower AUC secretion compared to the T-female lambs (Kruskall Wallis-Dunnet). These results suggest that the alterations in the reproductive axis produced by the prenatal exposure to T begin early during development and in part may be consequence of its conversion to estradiol

(1) Recabarren SE et al., Biol Res 2005; 38:235

Sources of Research Support: Fondecyt Grant 1050915 and Fondecyt Grant 1090031.

Nothing to Disclose: MPR, PPR-G, MCV, SP, TS-P, SER

P1-343

Differential Metabolic Responses to Androgen Exposure at Critical Developmental Periods in Mice.

M Lasic BS¹, F Aird PhD¹, J E Levine PhD² and A E Dunaif MD¹.

¹Northwestern Univ Evanston, IL and ²Northwestern Univ Chicago, IL.

Polycystic ovary syndrome (PCOS), a common endocrine disorder of reproductive-aged women characterized by hyperandrogenemia and ovulatory dysfunction, is frequently associated with insulin resistance and visceral obesity. Prenatal androgen excess can produce a phenocopy of PCOS in female rats, sheep, and rhesus macaques. We transiently exposed female C57BL/6 mice to androgens at three developmental stages: prenatal (PA, gestational days, 16-18), equivalent in humans to the second trimester of gestation; early postnatal (MP, "minipuberty" of infancy, days, D10-13), equivalent to 3 to 6 months of age in humans; and peripubertal (PP, D29-35), equivalent to ~3 to 12 years of age in humans. "Triple androgenized" (TA) mice were generated by androgen exposure at all three developmental stages. For PA treatment, dams were subcutaneously (sc) injected with 500 µg/day of free testosterone (T), while for both postnatal treatments, female pups were sc injected with 25 µg T/g body weight (BW)/day. All treatment groups had their respective vehicle controls (C). Glucose homeostasis and insulin action were assessed at week (W) 20 by intraperitoneal glucose (IPGTT) and insulin tolerance tests. Body fat distribution was assessed by removal and weighing of fat pads at sacrifice at W22. TA and PA females gained ~12% more BW than C ($P < 0.001$), starting at W8, while there was no change in BW in MP and PP females. Insulin sensitivity was decreased ($P < 0.001$) and fasting insulin levels were increased ($P < 0.05$) in TA, but not in PA, MP, or PP females as compared to their respective C. Fasting glucose was slightly increased in PA females only ($P < 0.01$), but there was no significant difference in IPGTT in any of the groups. There were significant increases in gonadal and retroperitoneal fat pads ($P < 0.05$) in TA, and subcutaneous fat pads ($P < 0.01$) in PA as compared to their respective C. In conclusion, the timing of androgen exposure had differential effects on insulin sensitivity and body composition. In contrast to PA animal models in other species, only androgen exposure at multiple developmental periods produced insulin resistance and visceral adiposity in female mice. TA female mice thus represent a good model for studying the metabolic features of PCOS.

Nothing to Disclose: ML, FA, JEL, AED

P1-344

Steroidogenic Regulatory Factor C-FOS Is Underexpressed in Polycystic Ovary Syndrome (PCOS) Adipose Tissue and Genetically Associated with PCOS Susceptibility.

MR Jones BSc¹, G Chazenbalk PhD¹, KD Taylor PhD¹, C Wang MD, PhD¹, Y-H Chen PhD¹, J Xu MD, PhD¹, J-M Lee MD, PhD^{1,2}, R Mathur MD¹, M Pall MD, PhD¹, R Azziz MD, MBA, MPH¹ and MO Goodarzi MD, PhD¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA and ²The Catholic Univ of Korea Seoul, Korea.

Introduction: The pathophysiology of PCOS remains unknown; however, gene expression studies in PCOS tissues are identifying novel factors that may be relevant. PCOS women often have insulin resistance beyond what would be predicted considering age or adiposity. In subcutaneous (SC) adipocytes, stimulation of glucose transport, GLUT4 production, and inhibition of lipolysis are abnormal in PCOS, which promote insulin resistance (1-3). FOS (c-fos, FBJ murine osteosarcoma viral oncogene homolog) is a component of the transcription factor AP-1. FOS family members have been implicated as regulators of cell proliferation, differentiation, and apoptosis. FOS has been identified in the granulosa, but not theca, cells of the ovary, with reductions in FOS resulting in increases in *CYP17* mRNA, implicating the FOS family in regulation of ovarian androgen production (4). We proceeded to determine the role of FOS in the adipose dysfunction of PCOS.

Methods: We recruited 11 PCOS subjects and 12 matched controls (BMI range 21-26 kg/m²) for the collection of SC fat. RNA was isolated from SC abdominal adipose tissue samples, and gene expression profiled using Affymetrix Human Genome U133 arrays. We also genotyped 10 tag SNPs from the *FOS* locus in 333 PCOS and 154 controls. Genotypic association with PCOS was tested by logistic regression.

Results: *FOS* mRNA was significantly underexpressed in PCOS vs control adipose (-30.4 fold; P=0.0024). We identified three *FOS* SNPs associated with PCOS (rs8006998, rs8013918, rs8013942; P=0.0056, 0.0019, 0.010, resp.; **Figure 1**).

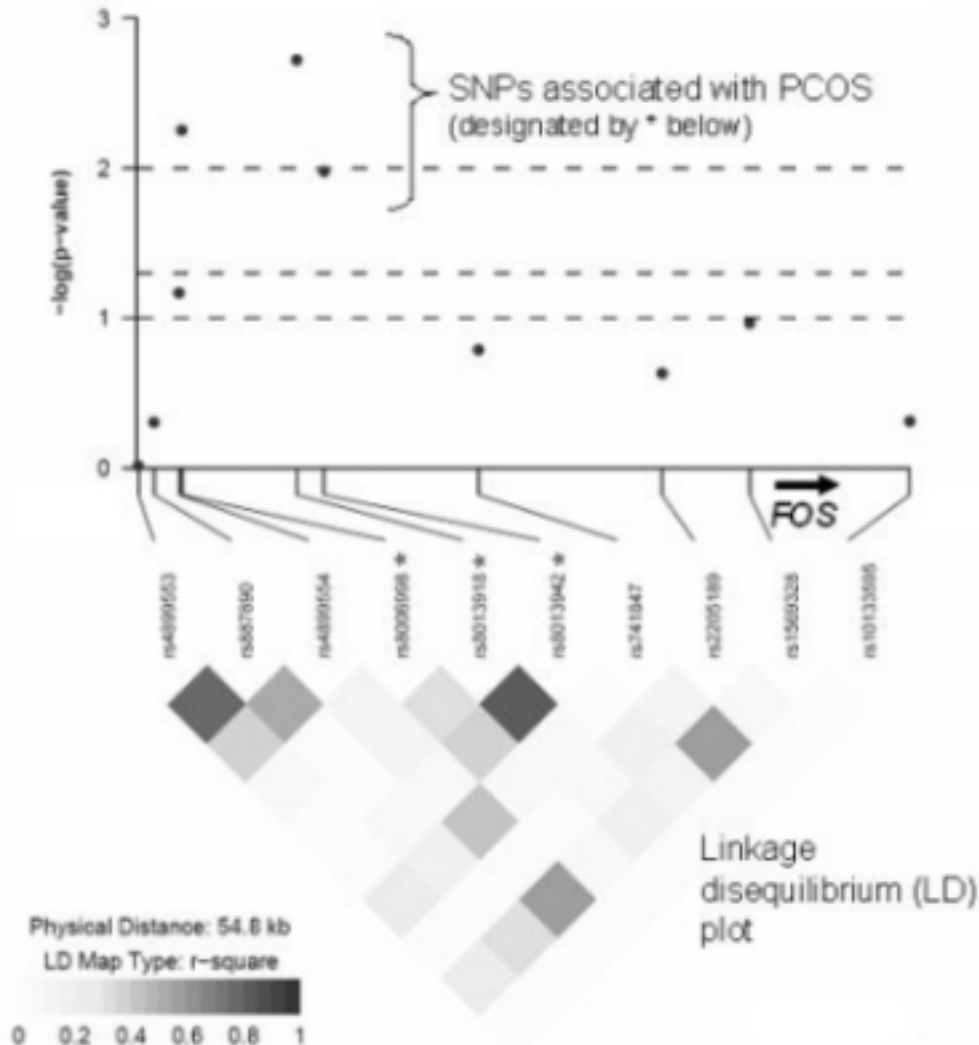


Figure 1. Regional Association and LD Plot for the *FOS* locus. P values ($-\log_{10}$) for association with PCOS for each SNP

(circles) genotyped from the gene region (50kb upstream/5kb downstream) are plotted along with LD structure for the gene region (r^2).

Conclusion: We have demonstrated differential gene expression and genetic association of the *FOS* locus in PCOS subjects, implicating it in the pathophysiology of PCOS. As *FOS* represses *CYP17* in the ovary, our finding of reduced *FOS* expression in adipose suggests *FOS* dysfunction may be a common factor between hyperandrogenism and insulin resistance.

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(2) Ciaraldi TP et al., *J Clin Endocrinol Metab* 2009;94:157

(3) Ciaraldi TP, *J Pediatr Endocrinol Metab* 2000;13(Suppl 5); 1291

(4) Patel SS et al., *J Clin Endocrinol Metab* 2009;94:5163

Sources of Research Support: NIH grant R01-DK073632 (to RA); NIH grant R01-HD029364 (to RA); NIH grant R01-DK079888 (to MOG); NIH grant M01-RR000425 (General Clinical Research Center Grant at CSMC); Helping Hand of Los Angeles, Inc.

Nothing to Disclose: MRJ, GC, KDT, CW, Y-HC, JX, J-ML, RM, MP, RA, MOG

P1-345

Resveratrol Inhibits HMG-CoA Reductase Expression and Cholesterol Synthesis in Rat Theca-Interstitial Cells: An Interaction with Simvastatin.

DH Wong BS¹, JA Villanueva DVM, PhD¹, AB Cress BS¹, A Sokalska MD, PhD^{1,2} and AJ Duleba MD¹.

¹UC DAVIS Davis, CA and ²Univ of Med Scis Poznan, Poland.

Objectives: Polycystic ovary syndrome (PCOS) is associated with ovarian theca-interstitial hyperplasia, systemic inflammation and oxidative stress. Resveratrol (RES) is a natural polyphenol with anti-carcinogenic, anti-inflammatory and anti-oxidant properties. Recently, we have shown that resveratrol inhibits rat ovarian theca-interstitial (T-I) cell proliferation and increases apoptosis. We have also shown that rat T-I cell growth was reduced by simvastatin (SIM), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). This study was designed to investigate the mechanisms of RES action in comparison to SIM.

Methods: Rat theca-interstitial cells were cultured in chemically-defined media for 24-48 hrs in the absence (control) or in the presence of RES (30-100 μ M) and/or SIM (0.1-10 μ M). DNA synthesis was determined by thymidine incorporation assay and the total viable cell number was estimated using the MTS assay. HMGCR mRNA level was evaluated by quantitative rt-PCR and expressed as a ratio normalized to hypoxanthine guanine phosphoribosyl transferase (HPRT).

Cholesterol synthesis was determined by the conversion of [¹⁴C]-acetate to [¹⁴C]-cholesterol. Data analysis was carried out by ANOVA followed by post-hoc pairwise comparisons.

Results: RES and SIM inhibited DNA synthesis in a concentration-dependent manner ($P < 0.001$). Furthermore, RES potentiated the inhibitory effect of SIM on cell proliferation by 50-68% ($P < 0.001$) compared to the inhibition observed in the presence of SIM alone. These effects were accompanied by a concentration-dependent reduction in cholesterol synthesis by RES (by 37-92%; $P < 0.001$) and SIM (by 62-99%; $P < 0.005$). RES (50 μ M) in combination with SIM (0.1 μ M) synergistically inhibited cholesterol synthesis by 50% ($P < 0.001$) beyond the effect of SIM alone. SIM (10 μ M) increased HMGCR mRNA level by up to 2.6 fold above control level ($P < 0.001$). In contrast, RES reduced HMGCR mRNA level and attenuated the simvastatin-induced effect in a concentration-dependent manner, reducing HMGCR expression back to control levels after 48hrs.

Conclusions: RES inhibits HMGCR gene expression and reduces cholesterol synthesis. These effects may complement and enhance the effects of SIM (which inhibits HMGCR activity but increases its expression). The present observations underscore the potential translational/clinical relevance of RES interaction with SIM.

Sources of Research Support: R01-HD050656 (to AJD).

Nothing to Disclose: DHW, JAV, ABC, AS, AJD

P1-346

Inhibition of Matrix Metalloproteinases Improves Estrous Cyclicity in Prenatally Androgenized (PNA) Mice.

FH Morgan MD¹, SR Hammes MD, PhD² and SM Moenter PhD¹.

¹Univ of Virginia Charlottesville, VA and ²Univ of Rochester Rochester, NY.

Polycystic ovary syndrome (PCOS), a leading cause of female infertility, is characterized by hyperandrogenism and elevated LH/GnRH pulsatility. Increased GnRH pulse frequency favors LH over FSH, limiting follicular maturation and ovulation and stimulating LH-induced ovarian steroidogenesis. Elevated androgens reduce negative feedback on GnRH neurons, further perpetuating these stimulatory interactions. Matrix metalloproteinases (MMPs) have two important roles in the ovary: determining the biomechanics of the ovarian matrix and acting downstream from LH to release epidermal growth factor (EGF), upon which LH-induced cumulus cell expansion, oocyte maturation, and androgen synthesis are dependent. To test if MMP inhibition restores estrous cyclicity in PNA mice, which exhibit a PCOS-like phenotype, estrous cycles were monitored in control and PNA mice for 2wks before and 2wks during treatment with an MMP inhibitor (galardin 0.04mg/g/day IP), or vehicle (0.05%DMSO). PNA phenotype was confirmed by increased ($P<0.01$) anogenital distance at 21d (6.0 ± 0.5 mm) vs controls (5.5 ± 0.4 mm) and advanced ($P<0.0001$) vaginal opening, an early external marker of puberty, in PNA mice (22 ± 3 d) vs controls (35 ± 6 d). First estrus was delayed or absent in PNA mice (59 ± 8 d) vs control mice (41 ± 3 d, $p<0.0001$). Body mass was not different between PNA (17.1 ± 1.6 g) and control mice (17.2 ± 0.8 g) and was not affected by galardin. Estrous cycles prior to initiation of therapy were absent or disrupted in PNA mice, as evidenced by increased time in diestrus ($93\pm 3\%$ vs $63\pm 4\%$, $P<0.0001$). During therapy, galardin-treated PNA mice had improved estrous cyclicity ($P<0.01$) with reduced time in diestrus ($59\pm 8\%$) compared to vehicle-treated PNA mice ($85\pm 16\%$), whereas galardin-treated controls had no change in time in diestrus ($57\pm 6\%$) compared to vehicle-treated control mice ($65\pm 11\%$). Galardin treatment restored estrous cyclicity in PNA mice with a 31% reduction of diestrus ($P<0.05$) such that cycles were indistinguishable from those of controls. Preliminary data suggest preserved effects on cyclicity after cessation of galardin treatment. This suggests inhibition of MMPs with galardin is a novel mechanism for restoration of estrous cyclicity in the PCOS phenotype. Future studies will evaluate the hormonal changes that result from MMP inhibition in these animals, if MMP inhibition alters ovarian matrix and folliculogenesis, and if pre-pubertal treatment can prevent development of the PNA phenotype.

Sources of Research Support: NIH U54 HD28934.

Nothing to Disclose: FHM, SRH, SMM

P1-347

The Reproductive Axis Retains Normal Insulin Sensitivity in the Setting of Diet-Induced Obesity and Infertility.

S Wu Ph.D¹, K Brothers BA¹, S Divall MD¹, F Wondisford Ph.D¹ and A Wolfe Ph.D¹.

¹Johns Hopkins Univ Sch of Med Baltimore, MD.

Reproductive hormone axis function is controlled by environmental, metabolic and genetic factors. In recent years, the increasing rate of obesity associated with type 2 diabetes and polycystic ovary syndrome (PCOS) has resulted in an increased prevalence of reproductive dysfunction. A fundamental and unanswered question is whether the cells of the reproductive hormone axis, located in the hypothalamus of the brain, the pituitary and the ovary, are resistant to the effects of the elevated insulin associated with obesity. Recently, our laboratory has developed a novel mouse model of diet induced obesity (DIO) that results in infertility. To investigate the relative insulin responses of reproductive and energy storage tissues, we conducted a dose response experiment in lean wild type mice treated with insulin at various doses for 10 minutes. pAKT, pERK, and total AKT were measured by a Luminex signaling assay. Pituitary and ovary pAKT were significantly elevated 1.67±0.15 and 1.83±0.03 fold, respectively, at 1.5 U/kg BW, but not at 0.5 or 1.0 U/kg BW, compared to PBS injected lean wild type mice. However, energy storage tissues such as liver, muscle and fat were more sensitive as pAKT was significantly upregulated by insulin at 0.5 U/kg BW, the lowest dose we tested. DIO mice were also examined for insulin signaling. Basal levels of pAKT in the pituitary and ovary were significantly elevated in DIO mice 1.41±0.16 and 1.36 ±0.03 fold respectively compared to age matched lean mice. No significant change in pERK levels was observed in response to insulin in either lean or DIO mice in reproductive tissues. Corresponding to the elevated basal pAKT levels in the pituitary, basal LH levels were also significantly elevated in DIO mice relative to lean controls. DIO mice exhibited an increase in pituitary and ovary pAKT levels (1.5 and 1.32-fold, respectively) in response to 1.5 unit/kg BW insulin. As in the lean mouse, the insulin induction of pituitary pAKT in the DIO mice was accompanied by a significant increase in circulating LH within 40 minutes. In these same mice we also observed a significant elevation of circulating testosterone levels in response to insulin in lean (16.12±2.12 ng/dl basal; 24.06±2.44 ng/dl stim) and DIO (16.81±1.19 ng/dl basal; 26.17±4.72 ng/dl stim) mice. These results indicate that excessive insulin signaling in pituitary and ovary, due to elevated insulin levels found in obesity, may contribute to the pathophysiology of PCOS in humans.

Sources of Research Support: Endocrine foundation grant awarded to S.Wu; National Institutes of Health (R01 HD44608 to A.W) and the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement (U54 HD58820 to A.W. and F.E.W.) as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

Nothing to Disclose: SW, KB, SD, FW, AW

P1-348

Predictors of Lipid and Lipoprotein Particle Number and Size in Women with PCOS.

Seema Sidhwani DO¹, Humberto Scoccia MD¹, Chantale Nicole Stephens BA¹, Theodore Mazzone MD¹ and Susan Sam MD¹.

¹Univ of Illinois Chicago, IL.

Polycystic Ovary Syndrome (PCOS) is associated with abnormalities in lipid profile characterized by increases in triglyceride and LDL cholesterol levels (1,2). However, analysis by NMR technique have revealed additional disturbances in lipoprotein particle number and size (3) that are strongly associated with cardiovascular disease (4,5). In addition, the role of hyperandrogenemia vs. insulin resistance in the pathogenesis of the lipid abnormalities in PCOS remains uncertain. In this study we evaluated the relationship between androgens and insulin resistance to lipid and lipoprotein abnormalities in PCOS. Fasting blood was obtained from 39 women with chronic anovulation and hyperandrogenemia fulfilling NIH criteria for PCOS (6). Lipoprotein particle size and number was determined using NMR technique.

Baseline Characteristics

| Variable | Mean ± Standard Deviation |
|-----------------------------------|---------------------------|
| Age | 28 ± 6 |
| BMI (kg/m ²) | 35.3 ± 8.5 |
| Waist (cm) | 97 ± 17 |
| Bioavailable testosterone (ng/dL) | 19 ± 12 |
| SHBG (nmol/L) | 25 ± 19 |
| HOMAIR | 3.1 ± 2.1 |

The relation between lipids, lipoprotein particle number and size and measures of adiposity, insulin resistance and androgens were examined using Spearman correlations. Higher SHBG levels were associated with higher HDL (rho=0.56, P<0.0001), lower triglyceride (rho=-0.36, P=0.03), lower VLDL particle number (rho=-0.49, P=0.003), lower LDL particle number (rho=-0.33, P=0.05), lower VLDL size (rho=-0.51, P=0.002), higher LDL size (rho=0.69, P<0.0001) and higher HDL size (rho=0.78, P<0.0001). There were no associations between bioavailable testosterone and any of the lipids or lipoprotein parameters. BMI was associated only with VLDL size (rho= 0.45, P=0.006). Waist circumference was associated only with VLDL size (rho=0.57, P<0.0001) and LDL size (rho= -0.34, P=0.04). We observed an association between HOMR IR and only VLDL particle size (rho=0.38, P=0.04). The associations between SHBG and the lipid and lipoprotein parameters remained significant even after adjustment for age, BMI and HOMR IR in general linear models. Our findings suggest that the atherogenic lipoprotein profile in PCOS is strongly associated with SHBG but not HOMA IR or bioavailable testosterone. These results suggest that SHBG is a better marker of insulin resistance in women with PCOS than HOMA IR or that another factor associated with SHBG is important in the pathogenesis of lipid abnormalities in this population.

- (1)Legro RS et al., Am J Med 2001; 111:607
- (2)Talbot E et al, J Clin Epidemiol 1998; 51:415
- (3)Garvey WT et al, Diabetes 2003; 52:453
- (4)Kuller L et al, Arterioscler Thromb Vasc Biol 2002; 22:1175
- (5)Blake GJ et al, Circulation 2002; 106:1930
- (6)Azziz R et al, J Clin Endocrinol Metab 2006; 91:4237

Sources of Research Support: NIH Grant (NIDDK) K23DK090988 awarded to Susan Sam; NIH Grant UL1RR029879 awarded to the University of Illinois at Chicago Center for Clinical and Translational Science.

Nothing to Disclose: SS, HS, CNS, TM, SS

P1-349

Differences in Lipoprotein Composition in PCOS and Healthy Control Women Matched for BMI.

Seema Sidhwani DO¹, Humberto Scoccia MD¹, Swetha Sunghay MD¹, Theodore Mazzone MD¹ and Susan Sam MD¹.

¹Univ of Illinois Chicago, IL.

Polycystic ovary syndrome (PCOS) is associated with insulin resistance and dyslipidemia characterized by raised concentrations of triglyceride and LDL cholesterol (1,2). In many insulin resistant states, Nuclear Magnetic Resonance Spectroscopy (NMR) has uncovered adverse changes in lipoprotein particle number and size not detected by conventional lipid panel (3) and associated with increased cardiovascular risk (4,5). Importantly, lipoprotein particle number and size using NMR has not been assessed in women with PCOS. We examined whether PCOS is associated with changes in these parameters independent of BMI. Fasting blood was obtained from 20 women with PCOS and 17 control women of comparable age and BMI. Lipoprotein particle number and size was examined using NMR and compared by independent *t*-test. Women with PCOS had higher WHR, bioavailable testosterone (Bio T), homeostatic model assessment of insulin resistance (HOMAIR) and lower level of SHBG compared to control women.

Baseline Characteristics

| Variable | PCOS | Control | P-Value |
|--------------------------|--------------|--------------|---------|
| Age | 27.6 ± 6.5 | 27.6 ± 6.3 | 0.9 |
| BMI (kg/m ²) | 28.5 ± 3.9 | 26.5 ± 7.4 | 0.3 |
| Waist (cm) | 84 ± 19 | 77 ± 14 | 0.06 |
| WHR | 0.79 ± 0.064 | 0.74 ± 0.056 | <0.05 |
| Bio T (ng/dL) | 19.6 ± 15.5 | 6.1 ± 2.0 | <0.0001 |
| SHBG (nmol/L) | 30 ± 23 | 43 ± 16 | <0.01 |
| HOMAIR | 2.3 ± 2.0 | 1.0 ± 0.8 | <0.01 |

Data presented mean±SD

Women with PCOS also had higher VLDL and LDL particle numbers in addition to higher plasma levels of triglyceride and total cholesterol. There were no differences in HDL particle number or any lipoprotein particle size.

Lipid and Lipoprotein Particle Number and Size

| Variable | PCOS | Control | P-Value |
|-------------------------------|-------------|-------------|---------|
| Total Cholesterol (mg/dL) | 184 ± 42 | 156 ± 44 | 0.05 |
| HDL Cholesterol (mg/dL) | 54 ± 16 | 59 ± 13 | 0.3 |
| LDL Cholesterol (mg/dl) | 109 ± 36 | 91 ± 23 | 0.07 |
| Triglyceride (mg/dL) | 106 ± 36 | 69 ± 23 | <0.01 |
| VLDL particle number (nmol/L) | 69.8 ± 43.4 | 43.2 ± 24.7 | <0.05 |
| LDL particle number (nmol/L) | 1126 ± 470 | 852 ± 254 | <0.05 |

Data presented mean±SD

We conclude that PCOS is associated with an atherogenic lipoprotein profile characterized by an increase in VLDL and LDL particle number independent of BMI. These changes are strongly linked to cardiovascular disease (4,5). Consistent with previous findings, our data confirms that PCOS is associated with abdominal obesity and insulin resistance independent of BMI.

- (1)Legro RS et al., Am J Med 2001; 111:607
- (2)Talbot E et al, J Clin Epidemiol 1998; 51:415
- (3)Garvey WT et al, Diabetes 2003; 52:453
- (4)Kuller L et al, Arterioscler Thromb Vasc Biol 2002; 22:1175
- (5)Blake GJ et al, Circulation 2002; 106:1930

Sources of Research Support: NIH Grant (NIDDK) K23DK090988 awarded to Susan Sam; NIH Grant UL1RR029879 awarded to University of Illinois at Chicago Center for Clinical and Translational Science.

Nothing to Disclose: SS, HS, SS, TM, SS

P1-350

Insulin Potentiates Androgen Receptor Activity.

T Patel MD¹, H Sun MDMS¹, S Mediwala MD^{1,2}, JE Dietrich MD¹, LP Karaviti MD¹ and M Marcelli MD^{1,2}.

¹Baylor Coll of Med Houston, TX ; ²Baylor Coll of Med Houston, TX ; ³Michael E DeBakey VA Med Ctr Houston, TX and ⁴ Baylor Coll of Med Houston, TX.

Background: Polycystic ovary syndrome (PCOS) is a common endocrinopathy, affecting 5-10% of reproductive age women. It is a heterogeneous condition, characterized by hyperandrogenemia/ clinical hyperandrogenism, ovulatory dysfunction, and infertility. A large percentage of these females are insulin resistant and suffer from metabolic alterations. A complex interplay between androgens and insulin is involved in the pathogenesis of PCOS, whereby hyperinsulinemia promotes androgen production which in turn contributes to insulin resistance. However, nearly 25% of PCOS females are found to have normal serum androgen levels despite clinical evidence of hyperandrogenism (e.g. hirsutism). In a study of 749 women with PCOS, fasting insulin levels were found to correlate most with the severity of hirsutism, independent of androgen levels¹. Similarly, a study of women with idiopathic hirsutism, hirsutism with normal androgen levels and ovulatory function, reported an association with insulin resistance². Given the data, we believe that insulin has a role in modulating the effect of androgens at the level of the androgen receptor (AR).

Hypothesis: Insulin increases the effect of androgens at the level of the AR.

Methods: HeLa cells, which endogenously express insulin receptors, were used to assess the effect of insulin on AR activity at the cellular level. Cells were transfected with AR and the AR responsive promoter ARR2PB driving the firefly luciferase reporter, and then seeded in a 96 well plate. Cells were either treated with 10 nM insulin or untreated, and stimulated with logarithmic concentrations of DHT (2×10^{-3} – 10^2 nM) for 24 hours. Relative luciferase activity (RLA) was measured and analyzed by ANOVA followed by post hoc multiple comparison analysis (Bonferroni).

Results: At low doses of DHT, treatment with insulin did not produce a significant difference in RLA. However, at physiologic and supraphysiologic doses of DHT, cells treated with insulin had a statistically significant increase in AR induced RLA ($p < 0.0001$).

Conclusions: Insulin potentiates the effect of androgens at the level of the AR. This novel finding adds a new facet to our knowledge of the complex interplay between androgens and insulin in PCOS. The exact mechanism of this interaction remains to be elucidated. Future studies dissecting the AR signaling pathway and investigating the interactions with insulin will provide an understanding of the androgenic effects seen in PCOS.

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2. Unluhizarci K, Karababa Y, Bayram F, Kelestimur, F. The investigation of insulin resistance in patients with idiopathic hirsutism. *J Clin Endocrinol Metab* 2004; 89: 2741-2744.

Nothing to Disclose: TP, HS, SM, JED, LPK, MM

P1-351

Metabolic Effects of Androgen and FSH in a Mouse Granulosa Cell Line.

J Joharatnam¹, P Sivanandarajah¹, K Hardy¹ and S Franks¹.

¹Imperial Coll London London, UK.

Insulin resistance and hyperandrogenism may contribute to the mechanism of anovulation in polycystic ovary syndrome. Impairment of glucose metabolism in granulosa cells via the PI3-K pathway is a possible mechanism contributing to subfertility. We have shown that, paradoxically, androgen augments insulin-stimulated glucose metabolism via the PI3-K pathway in a mouse granulosa cell line and in human granulosa cells. Gonadotrophins are also known to be important in the regulation of glucose metabolism of granulosa cells. In granulosa-lutein cells, LH has previously been shown to have a dose dependent effect on lactate production in both women with normal and polycystic ovaries (Rice et al. 2005). FSH stimulates glucose uptake in mouse cumulus oocyte complexes, which is significantly reduced by PI3K inhibitor, LY294002 (Roberts et al. 2004). Therefore, the effects of FSH in the presence and absence of androgen on the PI3-K pathway and MAPK pathway in KK-1 cells were explored.

Immortalised mouse granulosa cells (KK-1 cells) were transfected with the FSH receptor (FSH-R). For protein expression studies, KK1 cells were cultured in serum-supplemented medium 199 for 24 hours; medium was removed, the cells washed, then incubated in serum free medium for 8 hours. The cells were then exposed to FSH (2, 20 and 50ng/ml) with or without dihydrotestosterone (DHT, 0, 5, 10 and 25nM). Cell lysates were subjected to PAGE and Western immunoblotting was performed for phospho-AKT and phospho-p42-44MAPK in the insulin-signalling pathway. Imaging and densitometry were performed using the LICOR odyssey system. Glucose uptake and lactate production after 48h were measured in conditioned medium using a COBAS bioanalyser.

FSH increased levels of phosphorylated AKT and MAPK in a dose and time dependent manner. Androgen alone increased levels of phosphorylated AKT and glucose uptake ($p=0.0022$) and lactate production ($p<0.0001$) by the KK-1 cells, but this was not augmented by FSH addition. However, androgen did attenuate FSH-stimulated expression of phosphorylated MAPK. In summary we have characterised FSH action in a mouse granulosa cell line. FSH does activate the PI3K pathway and glucose metabolism but this is unaffected by short-term androgen exposure. Significantly, androgen alone stimulated both AKT phosphorylation and glucose metabolism.

Sources of Research Support: Wellbeing of Women.

Nothing to Disclose: JJ, PS, KH, SF

P1-352

Mechanisms for Varied Hypothalamic Progesterone Sensitivity in Hyperandrogenic Adolescent Girls: Role of Androgen Receptor CAG Repeat Polymorphism Length.

SK Blank MD¹, S Chhabra MD¹, CR McCartney MD¹, CA Eagleson MD¹, RJ Chang MD² and JC Marshall MD, PhD¹.

¹Univ of Virginia Charlottesville, VA and ²Univ of California, San Diego La Jolla, CA.

Adult women with PCOS have decreased GnRH pulse generator sensitivity to progesterone(P)-mediated slowing. This hypothalamic P insensitivity can be reversed with the androgen receptor blocker flutamide, indicating that androgens mediate the P insensitivity. Adolescent hyperandrogenism (HA) often progresses to adult PCOS and is variably associated with decreased hypothalamic P-sensitivity (1). Some HA girls have decreased P-sensitivity similar to adult women with PCOS, while others maintain P-sensitivity similar to that of adolescent normal controls (NC) despite an equivalent degree of HA. The androgen receptor (AR) gene contains a polymorphic trinucleotide (CAG) repeat sequence. CAG repeat length has been inversely correlated with transactivation of the AR, with longer CAG repeat length conferring reduced receptor activity. Some, but not all, studies have shown an association between shorter CAG repeat length and increased risk of PCOS in adult women.

To assess whether the effects of excess androgens on hypothalamic P sensitivity in adolescence are modulated by the length of the AR CAG repeat polymorphism, we measured AR CAG repeat length in 25 subjects (13 NC, 12 HA) who had undergone assessment of hypothalamic P sensitivity. DNA was isolated from peripheral blood leukocytes. The AR CAG repeat segment was amplified with PCR, and size determination made by fragment analysis. Hypothalamic P sensitivity was determined by assessing LH pulse patterns (via frequent blood sampling for 11 h) before and after treatment with oral estradiol and micronized P for 7 d. The slope of the percent reduction in LH pulses per 11 hours as a function of day 7 P concentration was used as a measure of hypothalamic P sensitivity. Girls with slopes that fell within the range seen in NC were defined as P-sensitive, while those with slopes below the normal range were defined as P-insensitive. No significant differences were seen in the AR CAG repeat lengths (biallelic, short allele, or long allele) between the HA P-sensitive subjects (n=6) and HA P-insensitive subjects (n=6) (TABLE 1).

Table 1

| | AR CAG Repeat Length | | |
|--------------------------|----------------------|-------------|----------------|
| | Short Allele | Long Allele | Biallelic Mean |
| HA - P Sensitive (n=6) | 18.2±1.5 | 23.0±1.3 | 20.6±1.3 |
| HA - P Insensitive (n=6) | 18.2±1.4 | 24.2±1.2 | 21.2±0.7 |
| Normal Control (n=13) | 19.5±0.6 | 23.6±0.6 | 21.5±0.4 |

In conclusion, AR CAG repeat length does not appear to mediate the variability in hypothalamic P-sensitivity seen in adolescent girls with HA.

(1) Blank SK et al., JCEM 2009; 94(7):2360

Sources of Research Support: NIH Grant F32 HD055014 awarded to SKB.

Nothing to Disclose: SKB, SC, CRM, CAE, RJC, JCM

P1-353**Metformin May Induce the Emergence of Luteal Body in Andronized Rats.**

RR Mahamed MD¹, CC Maganhin PhD¹, MA Haidar PhD¹, GAR Maciel MDPH², SAY Hayashida MDPH², CS Ferreira MS¹, RS Simoes MD¹, EC Baracat MD, PhD¹ and JM Soares, Jr MDPH¹.

¹Escola Paulista de Med Sao Paulo, Brazil and ²Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

Objective: to evaluate the histological changes of metformin treatment effects on ovaries of testosterone-induced permanent estrous rats. Design: 20 rats submitted to testosterone treated after five days of born. After three months of life, the animals were randomly divided into two groups: GI (n=10)- received 0,5 ml of water (vehicle) and GII (n=10) - received metformin (7.0 mg/kg/per day). The length of treatment was 30 days through gavage. At the end of treatment, all animals were sacrificed and ovaries were submitted to histological routine. Also, we collected blood for fasting glucose and insulin determination. The 5 um histological sections were stained by HE and analyzed under light microscope for histometrical analysis. The data were statistical evaluated using ANOVA and Tukey tests. Results: The number of ovarian cysts, atresic follicles and interstitial cells of GI were statistically superior compared to GII (p<0.01). In GI, we did not identified luteal bodies in any of animals. In GII, we found luteal body in 50% of animals (n=5). The metformin determine a reduction in fast insulin.

Tabel 1 – The biochemical and weight after the treatment in both groups.

| Variável | GI | GII | P |
|--------------------------|---------------|--------------|--------|
| Fasting Glucose (mmol/L) | 6,86 ± 0,86 | 5,72 ± 0,49 | 0,058 |
| Fasting Insulin (mU/mL) | 36,29±14,60* | 26,16±14,34 | 0,002 |
| HOMA IR | 11,06 ± 1,39* | 6,66 ± 0,57 | 0,0001 |
| Weight (g) | 263,20±18,28 | 264,50±23,09 | 0,004 |

HOMA = Homeostatic Model Assessment.

Conclusion: the metformin may induce the emergence of luteal body in andronized rats with permanent estrous (chronic anovulation).

Nothing to Disclose: RRM, CCM, MAH, GARM, SAYH, CSF, RSS, ECB, JMS

P1-354

Effect of Rosiglitazone on Circulating Adiponectin and Leptin Levels in Chinese Polycystic Ovary Syndrome Women with and without Peroxisome Proliferator-Activated Receptor γ 2 Gene C1431T Polymorphism.

Tao Tao MD^{1,2}, Wei Liu PHD¹, ShengXian Li MD¹, Xiuying Mao MD¹ and Jiejun Yang MD¹.

¹Shanghai Renji Hosp affiliated Shanghai Jiaotong Univ Sch of Med Shanghai, China and ²Med Coll of Virginia, Virginia Commonwealth Univ Richmond, VA.

Context: Genetic factors and hyperinsulinemia/ insulin resistance are involved in the pathogenesis of PCOS. Rosiglitazone, a PPAR γ 2 agonist, is known to improve the endocrine and ovulatory function of most women with PCOS. Adiponectin and leptin are two abundant adipokines with insulin sensitizing properties. However, the effect of PPAR γ 2 gene polymorphisms on serum concentrations of these adipokines on in women with PCOS has not been established.

Objective: To determine whether the presence of the C1431T polymorphism of the PPAR γ 2 gene influences the effects of rosiglitazone treatment on insulin sensitivity and serum levels of adipokines in women with PCOS .

Design: One hundred PCOS women, including 50 women with the C1431T polymorphism of the PPAR γ 2 gene and 50 women without C1431T polymorphism of the PPAR γ 2 gene , were studied before and after six months of treatment with rosiglitazone at a dose of 4 mg daily.

Main Outcome Measure(s): Insulin sensitivity was estimated using the homeostasis assessment insulin resistance index (HOMA-IR). Circulating adiponectin and leptin were determined by ELISA and RIA, respectively .

Results: (1) Rosiglitazone treatment significantly lowered circulating leptin (20.01 ± 4.8 vs 17.38 ± 6.81 ng/ml, $P < 0.05$) in women with CT or TT genotype; but it had little or no effect in women with CC genotype (14.26 ± 4.3 vs 14.61 ± 6.94 ng/ml, $P > 0.05$).

(2) Circulating adiponectin increased significantly (16.25 ± 6.27 vs 24.26 ± 7.36 ug/ml, $P < 0.05$) in women with CT or TT genotype after rosiglitazone treatment, but remained unchanged in women with CC genotype (23.12 ± 5.18 vs 24.01 ± 7.28 ug/ml, $P > 0.05$)

(3) Rosiglitazone treatment lowered HOMA-IR (3.28 ± 1.24 vs 2.59 ± 1.71 ; 2.81 ± 1.95 vs 2.21 ± 1.67) in both PCOS women with and without the CT or TT genotype.

Conclusion: Our findings demonstrate that rosiglitazone treatment improved the insulin resistance in PCOS women in China with and without the C1431T polymorphism in the PPAR γ 2 gene. Our data further suggest that these polymorphisms might influence the circulating levels of leptin and adiponectin in Chinese women with PCOS at baseline and after treatment with rosiglitazone. Future consideration of rosiglitazone as a therapeutic remedy for women with PCOS merits further investigation.

Sources of Research Support: Shanghai Science and Technology Development Fund(08411953000).

Nothing to Disclose: TT, WL, SXL, XM, JY

P1-355

Effect of Some Metabolic Modulators on 11beta-Hydroxysteroid Dehydrogenase (11b-HSD) Activity and mRNA of Rats with Polycystic Ovary.

MA Corona-Figueroa CsM¹, JM Malacara-Hernandez MD¹, G Barbosa-Sabanero PhD¹, EL Perez-Luque PhD¹ and K Wrobel-Zasada PhD¹.

¹Univ de Guanajuato León, Mexico.

Introduction: The polycystic ovary syndrome (PCOS) is a common endocrinopathy that affects 7-10% of the reproductive-aged females, best described as unexplained hyperandrogenic chronic anovulation; which implies other complications besides the related to infertility. Some cases present hyperinsulinemia, insulin resistance and obesity. It has been considered alterations of 5 α -reductase, 11 β -hydroxysteroid dehydrogenase(11 β -HSD), 17 β -hydroxysteroid dehydrogenase and insulin as mechanisms of the disease. At date its origin is unknown and its treatment is not well established.

Objective: To evaluate if the 11 β -HSD participates in PCOS and how it is affected by different metabolic modulators in a rat model.

Material and methods: Polycystic ovary disease was induced administering IM estradiol valerate (4 mg/0.3 ml oil) to adult Wistar females rats. The development of polycystic ovary was histologically evaluated. Different drug was assigned to each group of rats (n=6): **metformin(M)**, **rosiglitazone(R)**, **carbenoxolone(CBX)**, and **GW9662(GW)** (irreversible PPAR γ antagonist); a fifth group was only induced to polycystic ovary disease without extra treatment (**P**); sixth group was **control** group(**C**), without polycystic ovary disease and without treatment. After 16 days of treatment ovaries were obtained to evaluate 11 β -HSD1 activity by UV HPLC method and 11 β -HSD1 and 2 mRNA levels by RT-PCR technique. The data were analyzed by ANOVA or Kruskal Wallis with Statistic 6.0 program and a p value <0.05 was considered to be significant.

Results: The 11 β HSD1 activity (3.79 \pm 0.63%) and mRNA levels (0.84 \pm 0.17) were reduced in **P** group respect to the **C** group (4.67 \pm 0.6%* and 1.0 \pm 0.0 respectively). **R** group showed the highest inhibitory effect on 11 β HSD1 activity (1.32 \pm 0.2%) and mRNA levels (0.47 \pm 0.09). **CBX** increased 11 β HSD1 activity (6.54 \pm 0.97%*) and mRNA levels (1.58 \pm 0.13). The **C** and **CBX** groups showed similarity for 11 β HSD1 activity and mRNA levels. **M** group activity (3.63 \pm 0.5%) and mRNA levels(0.62 \pm 0.2) were reduced. **P** group showed 11 β -HSD2 mRNA levels increased (2.07 \pm 0.9) respect to the **C** group (1.0 \pm 0.0). The 11 β -HSD2 mRNA was increased by **GW9662** (6.71 \pm 2.3) action and decreased by **CBX** action (1.85 \pm 0.53). *p< 0.05 vs **R** group.

Conclusion: These findings open new implications on the 11 β HSD participation in PCOS pathology and treatment and suggest 11 β -HSD1 could be a necessary element in the ovarian normal physiology.

Sources of Research Support: Direccion de Investigacion y Postgrado, Universidad de Guanajuato.

Nothing to Disclose: MAC-F, JMM-H, GB-S, ELP-L, KW-Z

P1-356

Impact of Free Fatty Acids on Androgen Biosynthesis in Normal Human Fasciculata Cells.

CG Baptiste MSc¹, MC Battista PhD¹, S Bellanger MSc¹ and JP Baillargeon MD, MSc¹.

¹Univ of Sherbrooke Sherbrooke, Canada.

BACKGROUND: Hyperandrogenemia of both adrenal and ovarian origin is one of the main features of the polycystic ovary syndrome (PCOS). PCOS women often show insulin resistance and increased circulating free fatty acid (FFA) levels. It was suggested that FFA accumulation may stimulate androgenic biosynthesis following either direct alterations or through the insulin signalling pathway. Based on these findings, we hypothesized that increased *in vitro* exposure of human adrenal fasciculata cells to FFA would increase their androgenic secretion and response to ACTH stimulation. We also hypothesized that increased intra-cellular metabolism of FFA following treatment with a PPAR γ agonist, in combination with high FFA exposure, would restore normal androgenic biosynthesis.

METHODS: In order to test our hypothesis, human primary fasciculata cells were cultured and either left untreated (control) or treated for 3 days with palmitate (FFA; 100 and 200 μ M, twice daily) in the absence or presence of ACTH (10nM, twice daily) or rosiglitazone (1 μ M, once daily; a PPAR γ agonist). After 3 days in culture, cell count was performed using a hemocytometer (to estimate toxicity), DHEA secretions were measured by radioimmunoassay and corrected for cell count, while P450c17 expression (key steroidogenic enzyme involved in DHEA production) was evaluated by western blot.

RESULTS: For each experiment (\pm ACTH, \pm rosiglitazone), palmitate exposure did not alter cell counts after 3 days. Fasciculata cells exposed to palmitate did not show any modification with regard to DHEA secretion and P450c17 expression when compared to untreated cells. However, while ACTH stimulation increased both DHEA secretion and P450c17 expression, these responses were further potentiated following combination with palmitate. Rosiglitazone alone did not impact on any parameter. However, the addition of rosiglitazone to ACTH/palmitate stimulation partially normalized DHEA response and P450c17 expression to levels observed with ACTH alone.

CONCLUSION: These preliminary results suggest that under ACTH stimulation, fasciculata cells produce more androgens when exposed to FFA. Moreover, results obtained with rosiglitazone suggest that intra-cellular FFA metabolism or the insulin pathway may be involved in this FFA-induced hyperandrogenism. Finally, these results suggest that FFA may play a role in PCOS hyperandrogenemia by exacerbating androgen production from androgen secreting cells.

Sources of Research Support: Canadian Institutes of Health Research (MOP-97965). JPB is Junior 2 clinical investigator of the Fonds de Recherche en Santé du Québec (#12131).

Nothing to Disclose: CGB, MCB, SB, JPB

P1-357

Insulin Resistance and Oxidative Stress in Obese PCOS, Nonobese PCOS and Controls.

LW Cho MD^{1,2}, ES Kilpatrick MD³, J Shepherd³, AM Coady MD³ and SL Atkin MD¹.

¹Univ of Hull Hull, UK ; ²Changi Gen Hosp singapore, Singapore and ³Hull Royal Infirmary Hull, UK.

Hyperglycemia-induced oxidative stress is thought to be central to the development of micro- and cardiovascular complications in diabetes. Polycystic ovary syndrome (PCOS) is associated with insulin resistance and is also thought to be associated with increased cardiovascular risk. This study has compared malondialdehyde (MDA) concentrations, an established biomarker for oxidative stress, in obese and nonobese women with PCOS as compared to controls to see if insulin resistance alone is associated changes in free radical formation.

Eleven nonobese [BMI 22.7±0.13 kg/m² (mean ± SEM)] and 34 obese [BMI 36.0±1.2 kg/m²] PCOS women were recruited using the Rotterdam criteria for diagnosis of PCOS. Diabetes was excluded by a 75g oral glucose tolerance test. Twelve women with normal menstrual cycle and free androgen index were recruited as controls [BMI 29.0±1.4 kg/m²]. Fasting serum were taken at the same time each day on two occasions at 1 month apart for measurement of insulin, glucose and MDA and mean of the two values used for calculations. HOMA-IR was calculated from formula [(insulin x glucose)/22.5]. All subjects gave their informed consent prior to entering the study and local ethical committee approval had been obtained.

Non parametric distribution was assumed and non parametric calculations adopted. HOMA-IR was significantly higher in obese compared to nonobese PCOS [3.80(2.80-5.51) vs 1.64(1.30-1.93)], p<0.0001 but no significant difference in MDA level was noted [0.81(0.68-1.04) vs 0.85(0.64-0.99)], p=1.0. There was also a significant difference in HOMA-IR between obese PCOS and controls, p<0.0001 and again no significant difference in MDA p=0.556. No significant difference was noted in both HOMA-IR and MDA between nonobese PCOS and obese controls. No significant correlation was observed between MDA and HOMA-IR.

Oxidative stress, measured by MDA, in these obese and nonobese PCOS and controls were unchanged despite significant difference in HOMA-IR. It suggests that insulin resistance in the absence of hyperglycemia is not associated with increased free radical formation. In turn, any excess cardiovascular risk in this group of patients must be through mechanisms independent of oxidative stress.

Sources of Research Support: University of Hull.

Nothing to Disclose: LWC, ESK, JS, AMC, SLA

P1-358

Abnormal Glucose Transporter Isoform Type 4 (GLUT4) Expression in Adipocytes of Women with Polycystic Ovary Syndrome (PCOS).

Yen-Hao Chen PhD¹, Jung Lee MD^{1,3}, Gregorio Chazenbalk PhD^{1,2} and Ricardo Azziz PhDMD, MPH, MBA^{1,2}.

¹Cedars Sinai Med Ctr Los Angeles, CA ; ²The David Geffen Sch of Med at UCLA Los Angeles, CA and ³The Catholic Univ of Korea Seoul, Korea.

Introduction:

PCOS is a common endocrine-metabolic abnormality of women, and ~70% of affected women demonstrate clinically measurable insulin resistance above and beyond that determined by their degree of obesity. The glucose transporter type 4 isoform (GLUT4) plays a pivotal role in insulin-induced glucose uptake in adipocytes. In the present study, we have hypothesized that GLUT4 expression is abnormal in adipocytes of PCOS vs. matched controls.

Methods: Lower abdominal subcutaneous adipose tissue was obtained from 13 PCOS subjects and 10 body mass index (BMI)-matched controls. The tissue was digested with collagenase, filtered and centrifuged to isolate adipocytes. Insulin-stimulated 3-O-methyl glucose transport, GLUT4 protein and RNA expression were determined in adipocytes following standard protocols.

Results: Insulin-stimulated glucose transport was significantly decreased in PCOS vs. controls. In controls, GLUT4 protein expression was significantly higher in non-obese vs. obese subjects (4.2 fold, $p < 0.03$). GLUT4 protein expression was significantly lower in adipocytes from non-obese PCOS vs. non-obese controls (2.8 fold, $p < 0.02$); however, there were no significant differences in GLUT4 protein expression between non-obese and obese PCOS women. Real-time PCR indicated no difference in GLUT4 gene expression between non-obese PCOS and matched controls. There were also no differences in the gene expression of transcription factors which regulate GLUT4 expression, including PPAR- α , C/EBP α , and SREBP-1.

Conclusions: These results demonstrate a lower constitutive level of GLUT4 protein expression in non-obese PCOS that could in part, account for the insulin resistance present in non-obese PCOS patients. The decreased GLUT4 protein content identified in non-obese PCOS adipocytes does not result from altered gene expression, and may instead result from differences in mRNA translation or in the stability of the GLUT4 protein. Finally, the absence of a difference in GLUT4 levels in adipocytes between obese PCOS and matched controls suggests that different mechanisms may regulate GLUT4 expression in obese and non-obese PCOS individuals.

Sources of Research Support: R01-DK073632 (to RA), M01-RR00425, and the Helping Hand of Los Angeles, Inc.

Nothing to Disclose: Y-HC, JL, GC, RA

P1-359

Peroxisome Proliferator-Activated Receptor γ 2 Gene C1431T Polymorphism in Chinese Women with Polycystic Ovary Syndrome and Relation to Serum Adiponectin and Leptin.

T Tao MD^{1,2}, W Liu MD,PHD¹, JJ Yang MD¹, SX Li MD¹ and XY Mao MD¹.

¹Shanghai Renji Hosp Affiliated Shanghai Jiaotong Univ Sch of Med Shanghai, China and ²Virginia Commonwealth Univ Richmond, VA.

Context: Genetic factors and hyperinsulinemia/ insulin resistance are involved in the pathogenesis of PCOS. The PPAR γ 2 gene has been identified as a candidate gene for PCOS. Adiponectin and leptin are two abundant adipokines with insulin sensitizing properties, and alterations in serum levels of adiponectin and leptin and their relationship with PPAR γ 2 gene polymorphisms in women with PCOS remain unclear.

Objective: To determine whether the prevalence of the C1431T polymorphism of the PPAR γ 2 gene is increased in women with PCOS women, and to assess the relationship of this PPAR γ 2 polymorphism with serum levels of adiponectin and leptin .

Design: Cross-sectional study involving 200 women with PCOS and 150 normal women from Han Province in China.

Main Outcome Measure(s): The PPAR γ 2 gene C1431T polymorphism was determined by sequence analysis of DNA . Circulating adiponectin and leptin levels were determined by ELISA and RIA, respectively.

Results: (1) The frequencies of CT genotype (0.19 vs 0.14) and the T allele (0.155 vs 0.10) were increased in the PCOS group compared with the control group (P<0.05).

(2) In the subgroup of women with CT or TT genotype, those with PCOS had an increased BMI (33.3 \pm 5.4 vs 26.5 \pm 6.2 kg/m²) and leptin (20.0 \pm 4.8 vs 16.2 \pm 4.7 ng/ml) than the control women (P<0.05).

(3) Among women with PCOS , those with the CT or TT genotype had increased BMI (33.3 \pm 5.4 vs 28.3 \pm 5.1 kg/m²), HOMA-IR (3.28 \pm 1.24 vs 2.81 \pm 1.95), leptin (20.01 \pm 4.8 vs 14.26 \pm 4.3ng/ml) and decreased adiponectin (16.25 \pm 6.27 vs 23.12 \pm 5.18 ug/ml) (P<0.01) compared with the CC genotype. However, there were no significant differences in these parameters in the group of control women.

Conclusion: Women with PCOS had a higher prevalence of the C1431T polymorphism in the PPAR γ 2 gene than normal women from a population of Han Chinese. In women with PCOS the presence of this polymorphism was associated increased BMI, HOMA-IR and serum leptin and decreased serum adiponectin. This effect of the polymorphism might be unique to PCOS since it was not observed in control women.

Nothing to Disclose: TT, WL, JJY, SXL, XYM

P1-360

LHB Gene Analysis in Brazilian Women with Polycystic Ovary Syndrome.

A Lofrano-Porto MD, PhD^{1,2}, GB Barra PharmD, PhD³, MDR Borba MD², JP Mangussi-Gomes², P Godoy³, BF Luitgards², VM Caldas², AJR Queiroz², LDCR Motta MD, PhD⁴, LA Casulari MD, PhD² and FAR Neves MD, PhD¹.

¹Fac of Hlth Scis, Univ of Brasilia Brasilia, Brazil ; ²Ambulatory of Gonadal and Adrenal Diseases, Univ Hosp of Brasilia Brasilia, Brazil ; ³Sabin Lab and Inst Brasilia, Brazil and ⁴Fac of Med, Univ of Brasilia Brasilia, Brazil.

Abnormalities at different levels of the gonadotrophic axis, including those involving LH secretion or action, have been implicated on the pathogenesis of PCOS. The roles of *LHB* gene polymorphisms have been studied, however, their functional and clinical significance are still controversial. We aimed to compare the distribution of *LHB* polymorphisms in Brazilian women with PCOS and controls, and its association with clinical parameters. 43 PCOS and 41 control women were included. PCOS was defined in accordance with Rotterdam criteria. Weight, height, BMI, Ferriman-Gawley index, FSH, LH, testosterone levels, pelvic ultrasonography were analysed. Genomic DNA was extracted from blood leucocytes and the region spanning exon 2, intron 2 and exon 3 of *LHB* was sequenced. Haplotype and allelic frequencies for each polymorphism were determined for both groups, as shown in tables 1 and 2.

Table 1

| SNP | CONTROLS n (%) | | PCOS n (%) | | p | nucleotide change |
|------------|----------------|----------|------------|----------|----|-------------------|
| | Allele 1 | Allele 2 | Allele 1 | Allele 2 | | |
| RS34247911 | 80 (98) | 2(2) | 85(99) | 1(1) | NS | G/A |
| RS35270001 | 79(96) | 3(4) | 82(95) | 4(5) | NS | T/C |
| RS34349826 | 79(96) | 3(4) | 82(95) | 4(5) | NS | T/C |
| RS6521 | 50(61) | 32(4) | 47(55) | 39(45) | NS | C/G |
| RS1056914 | 54(66) | 28(34) | 47(55) | 39(45) | NS | A/C |
| RS2387588 | 57(70) | 26(30) | 50(58) | 36(42) | NS | C/T |
| RS4287687 | 57(70) | 26(30) | 50(58) | 36(42) | NS | A/- |
| C1430A* | 81(99) | 1(1) | 84(98) | 2(2) | NS | C/A |
| RS1056917 | 56(68) | 26(32) | 54(63) | 32(37) | NS | C/T |

Table 2

| HAPLOTYPE | TOTAL % | CONTROLS % | PCOS % |
|-----------|---------|------------|--------|
| GTTGCCTCC | 50,58 | 53,66 | 48,84 |
| GTTCATACT | 32,58 | 29,22 | 34,75 |
| GTTCCCTCC | 6,55 | 8,54 | 4,65 |
| GCCGACTCC | 3,73 | 3,66 | 3,62 |
| GTTCATACC | 1,79 | 0 | 3,49 |
| ATTGCCTCC | 1,79 | 2,44 | 0 |
| GTTCATAAT | 1,34 | 1,22 | 1,3 |
| Other (5) | 1,63 | 1,26 | 3,35 |
| | 100 | 100 | 100 |

We found 8/12 *LHB* variants described in pubmed database. A new mutation was identified in exon 3, in heterozygosity, in 3 PCOS and 1 healthy woman (*C1430A). This mutation would theoretically generate a T78N change in the LHB protein, making it similar to hCG-beta at this position. No significant association was noted between genotype and clinical parameters in these group of women. CONCLUSION: We describe a novel LH variant C1430A, which is probably rare. Other studies are necessary to clarify the resulting T78N effect on LHB secretion and/or action. The RS 35270001/34349826 (Trp8Arg/Ile15Thr) was not very common both in the PCOS and control group. Our still ongoing studies with a larger sample should adress these issues.

Nothing to Disclose: AL-P, GBB, MDRB, JPM-G, PG, BFL, VMC, AJRQ, LDCRM, LAC, FARN

P1-361

Corpus Luteum Production of Gonadotropin Releasing Hormone (GnRH) Regulates Luteal Responsiveness to Prolactin in the Estrous Cycle of Rats.

T Yonezawa Ph. D.¹, K Sakata¹, S Kurusu Ph. D.¹ and M Kawaminami Ph. D.¹.

¹Kitasato Univ Towada, Japan.

We have already reported that local GnRH, expressed in the corpus luteum, induces luteal regression in the afternoon of diestrus 2 (D2) of rats. When GnRH antagonist, Cetrorelix, was administered into ovarian bursa with osmotic mini-pumps from estrus, prolactin-reactive period of newly formed corpus luteum was prolonged. In this study, we hypothesized that GnRH would not only promote apoptosis of luteal cells but also suppress their responsiveness to prolactin. First, the effect of GnRH on prolactin- responsiveness of the corpus luteum was determined. GnRH agonist (GnRH_a, Des-Gly(Pro9)-GnRH Ethylamide) administration into ovarian bursa from estrus induced earlier refractoriness to prolactin. The effect of GnRH on the anti-apoptotic action of prolactin was examined with the primary culture of corpora lutea. After 24-h pretreatment with GnRH_a or Cetrorelix, prolactin was added to the medium for 48 h. Although cleaved caspase 3 levels were inhibited by prolactin in Cetrorelix-pretreated cells, its effect was not observed by GnRH_a-pretreatment. To see the effect of prolactin on steroidogenesis, 3 β -hydroxysteroid dehydrogenase (HSD) transcription and progesterone levels were measured. Although 3 β -HSD transcription and progesterone levels were increased by prolactin in the Cetrorelix-pretreated group, its action was not observed by GnRH_a. As these effects could be caused by alteration of prolactin signaling, we determined transcription levels of prolactin receptors in the corpus luteum. During the estrous cycle, the long form of prolactin receptor was increased in corpora lutea especially in the afternoon of D2, similarly to the pattern of luteal GnRH expression, while the short form was rather decreased. GnRH_a administration into ovarian bursa stimulated transcriptional activity of prolactin receptor long form, but reduced that of short form. These results demonstrate that GnRH exerts multiple suppressive effects on progestational function of corpus luteum during estrous cycle. It is suggested the suppressive effects were attained by modifying the responsiveness of corpus luteum to prolactin at least partly through prolactin receptor.

Nothing to Disclose: TY, KS, SK, MK

P1-362

Y Chromosome Mosaicism in 45, X Turner Syndrome.

M Ahmed MD¹, H Y AlHashem MBBS¹, A Rahmatullah MBBS¹ and C Walter Ph D¹.

¹King Faisal Specialist Hosp and Res Ctr Riyadh, Saudi Arabia.

Introduction

Turner syndrome(TS) is caused by X-chromosome monosomy (45,X) & is frequently ass'td w/chro. mosaicism. In addition to 45,X cell line, mosaic karyotypes have abnormal cell line(s) w/either X or Y chro. abnormalities. Y chro. mosaicism (YCM) in 45,X TS has been described in up to 60% pts. Y-specific sequences are described in 7%TS pts. A dic(Yq) is the most common structural Y chro. abnormality & virtually all 46,X,dic(Y) karyotypes are found in mosaicism with 45,X. Discrepancy of individual cell lines between lymphocytes & gonadal tissue is common & remains often undetected. The phenotype of pts. w/mosaicism depends on the individual proportions of gonadal cell lines as shown by cytogenetics, FISH & molecular studies. Detection of Y material in TS is important, since it is a risk factor for the development of gonadoblastoma.

Clinical Cases

In our series, 35 TS pts were randomly selected. 7 (20%) pts. with YCM were identified & are presented to identify frequency of Y-material & clinical implications. Karyotyping & FISH was done on lymphocytes. 2 were 45,X/46,X, idic(Y), 1 was 45,X/46,X, der(X), & 4 were 45,X/46,XY. 5 had male phenotype, 1 had female phenotype & 1 ambiguous phenotype. 2 had Mullerian duct derivatives but no gonads. Three had rudimentary testicles & 1 of these had anlagen of the Wolffian/Mullerian ducts, 1 had mixed gonadal dysgenesis & 1 had Coarc. aota.

Conclusion

20% TS pts had Y chromosome mosaicism. Cytogenetics & FISH to detect Y material should be part of the work-up of all TS pts. It helps to understand phenotype variability & therefore has a fundamental role in the clinical management of TS patients.

Pts.' characteristics

| PT. | Age (yrs) | Sex/Phenotype | FISH | Chromosomal Analysis | Imaging Findings |
|-----|-----------|---------------------------------------|---------------------------|---|---|
| 1 | 4 | M/Amb. genitalia, scrotal hypospadias | X, SRY-(25%)/X, SRY+(75%) | 45,X[5]/46,X, idic(Y) [15] | Hypoplastic uterus, No gonads |
| 2 | 2 | M/scrotal hypospadias | X, SRY-(40%)/X, SRY+(60%) | 45,X[4]/46,X, idic(Y) [16] | Scrotal testes & Wolffian duct derivatives |
| 3 | 40 | M/Azoospermia | X, SRY-(70%)/X, SRY+(30%) | 45,X[19]/46,X, der(X)t(X;Y)(q13;q11.2)[1] | ND |
| 4 | < 1 | M/scrotal hypospadias | ND | 45,X[5]/46,XY[45] | Mullerian & Wolffian derivatives / immature testicle (Bx) |
| 5 | < 1 | M/Amb. genitalia | ND | 45,X[6]/46,XY[14] | Absent Mullerian duct structures / scrotal gonads |
| 6 | 11 | Ambiguous/Amb. genitalia | ND | 45,X[8]/46,XY[12] | Mullerian derivatives present, Mixed gonadal dysgenesis |
| 7 | 8 | F | ND | 45,X[4]/46,XY[16] | Hypoplastic uterus, No gonads |

ND=not done; Amb.=Ambiguous

Nothing to Disclose: MA, HYA, AR, CW

P1-363

Phenotype Variability in Turner Syndrome Patients: Clinical, Cytogenetic and Molecular Genetic Correlation.

M Ahmed MD¹, HY Alhashem MD¹, AA Rahmatullah MD¹ and C Walter MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Introduction:

Turner syndrome (TS) with 45, X is the only viable monosomy syndrome in humans. It is due to complete or a partial loss of one of the two sex chromosomes. About 98% of TS embryos either fail to implant or get miscarried. TS has a clinically variable phenotype, & varying cytogenetic & molecular features.

Clinical Cases:

We report karyotypic and phenotypic correlations in 28 TS pts including hormonal, pelvic imaging findings and associated systemic anomalies, fertility status, and treatment response. Three different karyotypic groups were seen: (Group 1: 21 pts) 45, X, (Group 2: 6 pts) 45, X/46, XX mosaicism, & (Group 3: 1 pt.) 45, X/46, XX/47, XXX mosaicism. Group 1: Age <1-33 yrs. FISH in 3 pts confirmed absence of SRY gene. All adolescent girls had classical TS features, and primary amenorrhea. In all cases studied (n=14), Bone age done in 14 showed retardation, pelvic imaging done in 9 showed hypoplastic uterus in 5, undetectable Mullerian anlagen in 4, streak gonads in 6, no detectable gonads in 3, primary hypogonadism in 11 with complete data, & primary hypothyroidism in 7. Nine received combination of HRT & GH, 3 had HRT only & other 3 GH only. Height improved significantly in 9, menses were established in 8 & satisfactory breast development occurred in 3. Associated systemic conditions consisted of coarctation aorta in 3, horse-shoe kidney in 1, Hodgkin's lymphoma in a 15-yr. old girl, virilizing adrenal tumor in a 3-yr. old child for which presence of Y material was not assessed & 1 had mitral valve prolapse.

Group 2: Age 28-43 yrs. FISH done in 5 confirmed 45, X/46, XX mosaicism. None had TS stigmata nor amenorrhea. Spontaneous pregnancy occurred in 4. However, hypogonadism was present in 2. & Premature ovarian failure occurred in 1. Group 3: The 40-year-old 45, X/46, XX/47, XXX mosaicism pt had full female phenotype, a normal pituitary-ovarian axis, but later developed premature ovarian failure & received HRT.

Conclusions:

TS pts showed variable clinical, hormonal, anatomic, reproductive, and systemic features depending on their karyotypic/molecular genetic findings. Those with the 45, X findings were younger, more severely affected & needed Rx with HRT/GH/thyroxine. Clinical findings in those with 45, X/46, XX or 45, X/46 XX/47, XXX karyotypes ranged from milder abnormalities to normal females with recurrent pregnancies & may not be easily diagnosed without routine karyotyping and FISH.

Nothing to Disclose: MA, HYA, AAR, CW

P1-364

Socio-Economic Factors and Mortality in Turner Syndrome.

K Stochholm MD, PhD¹ and CH Gravholt MD, PhD¹.

¹Aarhus Univ Hosp Aarhus, Denmark.

Hypothesis and aim: Turner syndrome affects 1 in 2,000 liveborn girls, and is characterized by hypogonadism, failure to reach expected adult height, and increased mortality. In Turner persons a few questionnaire surveys identified quality of life, perception of health and education to be at a similar or higher level compared to the background population. To shed further light on these seemingly paradoxical findings, we identified all diagnosed Danish Turner persons, and analyzed socio-economic parameters as marital status, education, retirement, income, unemployment, and children, compared to an age-matched female background population.

Methods: All diagnosed Turner syndrome women (n=977) were compared with an age-matched cohort of the female background population (n=94,850) identified in Statistics Denmark. The socio-economic parameters were related to age and time of diagnosis. Furthermore, we analyzed mortality adjusted for marital status and education.

Results: Our major finding was significantly different socio-economic parameters in Turner persons, only unemployment was comparable to controls. Being diagnosed with Turner syndrome had a clear association with marital status and retirement. In Turner persons, the educational level was significantly higher, whereas income and the number of children were significantly reduced. All-cause mortality adjusted for gender and age and calendar time was significantly increased with a hazard ratio (HR) of 3.2 (95% confidence interval: 2.6-3.9). Further adjusted for socio-economic parameters, the HR only changed little.

Conclusion: As previously reported, the mortality among Turner persons was increased; it did not change when adjusted for socio-economic parameters. Albeit the number of children born to Turner women was significantly reduced, it was surprisingly high.

Sources of Research Support: Kirstine Stochholm was supported by a research grant from Central Denmark Region; Kirstine Stochholm was supported by a research grant from the Danish Ministry of Science, Technology and Innovation.

Nothing to Disclose: KS, CHG

P1-365

Screening for Tissue-Specific Mozaicism and Y-Chromosomal Derivates in Turner Syndrome.

K Freriks MD¹, DFCM Smeets PhD¹, RT Netea MD,PhD¹, CCM Beerendonk MD,PhD¹, MAF Traas MD¹, H Mieloo¹, WHJFL van de Zande¹, ARMM Hermus MD,PhD¹ and HJLM Timmers MD,PhD¹.

¹Radboud Univ Nijmegen Med Ctr Nijmegen, Netherlands.

INTRODUCTION

Turner syndrome (TS) is the result of complete or partial absence of one X-chromosome. Besides short stature and gonadal dysgenesis, girls and women with TS are prone to a wide range of disorders, including cardiovascular abnormalities, the metabolic syndrome and autoimmune diseases. The phenotypic variability is partly related to differences in underlying genotypes, including tissue-specific mozaicisms and the presence of Y-chromosomes or derivatives thereof. The latter may be associated with an increased risk of gonadoblastoma.

AIM

The aim of this study was to investigate the yield of screening for tissue-specific mozaicism and Y-chromosomal material in patients with an apparent monosomy (45,X) on conventional karyotyping.

MATERIALS AND METHODS

We investigated 57 women (mean age 29.2±10.5 years) with TS who underwent evaluation at the multidisciplinary TS clinic of the Radboud University Nijmegen Medical Centre between 2005 and 2009. They were selected on the basis of an apparent 100% monosomy 45,X on standard karyotyping of ≥30 blood lymphocytes. Buccal smears were collected to perform fluorescent in situ hybridization (FISH) with X- and Y-specific centromeric probes and an additional probe of the SRY-region. In a subgroup of 28 randomly selected patients, blood samples were analyzed by PCR-Y (sY84, sY86, sY127, sY134, sY254, sY255). In 12 additional patients with mozaicism in blood lymphocytes, the same investigations were performed.

RESULTS

In patients with 100% monosomy 45,X on conventional karyotyping, buccal smear analysis revealed a tissue-specific mozaicism in 31.6% (n=18): 45,X/46,XX (n=11), 45,X/47,XXX (n=1), 45,X/46,XX/47,XXX (n=1) and 45,X/46,XY or 45,X,derivative Y (n=5). In 3 of the 5 Y-positive cases prophylactic gonadectomy was performed subsequently. FISH of the gonadal cells was positive for Y; pathology results revealed no gonadoblastoma.

PCR-Y analysis (n=28) was negative in all cases, including those which were Y-positive on FISH of buccal cells.

Lymphocyte mozaicisms (n=12) were all confirmed in buccal cells, although sometimes in different proportions.

CONCLUSION

Sex chromosome-specific FISH studies on buccal cells should be included as routine investigation in TS. It yields clinically relevant additional information on tissue-specific mozaicisms, including the presence of Y-derivates.

Nothing to Disclose: KF, DFCMS, RTN, CCMB, MAFT, HM, WHJFLvdZ, ARMMH, HJLMT

P1-366

The Yield of Multidisciplinary Evaluation of Adult Women with Turner Syndrome in the Netherlands.

K Freriks MD¹, RT Netea MD, PhD¹, CCM Beerendonk MD, PhD¹, J Timmermans MD¹, CM Verhaak PhD¹, BJ Otten MD, PhD¹, DFCM Smeets PhD¹, HPM Kunst MD, PhD¹, ARMM Hermus MD, PhD¹ and HJLM Timmers MD, PhD¹.

¹Radboud Univ Nijmegen Med Ctr Nijmegen, Netherlands.

INTRODUCTION

Turner syndrome (TS) is the result of complete or partial absence of one X-chromosome. Besides short stature and gonadal dysgenesis TS is associated with abnormalities affecting nearly every organ system. TS women have a reduced life expectancy due to an increased risk of structural abnormalities of the heart and aorta, and atherosclerosis. Atherosclerosis is caused by an increased prevalence of hypertension, diabetes and dyslipidaemia. Adult TS patients need adequate estrogen substitution and often deal with infertility. Concerning this morbidity, women with TS are expected to benefit from a coordinated care service.

AIM

To investigate the yield of initial standardized multidisciplinary screening for TS associated morbidity in adult patients.

MATERIALS AND METHODS

From 2005, 150 adult women with TS underwent a standardized medical evaluation at the multidisciplinary clinic for adult women with TS at the Radboud University Nijmegen Medical Centre (RUNMC). The screening consisted of consultation by an endocrinologist, gynaecologist, cardiologist, ear nose throat (ENT) physician and, when indicated, a psychologist. The screening includes MRI of the heart and aorta, echocardiography, bone mineral densitometry, renal ultrasound, audiogram and laboratory investigations according to international recommendations.(1)

RESULTS

The mean age was 31.0±10.4 years. Karyotype 45,X was found in 44.7%. Thirty percent of the women were not followed by any medical specialist. Twenty-two percent lacked estrogen therapy in the last years. The following disorders were newly diagnosed: bicuspid aortic valve (n=9), (mild) coarctation (n=5), combination of both (n=3), additional aortic abnormalities (n=49), osteoporosis (n=8), osteopenia (n=54), renal abnormalities (n=6), subclinical hypothyroidism (n=36), positive serology for celiac disease (n=5), glucose intolerance (n=2), random total cholesterol ≥ 5.5 mmol/liter (n=47) and hypertension (n=21). Psychological consultation was needed in 23 cases. 81 patients underwent ENT evaluation: hearing loss was found in 29 (8 needed a hearing aid). In patients with previously diagnosed disorders, treatment was adjusted in 11 patients with thyroid disease, 4 with osteopenia/osteoporosis and 1 with hypertension.

CONCLUSION

Standardized multidisciplinary evaluation of adult women with TS yields significant previously undiagnosed morbidity. Women with TS probably benefit from a coordinated care service, such as the TS clinic of the RUNMC.

(1)Bondy CA et al., J Clin Endocrinol Metab 2007;92(1):10-25

Nothing to Disclose: KF, RTN, CCMB, JT, CMV, BJO, DFCMS, HPMK, ARMMH, HJLMT

P1-367

The Phenotypes of Women with Spontaneous 46,XX Primary Ovarian Insufficiency (POI) Associated with a Premutation in the Fragile X Mental Retardation (*FMR1*) Gene (FXPOI) and Women with the Idiopathic Disorder (iPOI) Are Similar.

SD Sullivan MD, PhD¹, V Baker MD², A Hegde MD, MS², V Popat MD, MPH¹, V Vanderhoof CRNP¹, J Troendle PhD¹ and LM Nelson MD¹.

¹NICHD/NIH Bethesda, MD and ²Stanford Univ Stanford, CA.

Introduction: A pre-mutation in the *FMR1* (55-199 CGG repeats) gene is the cause of 5-10% of cases of spontaneous 46,XX POI. *FMR1* is highly expressed in the genital ridge during embryonic development, which raises the possibility that women with FXPOI might have an earlier onset of the disorder due to impaired folliculogenesis. It is not known if there are phenotypic differences between women with FXPOI and women with iPOI.

Aims: 1) To test the hypothesis that women with FXPOI have an earlier onset of menstrual irregularity and lower BMD compared to women with iPOI. 2) To assess differences in the reproductive phenotypes of women with FXPOI versus iPOI.

Design: Cross-sectional

Methods: Women with 46,XX POI (n=291) between the ages of 18 and 42 were recruited for evaluation at NIH. Data collected included medical history; BMI; BMD by DEXA at the L-spine, femur, and radius; reproductive hormones (FSH, LH, E2, P4, total and free testosterone); and antibody testing for autoimmune adrenal/ovarian, thyroid, GI, and rheumatologic diseases. *FMR1* CGG repeat number was determined by Southern blot and PCR. We excluded women with 21-hydroxylase or anti-adrenal antibodies, indicative of steroidogenic cell autoimmune POI (SCA-POI, N=3), and women with 45-54 *FMR1* CGG repeats, which is associated with an unclear risk of FXPOI (n=7). Statistics: Wilcoxon rank-sum and Fisher's Exact tests.

Results: Although women with FXPOI had an earlier mean (SD) age of onset of menstrual irregularity, the difference was not statistically significant (FXPOI, n=19, 22±7.4 yrs; iPOI n=272, 25±8.7 yrs, p=0.245). No women with FXPOI had primary amenorrhea, compared to 8.8% (24/272) of women with iPOI (P=0.38). BMD did not differ between groups at any site; of note, BMI was also similar (p=0.327). All serum hormone and antibody measures were similar between groups, except for an increased incidence of positive rheumatoid factor (RF) in women with FXPOI (26% vs 7.4%, P<0.02).

Discussion: We were unable to identify significant differences in phenotype between women with FXPOI and iPOI. The finding of a higher incidence of positive RF in patients with FXPOI is of unclear significance and may be a result of multiple comparisons. Further analysis with a larger number of women with FXPOI is warranted.

Nothing to Disclose: SDS, VB, AH, VP, VV, JT, LMN

P1-368

Serum Free Testosterone Levels Are Positively Associated with Perceived Stigma in Young Women with Primary Ovarian Insufficiency.

SD Sullivan MD, PhD¹, V Popat MD, MPH¹, M Davis PhD², S Covington LCSW¹, M Ryan MLS³, VH Vanderhoof CRNP¹, DE Koziol PhD¹, J Troendle PhD¹ and LM Nelson MD¹.

¹NICHD, Natl Inst of Hlth Bethesda, MD ; ²Arizona State Univ Tempe, AZ and ³NIH Bethesda, MD.

Women with primary ovarian insufficiency (POI) feel stressed by an inability to fulfill a basic drive: reproduction. Feelings of stigmatization (STIGMA) and illness uncertainty (IU) are known to be positively correlated with symptoms of anxiety and depression in women with POI (1). Androgen and glucocorticoid receptors, located in brain regions involved in the neuropsychological response to stress, act together to modulate the stress response. Serum testosterone (T) levels have been positively associated with social status-seeking behavior in women (2). Women with POI have lower free T compared to normally-cycling control women (3), prompting us to evaluate how free T and cortisol levels relate to affect in this population.

AIM: To test the hypothesis that serum free T and AM cortisol levels correlate with measures of affect, STIGMA and IU in women with POI.

DESIGN: Cross-sectional

METHODS: We recruited women ages 18-42 with spontaneous 46,XX POI (n=99) to a protocol assessing general and emotional health. As part of the evaluation, we administered the following psychological tests: Center of Epidemiologic Studies Depression Scale (CES-D), State-Trait Anxiety Inventory (STAI), Positive and Negative Affect Scale (PNAS), Mishel Uncertainty in Illness Scale (IU), and the Lennon Stigma Scale modified specifically for POI (STIGMA). Cortisol and total T were measured by chemiluminescent immunoassay. Free T was calculated from total T, albumin and steroid hormone binding globulin (SHBG). Statistics: Spearman rank correlation was used to assess correlations.

RESULTS: AM cortisol did not correlate with any measure of affect, STIGMA, or IU. However, free T was positively associated with scores of STIGMA ($r=0.30$, $P<0.01$) and IU ($r=0.33$, $P<0.01$), but not depression, anxiety or positive or negative affect.

CONCLUSIONS: Women with POI who have higher free T levels perceive more stigma. These findings suggest that physiologic levels of endogenous T are bioactive with regard to perceived self-image in women. Specifically, T-influenced status-seeking may be why women with higher free T perceive increased STIGMA.

(1)Davis M et al., Fertil & Steril 2009; Feb 24 epub

(2)Eisenegger C et al., Nature 2009; Dec 8 epub

(3)Kalantaridou SN et al., Fertil & Steril 2006; 86: 1475-82

Nothing to Disclose: SDS, VP, MD, SC, MR, VHV, DEK, JT, LMN

P1-369

Lower Bone Mineral Density in Women with Primary Ovarian Insufficiency: A Cross-Sectional Study.

F Amarante MSci¹, LC Vilodre MD¹, MA Maturana MD¹ and PM Spritzer MD, PhD^{1,2}.

¹Hosp de Clins de Porto Alegre Porto Alegre, Brazil and ²Univ Fed Rio Grande do Sul Porto Alegre, Brazil.

Primary ovarian insufficiency (POI) is defined as the premature cessation of ovarian function before 40 years of age, with findings of amenorrhea and levels of follicle-stimulating hormone (FSH) in the menopausal range. POI is associated with a more prolonged low estrogen state than what is usually observed in natural menopause. Consequently, POI is potentially associated with both more severe and different health risks than natural menopause. Although early age at menopause has been reported to be associated with the development of osteoporosis, few studies so far have assessed the prevalence and factors contributing to low bone density in patients with spontaneous POI. Therefore, the aim of the present study was to assess the prevalence of osteoporosis in a sample of 32 POI patients in comparison to reference groups of women in the pre- and post-menopause. The two reference groups included 25 age-matched premenopausal women and 55 postmenopausal women matched by menopausal status. Women from POI and reference groups had undergone evaluation of bone mineral density (BMD) using dual energy x-ray absorptiometry densitometer (DXA). Mean age of postmenopausal reference group was higher than POI and premenopausal groups ($p = 0.001$). Twenty-one (65%) of POI women sought medical advice previously and had taken a variety of estrogen and progestin regimens. In the postmenopausal reference group, 34% of women were current or previous users of hormone therapy. Bone mineral density measurements were significantly lower in POI group compared to the premenopausal reference group of similar age ($p = 0.040$). Moreover, 71% of POI women was found to have low bone density (osteopenia/osteoporosis by the World Health Organization [WHO] criteria) *versus* 52% in postmenopausal reference group ($p = 0.042$). In conclusion, data from the present study indicate that patients with spontaneous primary ovarian insufficiency present significantly lower values of bone mineral density than premenopausal women of similar age and have higher prevalence of osteopenia/osteoporosis than women after natural menopause. These young women need early medical strategies to maintain their bone mass and compliance with the treatment.

Sources of Research Support: National Institute of Hormones and Women's Health, CNPq, Brazil and Fundo de incentivo a Pesquisa HCPA (FIPE).

Nothing to Disclose: FA, LCV, MAM, PMS

P1-370

A Survey of Young Women with 46,XX Hypergonadotropic Hypogonadism Indicates a Strong Preference for the Term 'Primary Ovarian Insufficiency' as Compared to 'Premature Ovarian Failure.'

K Smith BS¹, SD Sullivan MD, PhD¹, V Vanderhoof CRNP¹, E Sterling PhD, MPH, CHES², L Abrams MS, CGC³, M Ryan MLS⁴ and LM Nelson MD¹.

¹NICHD/NIH Bethesda, MD ; ²Rachel's Well, Inc Marietta, GA ; ³Natl Fragile X Foundation Walnut Creek, CA and ⁴NIH Bethesda, MD.

Introduction: Hypergonadotropic hypogonadism in young women has been referred to by several names, including 'premature menopause,' 'premature ovarian failure' (POF), and 'primary ovarian insufficiency' (POI). Different terms are currently being used in the literature and by national organizations to describe this disorder, which is often confusing to both patients and clinicians.

Objective: To determine if, after an educational intervention regarding the pathogenesis of this form of infertility, women are amenable to accepting the term 'POF,' which is more commonly used, or 'POI,' to describe their condition.

Materials and Methods: We telephoned 30 women with hypergonadotropic hypogonadism who had been recently evaluated during a 4-day admission at the NIH Clinical Center (April 2008- July 2009). Inclusion criteria: age 18-42, ≥ 4 months of oligo-amenorrhea not due to pregnancy, ≥ 2 serum FSH levels in the menopausal range, and no evidence of karyotypic or iatrogenic cause of ovarian dysfunction. All women attended a 90 minute educational presentation explaining the physiology of the normal menstrual cycle and the known pathogenic mechanisms of this form of ovarian dysfunction. During the follow-up telephone contact, we read a structured script asking which term, 'POF' or 'POI,' each patient preferred to use to describe their condition, and why.

Results: The median (IQR) age of patients was 34 (27, 36) years, time since diagnosis was 16.1 (8.7, 45.2) months, and time between visit and telephone contact was 5 (2.5, 9) months. Seventy six percent of women preferred to use 'POI' to describe their condition (23/30, 95% confidence interval 57.7, 90.1), 20% (6/30) favored 'POF,' and 3% had no preference (1/30). Patients preferred the term 'POI' for the following reasons: less negative (n=15, 65%), more accurate (n=10, 43%), and more hopeful regarding chance for pregnancy (n=6, 26%). All patients who preferred 'POF' did so because this term is currently better recognized than 'POI.' The woman with no preference felt the two terms have the same meaning.

Conclusion: After an educational session, most women with 46,XX hypergonadotropic hypogonadism preferred the term 'primary ovarian insufficiency' over 'premature ovarian failure' as the most accurate to describe their disorder. These findings provide evidence that well-informed patients support using 'POI' as best to describe this condition.

Nothing to Disclose: KS, SDS, VV, ES, LA, MR, LMN

P1-371

Non Chromosomal Non Syndromic Premature Ovarian Failure in Two Exceptionally Tall Adolescent Females.

NB Zaidi MBBS¹, E Rome MD¹ and A Haider MD¹.

¹Cleveland Clin Cleveland, OH.

Background: Premature ovarian failure (POF) or premature cessation of ovarian function before age 40, characterized by amenorrhea, high FSH and low estrogen levels, may present with short stature due to haploinsufficiency of SHOX (short stature homeobox) gene or normal stature/normal chromosomes. Tall stature is an unusual finding. We describe 2 tall, non dysmorphic patients with primary ovarian failure. Both had normal 46XX karyotype and no family history of mental retardation, early menopause or infertility.

Case details: Patient A was a 16-year-old healthy girl with primary amenorrhea. She had adrenarche at age 12 years, thelarche at 14 years. She was born 4.5kg and academically successful. Physical examination (PE): height 180.5cm (5'11", 99%); weight 89.8kg (197lbs, 98%); mid-parental height (MPH) 5'5"; goiter absent; both breasts Tanner 3; pubic hair Tanner 4.

Patient B was an 18-year-old healthy college freshman with one episode of vaginal spotting lasting 3 days at age 16 years and no further periods. Birth weight was 3.6kg. She had adrenarche at 13 years, thelarche at 14 years. PE: height 182.9cm (6'0", 99%); weight 87.1kg (191.6 lbs, 97%); MPH 5'8"; goiter absent; right breast Tanner 3; left breast Tanner 4; pubic hair Tanner 3.

Microarray analysis and fragile X premutation did not identify abnormalities in either patient. Imaging studies demonstrated infantile uterus with no follicular ovaries. Laboratory data are detailed in Table 1.

Table 1

| Reference Range | Patient A | Patient B |
|---|-----------|-----------|
| FSH (mU/ml):Follicular:2-11; Luteal: 1-9 | 64.9 | 66.4 |
| Estradiol (pg/ml):Follicular:20-378; Luteal:50-150 | <12 | 13 |
| FT4 :0.7-1.8 ng/dl | 1.2 | 1.1 |
| TSH :0.4-5.5 µU/ml | 0.438 | 2.26 |
| Bone Age | 13 years | |
| Antibodies | | |
| ANA :1.5 OD Ratio | 1.2 | 0.6 |
| Adrenal :1:2 | <1:2 | <1:2 |
| Antithyroglobulin :10 IU/ml | 32.1 | 36.3 |
| Antimicrosomal :5 IU/ml | 16.3 | |
| Antiovarian | Negative | |

Conclusion: Etiology of non chromosomal and non syndromic ovarian failure remains obscure. Presence of thyroid antibodies in our patients suggest autoimmune ovarian failure; (autoimmunity in one gland increases the likelihood of autoimmune disease in other glands.) Evaluation of POF should include measurement of thyroid antibodies and monitoring of thyroid function.

The tall stature is unusual. Overdosage of SHOX gene expression and delayed epiphysis closure from lack of estrogen could cause tall stature. Our patients are currently being treated with birth control pills along with Calcium and Vitamin D supplements.

Nothing to Disclose: NBZ, ER, AH

P1-372

Predictive Factors of Intermittent Ovarian Function in Patients with Premature Ovarian Failure.

M Bidet M.D¹, E Bissauge M.D¹, J Dulong¹, A Bachelot M.D, Ph.D¹ and P Touraine M.D, Ph.D¹.

¹GH Pitié Salpêtrière Paris, France.

Objective. Premature ovarian failure (POF) is not an early menopause and intermittent ovarian function can be spontaneously observed in POF patients. We sought to characterize these patients and determine which factors were predictors of intermittent ovarian function.

Patients and Methods. Among 395 patients with idiopathic POF followed from 1997 to 2009, in our department. Sixty-seven (17%) patients (Group1) had POF fluctuating criteria: FSH <15 IU / L, resumed spontaneous menstrual cycles and / or spontaneous pregnancies. We compared the clinical criteria, biological, ultrasonographic, histological and genetic characteristics of these patients to other patients from the cohort of POF patients (Group2, N = 328). The mean follow-up period was 23.3 ± 38 months in Group1 and 28.5 ± 42 in Group2 (NS).

Results. Sixty-three percent of Group1 patients showed an intermittent ovarian function in the year following their diagnosis. Primary amenorrhea was infrequently in patients with fluctuating POF (2.98% vs. 24.7% in group 2, p <0.05). FSH was more variable in Group1 patients: 79.6 ± 38.3IU/l at POF diagnosis versus 51.4 ± 41 UI/l during hospitalisation and significantly lower than in Group2 (83.2 ± 37.9 UI/l). Estradiol and Inhibin B were also significantly higher in patients with fluctuating POF. Follicular structures were more frequently visualized on ultrasound in patients with fluctuating POF (74% versus 44%, p <0.05). However, AMH levels were not significantly different between the 2 groups. BMD alterations were found in 44% in Group2 vs 30% in Group1 patients. Eleven patients (2.7%) experienced a pregnancy after diagnosis of POF. Issues of pregnancies in POF patients were 9 deliveries, 2 miscarriages and 1 current pregnancy.

Conclusion. An intermittent ovarian function in POF patients is not a rare phenomenon and seems more prevalent within a short time from the installation of a secondary amenorrhea. Moreover, presence of follicles by ultrasound and FSH levels appear to be more predictive than the rate of AMH level.

Nothing to Disclose: MB, EB, JD, AB, PT

P1-373

Unexpected Finding of Intact Distal Vagina in an Infant with Mixed Gonadal Dysgenesis.

SM Cabrera MD¹, EA Eugster MD¹, M Kaefer MD¹ and TD Nebesio MD¹.

¹Riley Hosp for Children, Indiana Univ Indianapolis, IN.

Background: Mixed gonadal dysgenesis (MGD) is a form of sex chromosome DSD (Disorders of Sex Development) with large phenotypic variability. Patients with MGD typically have asymmetric and ambiguous genitalia, with a combination of Mullerian and Wolffian duct derivatives.

Clinical Case: A term infant was found to have ambiguous genitalia with a phallic structure measuring 2.0 x 0.8 cm, separate vaginal and urethral openings, and prominent mildly rugated labioscrotal structures without hyperpigmentation or posterior fusion. No gonads were palpable. The infant was otherwise well-appearing without dysmorphic features. A 17-hydroxyprogesterone level at 72 hours was normal. Labs obtained during the first week of life included: FSH- 4.2 mU/mL (M: 0.26-3.0, F: 1.0-4.2), LH- 0.2 mU/mL (0.02-0.3), Testosterone- 5.6 ng/dL (M:75-400, F:20-64) , and Mullerian Inhibiting Substance (MIS) - 4.7 ng/mL (M:15.5-48.7, F: <7.1). Pelvic ultrasound showed a normal appearing uterus, endometrial stripe, and no visible gonads. Genitogram revealed intact Mullerian structures with a normal-appearing uterus, cervix, and vagina, with no communication between these structures and the normally developed urethra. Karyotype was 45,X[7]/46,XY[14]. An echocardiogram was unremarkable, and renal ultrasound revealed structurally normal kidneys. Testosterone after hCG stimulation (1500 IU IM qod x 3) was 89 ng/dL (M:180-735 ng/dL). FSH on day 13 of life was 18 mU/mL (M: 0.26-3.0, F: 1.0-4.2). After multidisciplinary care conferences and discussions with the family, a female gender assignment was made. The infant underwent bilateral laparoscopic gonadectomy at 6 weeks of age.

Conclusion: In MGD, dysgenetic testicular tissue results in a spectrum of androgen exposure and virilization during the critical period of sexual differentiation. This case is unusual in that, despite phenotypic evidence of early testicular function, there is a completely normal distal vagina with no urogenital sinus present. To our knowledge, such a case has not previously been described. This could represent differences in sensitivity to androgens or variations in the timing of androgen exposure within different urogenital tissues. Interestingly, there was no evidence of external asymmetry as is usually seen in MGD. Whether routine echocardiograms and renal ultrasounds are needed in patients with MGD, as recommended in girls with Turner Syndrome with this same karyotype, is unknown.

Disclosures: EAE: Study Investigator, Abbott Laboratories; Medical Advisory Board Member, ENDO Pharmaceutical.

Nothing to Disclose: SMC, MK, TDN

P1-374

Blepharophimosis, Ptosis, Epicanthus Inversus Syndrome Associated with True Hermaphroditism.

RM Hirosawa MD¹, M Marivo MD¹, FG Neto MD¹, GL Souza MD¹, VS Nunes MD¹, ABA Santos MD¹, CIR Coelho MD¹, A Pontes MD¹, CR Nogueira MD¹, FC Galan MD¹, AC Oliveira MD¹ and DCL Oliveira MD¹.

¹São Paulo State Univ/UNESP Botucatu, Brazil.

Introduction: Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES; OMIM #U10100) is a rare autosomal dominant disorder associated with eyelid malformation and premature ovarian failure in the affected female. The mutations causing this disorder are found in the *FOXL2* gene, a forkhead transcription factor. **Clinical case:** An 11 year-old girl presented with primary amenorrhea and typical signs of BPES since birth. She had telarche and adrenarche at 9 years and 6 months. Physical examination showed bilateral blepharophimosis, ptosis, epicanthus inversus, telecanthus and hyperandrogenic signs (clitoral enlargement and deep voice); Tunner: P4M2. Early laboratory tests demonstrated low estradiol (37.6pg/ml, reference value: [RV]10 - 160pg/ml ng/dL), high LH (31.2mUI/mL, RV: 2.89 - 21.72mUI/ml), high FSH (35.5mUI/mL, RV: 3.3 - 21.6mUI/mL), high free testosterone (58.3ng/dL, RV: 0.69-12.78 ng/dL), high total testosterone (217ng/dL, RV <80ng/dL), and normal thyroid function, DHEA-S and prolactin. Ultrasonography and magnetic resonance of the pelvis showed a hypoplastic uterus and no gonads. Chromosome analysis on 25 cells revealed a 46 XX karyotype. As virilization rapidly developed, the patient underwent gonadectomy. Structures resembling gonadal strips were found, but anatomopathological findings were compatible with bilateral ovotestis indicating true hermaphroditism. Microscopic examination evidenced hypoplastic uterine tubes, ovarian tissue and primordial follicles, as well as testes containing Leydig cells and seminiferous tubules bilaterally. Testosterone measured 24 hours after surgery showed a significant decline reaching normal levels (5.24ng/dL). Currently, the patient is undergoing estrogen hormone replacement. **Conclusion:** Mutations of the *FOXL2*, involved in cranio-facial and ovarian development, lead to the BPES and female infertility. A unique case of BPES associated with true hermaphroditism was described herein. The molecular biology approaches aimed at finding new *FOXL2* mutations are expected to reveal mechanisms that explain how this gene acts on gonadal differentiation and development.

Nothing to Disclose: RMH, MM, FGN, GLS, VSN, ABAS, CIRC, AP, CRN, FCG, ACO, DCLO

P1-375

A Case of Primary Ovarian Insufficiency in a Woman with an *FMR1* Full Mutation.

T Prodanov MD¹, M Raygada PhD, MSc, CGC², S Sullivan MD, PhD¹, V Vanderhoof RN, MHA, CCRP¹ and L. M. Nelson MD¹.

¹NIH/NICHD Bethesda, MD and ²NIH Bethesda, MD.

Introduction: Fragile X Syndrome (MIM# 309550), a cause of hereditary intellectual disability, is an X-linked disorder resulting from a mutation in the *FMR1* gene located at Xq27.3. Approximately 99% of cases of Fragile X are caused by CGG expansions in the 5' untranslated region. This unstable expansion in *FMR1*, accompanied by abnormal methylation, leads to suppression of *FMR1* transcription and decreased FMRP protein. Boys and men with a full *FMR1* mutation (>200 repeats) and the associated methylation express the full Fragile X phenotype. Approximately 50% of girls and women with a full mutation have intellectual disability and other associated behavioral problems. The *FMR1* premutation (55-199 CGG repeats) has been associated with the development of Fragile X associated primary ovarian insufficiency (FXPOI) and a tremor ataxia disorder (FXTAS). Individuals with the premutation express normal amounts of FMRP but increased amounts of mRNA, which is believed to lead to a gain of function toxicity. Generally, women with the full mutation have normal ovarian function and are not believed to be at increased risk of FXPOI.

Clinical Case: A 26 year old woman was determined to have a full mutation at the age of 10 as a result of cascade testing when her brother was diagnosed with Fragile X Syndrome. She had a normal menarche at age 13 and established regular menstrual cycles. At age 24 she developed amenorrhea and at age 25 was found to have menopausal level serum FSH on two occasions. She had no signs or symptoms of Fragile X Syndrome. Our evaluation showed: FSH 49.8 U/L (3-21), LH 12.4 U/L (1-77), estradiol 32.7 pg/ml (30-400), and progesterone 0.4 ng/ml (<1-20). Southern blot and PCR-based assays were used to determine the size and methylation status of the CGG repeat within the *FMR1* gene. The Southern blot assay utilized the DNA probe StB12.3 and an EcoR1/Nru I double digest. *FMR1* molecular analysis was performed twice confirming CGG repeats of 202 and 23. The gene was fully methylated, and there was no evidence of either repeat number or methylation mosaicism on the Southern blot.

Conclusion: To our knowledge this is the first report of FXPOI developing in a woman with a methylated full mutation in *FMR1*. Clearly, this case is an exception to current theory regarding the pathogenesis of FXPOI. Possibly the POI is unrelated to the *FMR1* mutation, or there is a premutation in the ovary that is not methylated and thus expressing high levels of mRNA.

Nothing to Disclose: TP, MR, SS, VV, LMN

P1-376

Postmenopausal Ovarian Virilization: Persistent Therapeutic Response to a Single Injection of a Long-Acting GnRH-Analog.

R Raj², G Guerrero MD¹, LM Bui MD¹ and M Karl MD².

¹Chen Med Associates Miami, FL and ²FIU Herbert Wertheim Coll of Med Miami, FL.

A 69-year-old woman was referred for evaluation of severe hyperandrogenism that had begun after menopause and progressively worsened for the last several years. She displayed excessive terminal hair growth on her face, chest, abdomen and extremities (Ferriman & Gallwey Score: 25) with mild diffuse alopecia and some frontal balding, slightly masculine facial features and a well-developed and accentuated muscle mass. Her reproductive history was rather unremarkable: menarche at the age of 10, followed by regular menstrual cycles, an uneventful pregnancy and delivery at the age of 30 and menopause around age 55. Her total serum testosterone measured 481 ng/dL and 586 ng/dL (0-76), her free testosterone was elevated at 13.6 pg/dL (0-1.8); concomitant LH and FSH levels measured 22.2 and 23.8 mIU/L, respectively. Additional biochemical tests revealed a high serum 17-OHP level of 457 ng/dL (<50) and an elevated DHEA concentration of 730 ng/dL (60-379), while her DHEA-S of 98 mcg/dL was within the age-adjusted normal range; her serum E₂ was slightly elevated at 56 pg/mL (0-19). Imaging of her adrenals revealed a 1.2-cm mass in her right and a 0.6 cm lesion in the left adrenal that were benign based on their radiological characteristics. Ultrasound imaging demonstrated a normal 0.5 x 2.8 cm right ovary; the left ovary measured 3.1 x 2.8 cm and contained a simple 1.8 cm cyst. To differentiate between gonadotropin-dependent and gonadotropin-independent hyperandrogenism, she received a single intramuscular injection of a long-acting GnRH-agonist (Lupron 3.75 mg). As expected, LH and FH decreased for about 3 months; this was accompanied by the fall of her serum testosterone levels. About 10 months after the one-time application of the GnRH agonist, her serum testosterone of 31 ng/dL has remained within the normal post-menopausal range while her E₂ concentrations have been low at 7 pg/mL; in contrast, LH and FSH levels have risen to 67.8 and 133.6 mIU/mL, respectively. The normalization of her serum testosterone led to a substantial reduction in her facial and body hair. Taken together, the extremely high and gonadotropin-dependent testosterone levels, associated with elevated 17-OHP and DHEA but normal DHEA-S concentrations, were most consistent with ovarian androgen secretion rather than being of adrenal origin. Importantly, the ovarian responsiveness to LH and FSH appears to be lost after a single GnRH-agonist injection suggestive of a persistent therapeutic effect.

Nothing to Disclose: RR, GG, LMB, MK

P1-377

Spontaneous Menses Post-Splenectomy in a Patient with Secondary Amenorrhea and Hereditary Spherocytosis.

CH Meyers-Seifer¹, P Agarwala² and MA Masterson².

¹Jersey Shore Univ Med Ctr Neptune, NJ and ²UMDNJ-Robert Wood Johnson Med Sch New Brunswick, NJ.

A 14 year old girl with a history of hereditary spherocytosis (HS) presented to pediatric endocrinology with a complaint of irregular menses. Menarche was at age 12. Menses had always been irregular. She reported no menses in over 6 months. She had been diagnosed with HS at age 3 and was status post cholecystectomy at age 8. Medications included folate, 1 mg per day. Past medical history and review of systems were otherwise unremarkable. Physical examination was remarkable for height, weight and body mass index in the 50th percentile for age, scleral icterus, and pubertal development at stage V according to the method of Tanner. Laboratory studies revealed hematocrit 34 %, hemoglobin 12.3 g/dl, MCV 88.9 fL and reticulocyte count 12.67%. Moderate anisocytosis and slight spherocytes were seen on peripheral smear. Total bilirubin was 4.56 mg/dl, estradiol 36 pg/ml (40 - 410), follicle stimulating hormone (FSH) 1.88 mIU/ml, luteinizing hormone (LH) 4.2 mIU/ml, and free testosterone 1.3 pg/ml (0.5-3.9). A lipid profile showed total cholesterol 74 mg/dl, HDL cholesterol 32 mg/dl, LDL cholesterol 20 mg/dl, and triglycerides 108 mg/dl. She responded to medroxyprogesterone acetate 10 mg po daily for 10 days on two occasions, but did not sustain regular menses. She underwent splenectomy at age 16 2/12. Nine months after the splenectomy she had spontaneous menses with subsequent regular cycles. At that time laboratory studies revealed hematocrit 46.6 %, hemoglobin 16.1 mg/dl, MCV 88.5 fL, estradiol 72 pg/ml, LH 2.27 mIU/ml, FSH 0.55 mIU/ml, free testosterone 3.2 pg/ml, total cholesterol 94 mg/dl, LDL cholesterol 49 mg/dl, HDL cholesterol 31 mg/dl and triglycerides 59 mg/dl. Low cholesterol has been reported in association with HS since the 1980's (1). It has been hypothesized that increased cholesterol consumption is required for red blood cell membrane production in patients with hemolytic anemia. To our knowledge, this is the first reported case of secondary amenorrhea in a patient with hereditary spherocytosis and hypocholesterolemia. Splenectomy appears to have decreased hemolysis, restored cholesterol precursors for sex steroid biosynthesis and allowed for regular menses.

(1)Sugihara T et al., Clin Chim Acta 1984;137:227

Nothing to Disclose: CHM-S, PA, MAM

P1-378

Virilizing Sclerosing-Stromal Tumor of the Ovary in a Young Woman with McCune-Albright Syndrome.

Kahina Boussaid M.D.¹, Jean-Christophe Maiza M.D.¹, Pierre Leguevaque M.D.², Isabelle Gennero M.D.³, Ghislaine Escourrou M.D.², Geneviève Motton M.D.², Antoine Bennet M.D.¹ and Philippe Caron M.D.¹.

¹CHU Larrey Toulouse, France ; ²CHU Rangueil Toulouse, France and ³CHU Purpan Toulouse, France.

McCune-Albright Syndrome (MAS) is characterized by polyostotic fibrous dysplasia, café-au-lait skin pigmentations and hyperfunctioning endocrinopathies. Precocious puberty is common in girls and represents with fibrous dysplasia of bones and skin spots the classical triad of MAS. Other endocrinopathies such as growth hormone excess, hyperthyroidism, Cushing's syndrome and renal phosphate wasting may also occur. These clinical manifestations result from a somatic post-zygotic activating mutation of GNAS1 gene, which encodes the Gs alpha protein involved in the AMP cyclic signaling pathway. We report the case of a virilizing sclerosing-stromal tumor of the ovary in a young female with MAS. The patient presented polyostotic fibrous dysplasia of the left upper and lower limbs, and a café-au-lait skin spot in the posterior area of the neck. She had a history of precocious puberty diagnosed at the age of 6 years and treated by cyproterone acetate until the age of 10 years; then she developed a central puberty with severe oligomenorrhea. At the age of 23 years, she was hospitalized for investigation of a virilization syndrome including hirsutism, increased acne, deepening of the voice, amenorrhea and clitoromegaly. Pre-operative serum level of testosterone was dramatically increased (1293 ng/dl; normal range: 10-80). Abdominal CT scan revealed a solid mass located on the left ovary. An ovariectomy was performed and histological features revealed a sclerosing-stromal tumor with characteristic pseudolobular pattern. Molecular analysis revealed that the ovarian tumor cells harbored the Arg 201 activating mutation in the GNAS1 gene. After surgery, testosterone levels returned to normal (25 ng/dL on the day after surgery), and the patient was able to become pregnant shortly. Therefore this observation extends the clinical spectrum of ovarian pathology encountered in MAS. The oncogene gsp is known to play a significant role in the pathogenesis of these tumors but the mechanisms remain unclear. Additional mutational events or involvement of other cellular signaling pathways that facilitate tumorigenesis remain to be elucidated.

Nothing to Disclose: KB, J-CM, PL, IG, GE, GM, AB, PC

P1-379

Cushing's Syndrome - The Forgotten Diagnosis?.

RG Paul¹, MS Elston¹ and JV Conaglen¹.

¹Univ of Auckland Hamilton, New Zealand.

Introduction: Cushing's syndrome (CS) is a known cause of PCOS with 85% of women with CS having polycystic ovaries sonographically. ⁽¹⁾ CS is associated with significant morbidity and mortality and unlike PCOS it may be cured. Although florid CS is rare, the prevalence of CS has been shown to be greater than previously thought, due to the detection of subtle hypercortisolism in high risk groups. ⁽²⁾ The NIH, Rotterdam and AE-PCOS Society diagnostic criteria all require the exclusion of CS for the diagnosis of PCOS. However most clinical guidelines do not recommend routine biochemical screening to exclude CS, therefore this is seldom done in clinical practice and may result in misdiagnosis. ⁽³⁾ ⁽⁴⁾ This series highlights 4 cases of CS which were misdiagnosed as PCOS by specialist endocrinologists with no appropriate screening tests for CS performed.

Case Series

| | Case 1 | Case 2 | Case 3 | Case 4 |
|--|---------------------------------|---------------------------------|---|--|
| Age at diagnosis | 39 years | 34 years | 27 years | 22 years |
| Time since diagnosis of PCOS | 9 years | 10 years | 2 years | 7 months |
| Initial clinical features | Hirsutism | Menstrual irregularity | Hirsutism & acne | Menstrual irregularity |
| Serum testosterone (RR 0-3nM) | 2.4nM | 3.3nM | 5.1nM | 3nM |
| U/S Ovaries | Polycystic ovaries | Polycystic ovaries | Polycystic ovaries | Polycystic ovaries |
| Screening 24hr urinary cortisol (RR <286nM/24hr) | 1181nM/24hr | 1200nM/24hr | 2100nM/24hr | 2282nM/24hr |
| CS diagnosis | Corticotroph adenoma | Corticotroph adenoma | Corticotroph adenoma | Ectopic ACTH producing bronchial adenoma |
| Definitive management | Trans-sphenoidal hypophysectomy | Trans-sphenoidal hypophysectomy | Trans-sphenoidal surgery x 2 | Attempted wedge resection of bronchial adenoma |
| | | | Bilateral adrenalectomy followed by pit. radiotherapy | Bilateral adrenalectomy |

Clinical Lessons: The diagnosis of PCOS involves the exclusion of related conditions. Routine biochemical screening to exclude CS is controversial and so frequently is not standard practice. We recommend routine biochemical screening for CS in PCOS as the clinical features may be inadequately assessed or may be too subtle to be clinically evident, as highlighted by this series of CS cases missed by trained Endocrinologists.

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Nothing to Disclose: RGP, MSE, JVC

P1-380

Case Report: Utility of Magnetic Resonance Imaging (MRI) in an Adolescent with Sertoli-Leydig Cell Tumour-Induced Virilization.

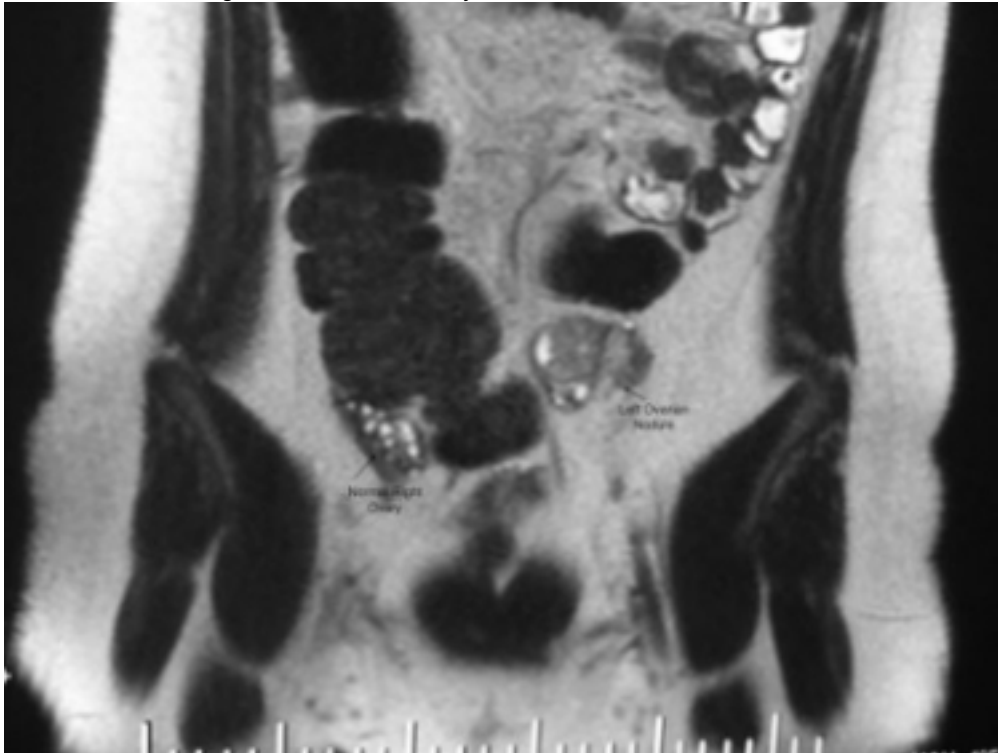
YC Kon FAMS, FRCPE¹, V Viardot-Foucault MD² and SY Loi MRCOG (UK), FAMS².

¹Tan Tock Seng Hosp Singapore, Singapore and ²KK Women's and Children's Hosp Singapore, Singapore.

A 15-year-old Chinese girl with previously regular menses (7/30 days) noted that 8 months ago, she had skipped a month, then had prolonged menses for 10 days, spotting for 10 days, then no periods. Her LMP was 5 months ago. She had gained 10 kg in weight. Her weight was 93.7 kg, height 170.5 cm, BMI 32.3 kg/m², BP 130/74 mmHg, Ferriman-Galway score 8. She had a deep voice, mild facial and truncal acne and cliteromegaly, but no Cushingoid nor acromegalic features, nor acanthosis nigricans.

Baseline investigations: Serum total testosterone (TT) 9.0 nM, E2 145 pM, LH 5.91 IU/L, FSH 3.6 IU/L, PRL 21.6 ug/L (6.98-33), Prog 0.9 nM (<0.32-52), fT4 13.3 pM (10.3-25.7), TSH 3.46 mIU/L (0.5-4.5), DHEAS 5.6 uM (1.1-11.8) and 17(OH) Prog 11.1 nM. Post-LDDST 8 am Cortisol <11nM, total testosterone 6.7nM; and SST 17OH Prog (nM) 0 min (8am) 7.7nM, 30 min 14.3nM, 60 min 14.8 nM. Thus both Cushing's syndrome and CAH were excluded.

Transabdominal pelvic USS was normal. On CT abdomen/pelvis, both adrenal glands were normal. Her left ovary (3.7 x 3.7 x 2.7 cm) was larger than her right ovary (3.7 x 3.6 x 1.6 cm), with a dense internal lesion (2.7 x 2.3 cm, 90 HU). A second transabdominal pelvic USS was again normal. Subsequently, MRI (pelvis) revealed a well defined, ovoid, 2.5 x 2.0 x 2.6 cm enhancing nodule in the left ovary.





Results: On laparoscopic left ovarian cystectomy, her left was found to be enlarged by a 2.5 cm cyst. There was no ascites, her liver was normal. Frozen section showed and histology confirmed a left ovarian Sertoli-Leydig cell tumour, grade 2, stage 1A (N:0/22, M:0). One month later, her menses had returned. Serum TT had normalized to 1.9 nM. Conclusion: In adolescents suspected to harbor ovarian neoplasm, further localization and characterization with MRI should be considered if initial trans-abdominal USS is negative.

Nothing to Disclose: YCK, VV-F, SYL

P1-381

Prolactinoma and Polycystic Ovary Syndrome: A Rare Association.

PS Gadelha¹, A Glezer¹, DB Paraiba¹ and MD Bronstein¹.

¹Univ of Sao Paulo Med Sch São Paulo, Brazil.

Background: Prolactinomas are the most common pituitary hormone secreting tumors. Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine diseases, affecting 5-10% of reproductive age women. PCOS diagnosis requires exclusion of hyperandrogenism due to hyperprolactinemia, nonclassic adrenal hyperplasia and androgen-secreting tumors. However, mild non-tumoral hyperprolactinemia could be present in 15-20% patients with PCOS, probably owing to hyperestrogenism. Nevertheless, the association of prolactinoma and PCOS is rarely described. We report 6 patients with prolactinomas who presented PCOS criteria during treatment that led to PRL normalization.

Case reports: We analyzed 147 age reproductive women with hyperprolactinemia from our Neuroendocrine Unit. Ninety three had prolactinomas (49 microprolactinomas - MIC, 44 macroprolactinomas - MAC). Of the 93 patients, 6 (4 MAC, 2 MIC) had clinical hirsutism, biochemical findings of hyperandrogenism and/or oligo-anovulation not completely resolved despite PRL normalization during treatment with dopaminergic agonists. Therefore, features not compatible with controlled prolactinoma. Hormonal profile of these patients was (median/range): pre-treatment serum PRL 147/79-552 ng/mL (nr <15 ng/mL), with exclusion of macroprolactinemia; post treatment PRL 18.9/3.8-28.8; serum testosterone 83/66-143 ng/dL (nr <98ng/dL); androstenedione 3.1/2.6-5.5 ng/mL (nr 0.7-2.8ng/mL); DHEA 9.1/4.8-19ng/mL (nr 2.5-6.4ng/mL) and normal basal 17OHP. Pelvic ultrasound was compatible with PCOS in 4 of 6 patients. Since PCOS diagnosis, these patients are on oral contraceptive pills, metformin or both, with improvement of symptoms.

Discussion and conclusion: Mild non-tumoral hyperprolactinemia (< 100 ng/mL) is found in 15-20% of PCOS patients. In these patients, higher levels of androgens and LH/FSH ratio could help to differentiate between hyperprolactinemia due to PCOS and to prolactinoma. We described cases with prolactinoma and PCOS association. Thereafter, we suggest that patients adequately treated for prolactinomas who maintain menstrual disorders, hyperandrogenism and/or anovulation should be searched for PCOS. The current PCOS diagnostic criteria which exclude hyperprolactinemia should be evaluated carefully, since the association of these two diseases exist and should be reminded.

Nothing to Disclose: PSG, AG, DBP, MDB

P1-382

An Unusual Cause of Galactorrhea in a Premenopausal Woman.

S Ali MD¹, SM Haggerty MD¹, AF Sabbagh MD¹, M Kaur DO¹ and DL Diab MD¹.

¹Univ of Cincinnati Cincinnati, OH.

Background:

Depot medroxyprogesterone acetate (DMPA) is a commonly available contraceptive medication. Galactorrhea is a rare and benign side effect associated with DMPA use. We report a case of galactorrhea from DMPA use that resolved after discontinuation of the drug.

Case Presentation:

A 36 year-old Caucasian female presented to the Endocrinology clinic with galactorrhea that started 2 months prior to presentation. This was associated with a 65-pound weight gain over the previous six months. Her increased weight was mostly described as an increase in abdominal girth and lower extremity swelling. Her medication list included DMPA which was initiated three years ago for contraception. She reported amenorrhea since starting DMPA. Physical examination revealed abdominal obesity and pedal edema. Significant milky, non-bloody discharge was expressed from both nipples. No palpable abdominal masses were appreciated. Biochemical work-up was essentially unremarkable. Multiple urine and serum β HCG levels were negative, ruling out pregnancy. Serum prolactin level was within normal at 9.4 ng/dL (2.8-29.2 ng/dL). Serum estradiol and testosterone levels were undetectable, ruling out potential sex-hormone secreting tumors. Serum LH, FSH and thyroid function tests were all normal. An overnight 1-mg dexamethasone suppression test done to evaluate for Cushing's syndrome was negative with an AM cortisol level of 0.4 mcg/dL (<1.8 mcg/dL). A pituitary MRI did not show any evidence of pituitary tumor. Abdominal ultrasound and CT were negative for intrauterine pregnancy and intra-abdominal tumor respectively. The patient's DMPA was stopped and on seven-month follow up her galactorrhea had resolved.

Conclusion:

Galactorrhea should be recognized as a rare and reversible side effect of DMPA use. The incidence varies in literature from 0.3% to 9% and the most likely mechanism is believed to be the direct effect of progesterone on proliferation of the acini of mammary glands. The time to onset of galactorrhea is variable, mostly within 5 to 17 months of therapy. Discontinuation leads to resolution of symptoms mostly within 12 to 18 months, with some cases taking longer to resolve. Patient education and reassurance is sufficient as most cases have been known to resolve after stopping DMPA. Given the social, physical and emotional implications associated with galactorrhea, clinicians are advised to counsel their patients of this anticipated side effect prior to starting therapy.

Nothing to Disclose: SA, SMH, AFS, MK, DLD

P1-383

Cushing's Disease Accompanying Polycystic Ovary Syndrome in Japanese Patients.

T Miyoshi M.D.¹, F Otsuka M.D., Ph.D.¹, M Takeda M.D.¹, J Suzuki M.D.¹, K Inagaki M.D.¹, N Tsukamoto M.D.¹, E Nakamura M.D.¹, Y Mimura M.D.¹, T Ogura M.D.¹ and H Makino M.D., Ph.D.¹.

¹Okayama Univ Graduate Sch of Med, Dentistry and Pharma Scis Okayama, Japan.

Patients with Cushing's syndrome (CS) and PCOS show similar clinical features, including menstrual abnormality, obesity, hirsutism and insulin resistance. It is often difficult to clinically distinguish these disorders, although PCOS complicated with CS have been rarely reported. We report two Japanese cases of PCOS with CS due to ACTH-producing pituitary tumors. Case 1: A 25-y.o. woman with central obesity and amenorrhea. When she was 16 y.o., plasma ACTH was moderately high (72 pg/ml, normal: 7-63). However, her ACTH secretions maintained normal fluctuation and cortisol levels were suppressed by dexamethasone (Dex) test. At her age of 24, plasma ACTH was further elevated (105 pg/ml) without circadian fluctuation, urinary cortisol levels became excessive (489.5 µg/d), and cortisol secretion was not suppressed by Dex. Serum testosterone (T) was also elevated (105.6 ng/dl: 9-56) and pelvic MRI detected PCO changes of the bilateral ovaries. GTT revealed increased insulin resistance (peak insulin: 341 U/l). Venous sampling of the cavernous sinus uncovered the presence of an ACTH-secreting pituitary microadenoma. After the transsphenoidal surgery, serum T was decreased (31.7 ng/dl). Serial pituitary function tests further revealed that LH hyperresponsiveness to GnRH was inversely correlated to hypercortisolemic status. Case 2: A 32-y.o. woman having general fatigue, hirsutism, menstrual irregularity, hypertension and weight gain. Her ACTH (97.8 pg/ml) and cortisol (24.4 µg/dl) levels were increased, in which circadian ACTH fluctuation disappeared and Dex test failed to suppress cortisol secretion. A pituitary macroadenoma was detected by cranial MRI. GTT revealed high insulin resistance (peak insulin: 280 U/l), serum T was elevated (95.9 ng/dl) and the ratio of LH/FSH to GnRH stimulation was found to be high (peak: 6.7). Her bilateral ovaries were enlarged into PCO features by ultrasounds. Following the transsphenoidal surgery of the ACTH-producing adenoma, serum T (36.9 ng/dl) and insulin resistance (peak: 178 U/l) were rapidly decreased, while the LH/FSH ratio in response to GnRH was further increased (peak: 9.8) in addition to remission of CS. Conclusion: CS patients should be examined for excluding the complication of PCOS. Hypercortisolemia and high insulin resistance in CS may directly affect gonadotropins pulsatility in the pituitary as well as androgen production in the ovary, which may relate to the clinical manifestation of PCOS.

Nothing to Disclose: TM, FO, MT, JS, KI, NT, EN, YM, TO, HM

P1-384

Apolipoprotein A-I Is Downregulated in the Granulosa Cells of Polycystic Ovary Syndrome Patients and Affects the Expression of Steroidogenic Enzymes.

MA Won¹, DH Choi², WS Lee³, MR Park¹, HO Park¹, EK Shin⁴, HY Ko⁴, KS Lee⁴ and JH Bae¹.

¹CHA Univ Seongnam, Korea ; ²Bundang CHA Women's Hosp Seongnam, Korea ; ³Gangnam CHA Hosp Seoul, Korea and ⁴Chung-Ang Univ Seoul, Korea.

Background: Polycystic ovary syndrome (PCOS) is the leading endocrine disorder found in women. The etiology of PCOS is still not clear, and there is no available study on proteome analysis of granulosa cells (GCs) in PCOS patients. To identify the pathogenic mechanisms and potential diagnostic markers for PCOS, we conducted proteomic profiling of GCs in PCOS patients

Clinical case: This study was performed at two referral centers. PCOS patients (n = 11) were recruited according to the 2003 Rotterdam criteria, and women with regular menstrual cycles were included as controls (n = 14).

In vitro studies: Proteomic profiles of GCs were obtained by two-dimensional gel electrophoresis (2-DE) and liquid chromatography coupled with mass spectrometry (LC-MS/MS) analyses, and the expression levels of apolipoprotein A-I (ApoA-I) were confirmed by western blot. Changes in steroidogenic enzyme expression based on altered levels of ApoA-I in a granulosa cell line (KGN) were also determined. Eight down-regulated spots and 12 up-regulated spots were found in PCOS patients compared to controls after proteomic analysis. Among these, a change in ApoA-I protein expression was profound, and a significant down-regulation of ApoA-I in GCs of PCOS women was confirmed. Knockdown of ApoA-I decreased the transcripts of steroidogenic enzymes in the GCs while its overexpression generally increased their expression.

Conclusions: We concluded that the disturbed expression of steroidogenic enzymes in PCOS patients may be related with down-regulated ApoA-I.

Nothing to Disclose: MAW, DHC, WSL, MRP, HOP, EKS, HYK, KSL, JHB

P1-385

Combination Therapy for Erectile Dysfunction with Testosterone and Vardenafil in Hypogonadal Patients Who Failed To Respond to Testosterone Treatment Alone.

AA Yassin MD, PhD^{1,2}, A Haider MD, PhD³ and F Saad DVM, PhD^{2,4}.

¹Segeberger Kliniken Norderstedt, Germany ; ²Gulf Med Univ Sch of Med Ajman, United Arab Emirates ; ³Private Urology Practice Bremerhaven, Germany and ⁴Bayer Schering Pharma Berlin, Germany.

Objectives: This study was undertaken to investigate the efficacy and safety of Vardenafil in combination with testosterone for the treatment of erectile dysfunction (ED) in hypogonadal patients who failed to respond to testosterone therapy (TT) alone.

Methods: This prospective study in hypogonadal patients investigated every 3 months non-responders to therapy with injectable long-acting testosterone undecanoate 1000mg alone. 122 testosterone deficient patients with average age of 56 ± 3.9 years received initially this preparation. 71 responded well within 3 months, 51 subjects did not. The non-responders showed lower T-levels, smoking and higher rates of concomitant diseases such as diabetes, hypertension, dyslipidemia and drugs. 34/51 non-responders received combination treatment with 20mg vardenafil on demand. Efficacy was assessed by the International Index of Erectile Function - Erectile Function domain (IIEF-EF) and a self-designed partner survey. The partners were asked 4 questions regarding erectile quality/rigidity, duration of erection, successful intercourse completion and frequency at baseline and after 4-6 weeks with at least 6 attempts of using vardenafil.

Results: 30/34 patients responded to this combination. IIEF-EF score improved from 12 to 24. These subjects reported to feel again spontaneous or nocturnal and morning erections or tumescence. No adverse effects were recorded. 10 patients who stopped TT relapsed to ED and decided to go back on TT. Partner survey showed significantly higher scores.

Conclusion: Data suggest that combination therapy of ED with testosterone and vardenafil in hypogonadal patients is safe and effective in a subset of hypogonadal patients who failed testosterone mono-therapy. TT led to recovery of erectile function in 54 per cent of patients after 12 weeks of treatment. In non-responders to TT alone, combination therapy with vardenafil improved results in the majority of patients.

Disclosures: AAY: Speaker, Bayer Schering Pharma, Berlin, Germany, Pfizer Germany, Ferring Pharmaceuticals. FS: Employee, Bayer Schering Pharma.

Nothing to Disclose: AH

P1-386

Testosterone Effect on Seminal Plasma Proteins: Initial Characterization by Top-Down Strategy.

G. Grande MD¹, F. Vincenzoni BD¹, D. Milardi MD¹, A. Bianchi MD¹, A. Giampietro MD¹, L. Tartaglione MD¹, M. Lo Russo MD¹, M. Castagnola BD¹, A. Pontecorvi MD¹, R. Marana MD¹ and L. De Marinis MD¹.

¹Catholic Univ Rome, Italy.

Testosterone directly regulate a vast number of physiological events. Transcriptional effects are mediated through binding of androgen-AR complexes to specific DNA sequences. To investigate the effect of testosterone on seminal protein expression, we performed proteomic studies on human seminal plasma by a top-down approach. Semen samples were collected by 3 fertile normospermic men (sperm concentration (mean±SD) $56.67 \pm 15.27 \times 10^6$ /ml; progressive motility $54.0 \pm 5.29\%$; normal morphology $39.0 \pm 6.56\%$) and 3 patients affected by secondary hypogonadism (sperm concentration $50 \pm 34 \times 10^6$ /ml; progressive motility $32.0 \pm 13.4\%$; normal morphology $15.3 \pm 6.21\%$). Hormonal blood assay were performed, with evidence of testosterone 1.59 ± 0.18 ng/ml (n.r. 3.5-8.0), estradiol 10.0 ± 2.0 pg/ml (n.r. 20-40), LH 1.57 ± 0.06 UI/l (n.r. 2.5-10.0), FSH 1.7 ± 0.1 UI/l (n.r. 2.5-8) in hypogonadal men and testosterone 4.9 ± 1.3 ng/ml, estradiol 25 ± 4.93 pg/ml, LH 5.1 ± 2.12 UI/l, FSH 4.2 ± 1.2 UI/l in controls. An aliquot of seminal plasma was mixed with aqueous trifluoroacetic acid, and centrifuged. The upper acidic supernatant was analyzed by an Ultimate 3000 Nano/Micro-HPLC apparatus equipped with an FLM-3000-Flow manager module coupled to an LTQ Orbitrap XL apparatus. The column was a Dionex C18 with 3 μ m particle diameter. The chromatography eluents were A TFA/H₂O 0.056% (v/v) and B CH₃CN+ 0.050% TFA. The applied gradient was linear from 0 to 50% of solvent B in 60 min, at flow rate of 4.5 μ l/min. The LTQ-Orbitrap mass spectrometer was operated in data dependent mode in which each full MS scan was followed by three MS/MS scans. The most abundant molecular ions were dynamically selected and fragmented by collision-induced dissociation (CID) using a normalized collision energy of 35%. Tandem mass spectra were searched against the Swiss-Human.fasta database using SEQUEST (Proteome Discoverer software, ThermoFisher). The results were filtered using the following criteria: XCorr versus charge 1.8, 2.5, for 2+, 3+ ions; mass accuracy 3 ppm; high value peptide confidence. A pattern of 114 proteins were present in normonadic patients and was compared with proteic spectrum of hypogonadal men. Among these 114 proteins, 20 were absent in hypogonadal men. This report is the first identification, based on new approach and stringent criteria, of seminal plasma proteome in hypogonadal men. This report support the role of the testosterone in the regulation of the proteic expression in seminal fluid.

Nothing to Disclose: GG, FV, DM, AB, AG, LT, MLR, MC, AP, RM, LDM

P1-387

The Dose-Response Relationship between Human Chorionic Gonadotropin and Intratesticular Testosterone in Normal Men with Experimentally-Induced Hypogonadism.

MY Roth MD^{1,2}, ST Page MD, PHD^{1,2}, K Lin MD¹, BD Anawalt MD^{1,2}, AM Matsumoto MD^{1,2,3}, CN Snyder MD^{1,2}, BT Marck BS³, WJ Bremner MD, PHD^{1,2} and JK Amory MD, MPH^{1,2}.

¹Univ of Washington Sch of Med Seattle, WA ; ²Univ of Washington Seattle, WA and ³Veteran Affairs Puget Sound Hlth Care Syst Seattle, WA.

Background: Intratesticular testosterone (ITT) is essential for spermatogenesis, yet the dose-response relationship between ITT and spermatogenesis in man is unknown. In addition, whether exogenous testosterone administration increases ITT in gonadotropin-suppressed men is also unknown. We experimentally induced low levels of ITT in normal men with the gonadotropin releasing hormone antagonist acyline, then either stimulated testosterone biosynthesis with graded doses of hCG to determine the dose-response relationship between hCG and ITT, or treated men with exogenous testosterone to determine whether exogenously administered testosterone would increase ITT.

Methods: We measured serum hormones and intratesticular hormones by testicular fine-needle aspiration in 31 normal men randomized to one of four doses of human chorionic gonadotropin (hCG) - 0 IU, 15 IU, 60 IU or 125 IU subcutaneously every other day for 10 days. A fifth group of 8 men was treated with acyline and testosterone gel 7.5gm daily to determine the impact of exogenous testosterone on ITT. Subjects received acyline to suppress gonadotropin production. Intratesticular hormones and corresponding serum hormones were measured at baseline and at the end of treatment on day 10. Intratesticular hormones were measured by mass spectrometry.

Results: Baseline ITT in all subjects averaged 3050 ± 1621 nmol/L. After ten days of treatment, there was a strong dose-response relationship between hCG and ITT. In addition, exogenous testosterone did not significantly increase ITT compared with acyline alone (Figure).

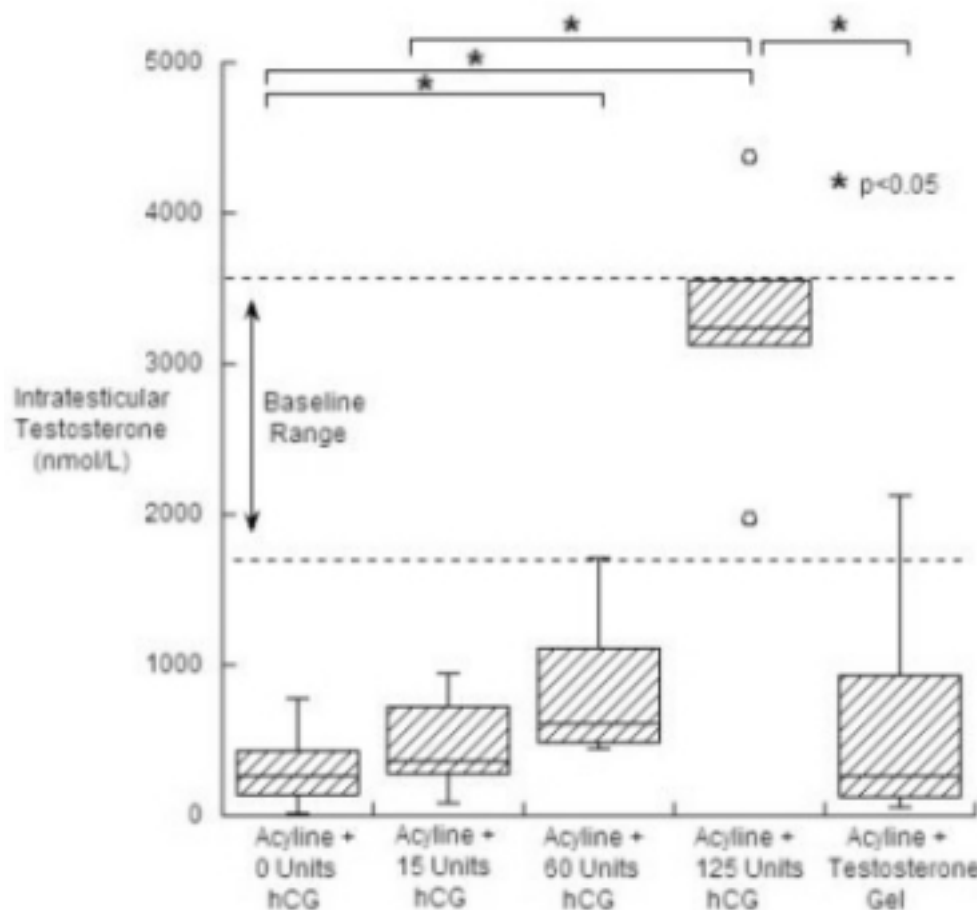


Figure: Boxplot of ITT in gonadotropin-suppressed subjects on day 10 by treatment group (n=6 for the 0 IU hCG group, n=7 for the 15 IU hCG group, n=5 for the 60 IU hCG and 125 IU hCG groups, n=8 for the testosterone group).

Conclusion: ITT has a clear dose-response relationship with exogenously administered hCG in normal men with experimentally-suppressed gonadotropins. In addition, treatment of men with testosterone gel does not increase ITT. Long term treatment with acyline and a low dose of hCG will allow for determination of the relationship between ITT and spermatogenesis.

Sources of Research Support: The National Institute of Child Health and Human Development supported this work through cooperative agreements U54-HD-12629 and U54 HD-42454 as part of the specialized Cooperative Centers Program in Reproductive Research and the Cooperative Contraceptive Research Centers Program.

Nothing to Disclose: MYR, STP, KL, BDA, AMM, CNS, BTM, WJB, JKA

P1-388

Exogenous Dihydrotestosterone Does Not Increase Intraprostatic Dihydrotestosterone Concentrations in Healthy Men.

ST Page MD, PhD¹, DW Lin MD¹, EA Mostaghel MD, PhD¹, B Marck², JL Wright MD¹, JK Amory MD¹ and AM Matsumoto MD^{1,2}.

¹Univ of Washington Sch of Med Seattle, WA and ²VA Puget Sound Hlth Care Syst Seattle, WA.

Objective: Since the prostate is an androgen-sensitive organ, concern exists that androgen replacement might adversely impact prostate health in older men. The principal circulating androgen in men is testosterone (T) but within the prostate dihydrotestosterone (DHT) predominates due to the high level of expression of 5 α -reductase enzymes that convert T to DHT. 5 α -reductase inhibitors, which lower intraprostatic DHT, have been shown to lower the risk of prostate cancer; therefore, hormonal replacement strategies that do not increase intraprostatic DHT might be desirable. We hypothesized that exogenous DHT, by providing negative feedback to the pituitary and thus suppressing gonadotropin and T production, might paradoxically lower intraprostatic DHT and androgen-action within the prostate.

Methods: 31 healthy men ages 35-55 were randomly assigned to receive either 10 grams daily transdermal 0.7% DHT gel (70 mg DHT) or placebo gel for 4 weeks. Blood was obtained bi-weekly throughout the study. Prostate volume was measured at baseline, on Day 28 when a prostate biopsy was performed, and on Day 56. Serum and prostate tissue DHT and T concentrations were measured by liquid chromatography-tandem mass spectrometry and androgen-regulated gene expression was assessed by laser-capture microdissection and microarray analyses.

Results: 28 men completed all study procedures. Serum DHT increased nearly seven-fold during treatment with DHT gel (baseline DHT 0.98 \pm 0.28; Day 28 6.99 \pm 1.18 ng/ml) and was significantly higher than in the placebo group (P<0.001). Serum T concomitantly decreased in the DHT-treated group compared to baseline and placebo treatment (P<0.05). In contrast, intraprostatic DHT and T concentrations on Day 28 were no different in the two groups (DHT: Placebo=2.89 \pm 1.03 vs DHT gel= 3.1 \pm 1.66 ng/g; T: Placebo=0.62 \pm 0.38 vs. DHT gel=0.39 \pm 0.27). Similarly, prostate volume, serum PSA and androgen-regulated gene expression were not different between groups.

Conclusions: Increases in serum DHT do not increase intraprostatic levels of DHT and are not associated with increases in PSA, prostate volume, or prostate epithelial cell androgen-regulated gene expression in healthy men treated for 4 weeks. Changes in serum androgen concentrations are not necessarily mimicked within the prostate. DHT gel might have utility as part of an androgen replacement regimen for men.

Sources of Research Support: National Institutes of Health through grants from the National Institute of Aging (K23 AG027238), the NIDDK Pacific Northwest Prostate Cancer SPORE (P50CA97186) and the The Eunice Kennedy Shriver National Institute of Child Health and Human Development (Co-operative agreement U54 HD-42454). BHR Pharma, LLC provided transdermal DHT gel and funds for the study biopsy procedure through an investigator-initiated grant.

Nothing to Disclose: STP, DWL, EAM, BM, JLW, JKA, AMM

P1-389

Testicular Function in Boys with 47,XYY.

MD Zeger MD^{1,2}, C Levy MD¹, N Lahlou MD³ and JL Ross MD^{1,2}.

¹Nemours Philadelphia, PA ; ²Thomas Jefferson Univ Philadelphia, PA and ³Hosp St Vincent Paul Paris, France.

Objective: To describe testicular function phenotype of boys with the karyotype 47,XYY.

Background: The karyotype 47,XYY is common (1/1,000 males). Boys with 47,XYY have been described as having tall stature, normal body proportions, and normal or delayed puberty with normal fertility. There are infrequent reports of individuals with 47,XYY with oligozoospermia related to Sertoli-cell-only syndrome or to spermatogenic arrest.

Methods: Clinical assessment was performed on males with 47,XYY and age-matched control boys. Measurement of hormonal indices of testicular function were performed for the boys with 47,XYY only. Results are presented as means \pm SD.

Results: This study included 26 boys with 47,XYY, ages 4.3-14.4 years (9.5 ± 2.8 yrs) and 57 age matched controls, ages 4.2-13.9 years (9.7 ± 2.8 yrs). Reasons for diagnosis of 47,XYY included routine prenatal for advanced maternal age (n=7), developmental or behavioral concerns (n=16), hypotonia (n=2), unknown (n=1). Compared with controls, the boys with 47,XYY were taller (1.0 ± 1.2 vs. 0.2 ± 1.0 height SDS) and had similar weight and BMI SDS. Testicular SDS values on average were increased in the 47,XYY group. The youngest child with testicular volume ≥ 4 ml was 11.5 years. However the frequency of testicular enlargement (>10 ml) in the pubertal age range of 11-13 years was increased in the 47,XYY versus control boys (4/8 47,XYY vs 0/8 controls). Testosterone, LH, FSH, estradiol, and inhibin B values were normal for age and pubertal status in 24/26 boys. Two boys had low inhibin B levels for age, one was 9.8 years old, had normal, prepubertal testicular size (2 ml), testosterone and LH values. The other boy was 13.6 years, had an advanced bone age (16 yrs), tall stature ($+3.6$ SDS), and had increased BMI ($+2.1$ SDS), 15 ml testicular volume, Tanner 5 pubic hair, low inhibin B (6 pg/ml), elevated LH (5.2 mIU/ml) and FSH (11.2 mIU/ml) and pubertal testosterone levels (403 ng/dl).

Conclusions: This study supports normal testicular function and onset of puberty in most boys with 47,XYY. While there was no increase in precocious puberty, enlarged testes for age were seen in 10-13 year olds. In contrast to the data regarding normal testicular function and fertility, the elevated FSH and low inhibin B in 1 out of 26 boys may be consistent with Sertoli-cell-only syndrome with predicted infertility.

Disclosures: JLR: Consultant, Novo Nordisk.

Nothing to Disclose: MDZ, CL, NL

P1-390

CAG Repeat Androgen Receptor Polymorphism and Skewed X-Chromosome Inactivation Have Little Impact on the Klinefelter Syndrome Phenotype.

A. Bojesen MD, PhD¹ and C. H. Gravholt MD, PhD, Dr. Med. Sci.².

¹Vejle Hosp, Sygehus Lillebaelt Vejle, Denmark and ²Aarhus Sygehus, Aarhus Univ Hosp Aarhus, Denmark.

Background: The phenotype in Klinefelter syndrome (KS) is extremely variable with small testes being the only feature shared by all KS[1]. Skewed inactivation of the supra-numerical X-chromosome (SkewX) and the androgen receptor polymorphism (CAG repeat length polymorphism, (CAG)n) has been suggested as a plausible causes of the variation and has been studied, but with somewhat conflicting results[2-4].

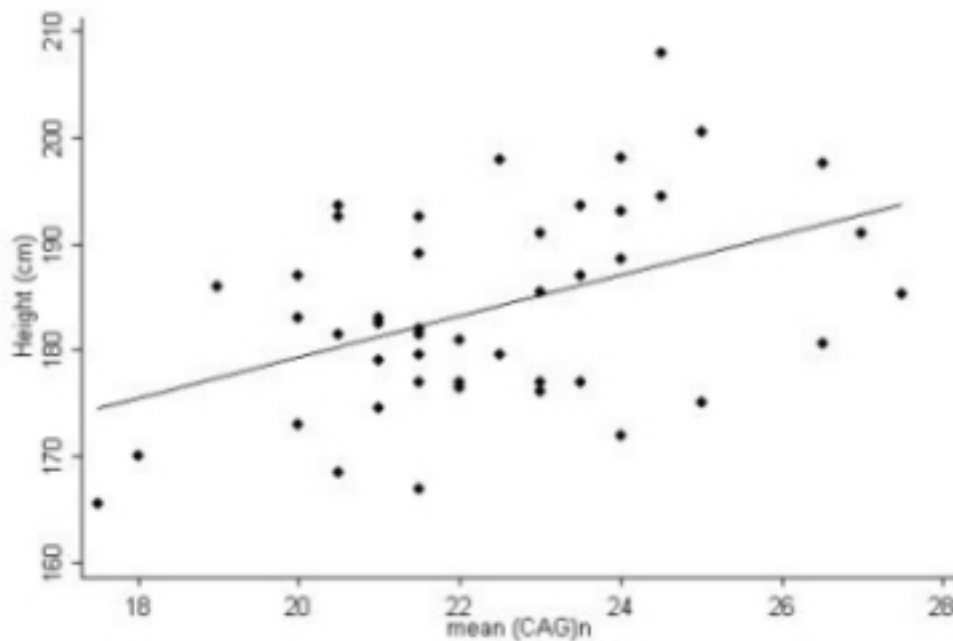
We therefore investigated the distribution of SkewX and (CAG)n in 70 KS patients and 70 age-matched controls and correlated the findings to anthropometrical, hormonal, metabolic and bone-related variables.

Materials and methods: SkewX and (CAG)n was analysed using a methylation sensitive PCR followed by capillary electrophoresis as described[2]. SkewX was defined as >80% of one X-chromosome inactivated. Anthropometric measures, whole body and regional DXA scans as well as sex hormones, insulin sensitivity and related hormones were measured.

Results: 46 of 70 KS men were heterozygous for (CAG)n. The mean CAG repeat length (CAG)n did not differ significantly from the (CAG)n from homozygous KS men or from the controls (22.4 vs. 22.5 vs. 22.0). SkewX was found in 12 of the 46 informative KS men (26%).

In KS, only height correlated to (CAG)n (fig. 1) ($r^2=0.20$, $p=0.002$), all other investigated parameters did not correlate to (CAG)n. In controls, bone mineral density at the spine and hip correlated positively with (CAG)n (spine: $r^2=0.11$, $p=0.006$ and hip: $r^2=0.09$, $p=0.01$).

SkewX did not correlate to any of the investigated parameters.



Discussion: In contrast to a previous report on adult KS men[2], (CAG)n did not explain much of the phenotypic variation in KS. The impact of (CAG)n on final height may be caused by later reactivation of the pituitary-gonadal axis as described [4] resulting in prolonged growth in KS men with longer (CAG)n. Skewed X-chromosome does not seem to influence the phenotype of KS, albeit the number of KS with SkewX was low.

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Nothing to Disclose: AB, CHG

P1-391

Socio-Economic Factors and Mortality in Klinefelter Syndrome.

A. Bojesen MD, PhD¹, K. Stochholm MD, PhD², S. Juul MD³ and C.H. Gravholt MD, PhD, Dr.Med.Sci².

¹Vejle Hosp, Sygehus Lillebaelt Vejle, Denmark ; ²Aarhus Sygehus, Aarhus Univ Hosp Aarhus, Denmark and ³Sch of Public Hlth, Aarhus Univ Aarhus, Denmark.

Introduction: Klinefelter syndrome 47,XXY (KS) is a relatively common cause of male infertility, hypogonadism and learning disability. Recent studies on morbidity and mortality showed a reduction in lifespan and increase in co-morbidity, the reasons however remains unknown; is it the chromosome aberration itself or is it caused by the poorer socio-economic status?

We therefore conducted an epidemiological study including all the KS patients diagnosed in Denmark and enrolling a 100 times larger control group of age-matched men, in order to study the socio-economic profile in KS and the impact of these factors on mortality.

Materials and methods: Register study using several Danish nationwide registries. 1,049 KS men and 100,824 controls were included. Information concerning marital status, fatherhood, level of education, income, and retirement were obtained from Statistics Denmark. 204 KS and 14,725 controls died during the study period. For the various socio-economic parameters, median age at first relevant episode was calculated. Marital status, educational level, unemployment, income, and retirement were analyzed using conditional logistic regression, where each case and his matched controls were one stratum. P-values <0.05 were considered significant.

Results: KS men had significantly fewer partnerships at a later age (27.1 years vs. 24.6 years), less fatherhoods at a later age (32.0 years vs. 27.0 years), lower educational level and lower income, but were retired at an earlier age (43.5 years vs. 60.3 years).

The mortality among KS men was significantly increased by a hazard ratio (HR) of 1.9, but even after adjustment for marital status and educational status the mortality was still significantly increased by HR of 1.5.

Discussion: This is the first study regarding the socio-economic outcome in KS. The results show a severely worse outcome in all investigated socio-economic parameters compared to the background population, even though the population in Denmark has equal and free admission to healthcare and education. The mortality was increased and may partially be caused by the poorer socio-economic status, but we cannot rule out an impact of the chromosome aberration itself.

We think that these results may become important tools in the clinical setting and in the counseling of future parents to a KS child, but the results also stress the need for more interventional studies in KS to improve the socio-economic and educational outcome in the future.

Nothing to Disclose: AB, KS, SJ, CHG

P1-392

Low Testosterone Is Associated with an Increased Risk of MACE Lethality in Subjects with Erectile Dysfunction.

G Corona MD^{1,2}, V Boddi MD¹, AD Fisher MD¹, G Forti MD¹ and M Maggi MD¹.

¹Andrology Unit Florence, Italy and ²Endocrinology Unit Bologna, Italy.

Introduction: Although testosterone (T) has been suggested to play a protective role against the development of atherosclerosis, studies demonstrating an association between low T, and incident major adverse cardiovascular events (MACE) are scanty in the general population and absent in subjects with erectile dysfunction (ED). The aim of this study is to investigate whether low T in subjects with ED predict incident fatal or non fatal MACE.

Methods: This is an observational prospective cohort study evaluating a consecutive series of 1687 patients attending our Andrological Unit for ED. Patients were interviewed using SIEDY and ANDROTEST structured interviews measuring components relative to ED and hypogonadal-related symptoms, respectively. Total testosterone was evaluated at baseline. Information on MACE was obtained through the City of Florence Registry Office.

Results: Among the patients studied 5.2, 13.8 and 22.4% were hypogonadal according to different thresholds (T < 8, 10.4 and 12 nmol/L or 230, 300 and 350 ng/dl, respectively). During a mean follow-up of 4.3±2.6 years, 139 MACE, 15 of which were fatal, were observed. Unadjusted incidence of MACE was not associated with T levels. Conversely, the proportion of lethal events among MACE was significantly higher in hypogonadal patients, using either 10.4 nmol/l (300 ng/dl) or 8 nmol/L (230 ng/dl) thresholds. However, after adjustment for age and Chronic Diseases Score in a Cox regression model, only the association between incident fatal MACE and T < 8 nmol/L (230 ng/dl) was confirmed (HR=7.1[1.8-28.6]; p<0.001). Interestingly, measuring hypogonadal-related symptoms and signs through ANDROTEST, only fatal MACE were also associated with a higher score (HR=1.2[1.0-1.5] for each ANDROTEST score increment; p=0.05 after adjustment for age and Chronic Diseases Score).

Conclusions: T levels are associated with a higher mortality of MACE. The identification of low testosterone levels should alert the clinician thus identifying subjects with an increased cardiovascular risk.

Nothing to Disclose: GC, VB, ADF, GF, MM

P1-393

Architectural Decay of the Skeleton and Accumulation of Visceral Abdominal Fat Following Androgen Deprivation Therapy for Prostate Cancer.

EJ Hamilton^{1,2}, EJ Gianatti^{1,2}, BJ Strauss³, J Wentworth¹, A Ghasem-Zadeh¹, R Zebase¹, D Lim Joon², D Bolton^{1,2}, E Seeman^{1,2}, JD Zajac^{1,2} and M Grossmann^{1,2}.

¹Univ of Melbourne Heidelberg, Australia ; ²Austin Hlth Heidelberg, Australia and ³Monash Univ Clayton, Australia.

Introduction: Androgen deprivation therapy (ADT) for treatment of prostate cancer reduces bone mineral density (BMD), but the structural basis of the BMD deficit has not been determined. ADT also increases fat mass, but the relationship between sex steroid deficiency and abdominal fat distribution is controversial.

Aim: We hypothesized that ADT reduces cortical and trabecular volumetric BMD (vBMD) and increases visceral abdominal fat following ADT for non-metastatic prostate cancer.

Methods: 26 males, mean age 70.6 (± 6.8) years, with non-metastatic prostate cancer were studied at baseline, 6 and 12 months after commencing ADT. Bone microarchitecture was assessed using high resolution peripheral quantitative CT (HR-pQCT), and abdominal fat distribution was assessed using Slice-O-Matic software analysis of L4-5 computed tomography images.

Results: ADT decreased total testosterone (12.8 to 0.7nmol/L), and estradiol (103.7 to 29.6pmol/L), all p<0.001. After 12 months of ADT, total vBMD at the distal radius decreased by 5.2%, p<0.001, due to a decrease in cortical vBMD (-11.3%, p<0.001) and trabecular vBMD (-3.5%, p<0.01). Changes at the tibia were similar, with a decrease in total vBMD (-4.2%, p<0.001), cortical vBMD (-6.0%, p<0.001) and trabecular vBMD (-1.5%, p<0.01). Osteocalcin (15.40 to 28.33ng/mL), P1NP (44.19 to 81.38mcg/L), and C-telopeptide (0.357 to 0.702ng/mL), increased significantly (all p<0.001). Subcutaneous fat (240.7 ± 107.5 to 271.27 ± 92.8cm², p=0.001) and visceral fat area (160.8 ± 61.7 to 195.9 ± 69.7cm², p=0.001) increased, as did total cholesterol (4.7 + 1.0 to 5.3, + 1.1 mmol/L, p=0.002) and triglycerides (1.1 + 1.5 to 1.6 + 1.1 mmol/L p=0.01). 12 months ADT increased Leptin (12.5 to 18.8ng/mL, p=0.007) whereas Adiponectin increased at 6 months (21.6 to 30.2mg/mL, p=0.025), but not 12 months.

Conclusions: ADT-induced sex steroid deficiency is associated with decay of both cortical and trabecular bone, as well as accumulation of visceral abdominal fat. Since fractures, insulin resistance and cardiovascular disease are major causes of morbidity and mortality in men with non-metastatic prostate cancer, a better understanding of physiological changes associated with ADT should improve the identification of those at highest risk of these complications in the future.

Sources of Research Support: NHMRC Health Professional Fellowship to M. Grossmann.

Nothing to Disclose: EJH, EJG, BJS, JW, AG-Z, RZ, DLJ, DB, ES, JDZ, MG

P1-394

Low Testosterone (T) Is Not Associated with Subclinical Cardiovascular Disease (CVD) in Men with or at Risk for HIV Infection.

AK Monroe MD, MSPH¹, AS Dobs MD, MHS¹, X Xu MS¹, FJ Pallela MD², LA Kingsley DrPH³, WS Post MD, MS¹, MD Witt MD⁴, S Bhasin MD⁵ and TT Brown MD, PhD¹.

¹The Johns Hopkins Univ Sch of Med Baltimore, MD ; ²The Johns Hopkins Univ Sch of Med Baltimore, MD ; ³Northwestern Univ Feinberg Sch of Med Chicago, IL ; ⁴Univ of Pittsburgh Graduate Sch of Public Hlth Pittsburgh, PA ; ⁵The Johns Hopkins Univ Sch of Med Baltimore, MD ; ⁶David Geffen Sch of Med at UCLA Torrance, CA and ⁷Boston Univ Sch of Med Boston, MA.

Background

Hypogonadism has been associated with CVD in the general population. Although lower T concentrations have been reported among HIV+ populations, the relationship between HIV, androgens, and subclinical CVD is unknown. We hypothesized that HIV+ men would have lower T and higher prevalence of subclinical CVD compared with HIV- men.

Methods

We analyzed data from the Multi-Center AIDS Cohort Study (MACS), which enrolled 6,973 HIV+ and HIV- men who have sex with men starting in 1984. The MACS CV Substudy is a nested cohort of men with no prior CVD (n=947, 856 with hormone data). To assess subclinical atherosclerosis, participants underwent assessment of far wall common carotid intima-media thickness (IMT) by B-mode ultrasound and coronary artery calcium (CAC) with computerized tomography. T and sex hormone binding globulin (SHBG) were measured from archived serum by liquid chromatography-tandem mass spectrometry and radioimmunoassay, respectively. Free T (FT) was calculated. We performed multivariable linear regression to examine the relationship between HIV status and sex hormones adjusting for age, race, BMI and clinic site. We also used multivariable regression to evaluate the association between sex hormones and subclinical CVD adjusting for the same covariates and smoking, diabetes mellitus, lipids (LDL and non-HDL cholesterol), and hypertension.

Results

Compared to the HIV- men (n=318), HIV+ men (n=538) were younger (48.8 v 52.6 yrs, p<0.0001), were more likely to be black (33.5% v 23.9%, p=0.003), and had a lower BMI (25.6 v 26.8 kg/m², p<0.001). Of the HIV+ men, 91.5% were HAART-experienced. HIV+ men had a significantly lower adjusted mean log FT than HIV- men (4.46 v 4.59 ng/dL, p<0.01). Adjusted mean log SHBG was significantly higher in HIV+ men than HIV- men (4.35 v 3.88 mg/dL, p<0.01). Overall, 31.1% of the study sample (HIV+ men: 29.0% v HIV- men: 34.6%, p=0.09) had CAC score >10 and mean IMT was 0.758 mm. There were no statistically significant associations between FT, SHBG, and prevalence of CAC or common carotid IMT in adjusted multivariable analyses.

Conclusions

HIV-infected men have a lower FT and higher SHBG compared to HIV-uninfected men. However, lower FT was not associated with subclinical cardiovascular disease.

Sources of Research Support: U01-AI-35042, 5-MO1-RR-00722 (GCRC), M01 RR00425 (GCRC-Harbor-UCLA), U01-AI-35043, U01-AI-37984, U01-AI-35039, U01-AI-35040, U01-AI-37613, U01-AI-35041. NIH (NCAAM) 5K23AT2862 (TTB). 2 T32 HP 10025-15-00.

Nothing to Disclose: AKM, ASD, XX, FJP, LAK, WSP, MDW, SB, TTB

P1-395

Long-Term Effects of Normalization of Testosterone on Variables of the Metabolic Syndrome in Hypogonadal Men.

SY Kalinchenko MD, PhD¹, YA Tishova MD, PhD¹, GJ Mskhalaya MD², LJG Gooren MD, PhD³, EJ Giltay MD, PhD⁴ and F Saad DVM, PhD⁵.

¹People's Friendship Univ of Russia Moscow, Russian Federation ; ²Scientific Ctr for Endocrinology Moscow, Russian Federation ; ³VU Univ Med Ctr Amsterdam, Netherlands ; ⁴Leiden Univ Med Ctr Leiden, Netherlands and ⁵Bayer Schering Pharma AG Berlin, Germany.

Objectives: Men with the metabolic syndrome have low plasma testosterone (T) levels. We aimed to study whether normalization of plasma T in such men improved features of the metabolic syndrome over the longer term.

Patients: 145 men, 35 to 70 years, with the metabolic syndrome (Alberti et al. *Circulation* 2009; 120:1640-5), and hypogonadism (baseline total testosterone level <12.0 nmol/l or calculated free T level < 225 pmol/L). They had received treatment for 30 weeks with either parenteral testosterone undecanoate (n=88); TU; 1000 mg IM) or placebo (n=57) with measurements at baseline, and after 6 and 18 weeks (reported elsewhere). After 30 weeks all men received TU for an additional 33 weeks. Main Outcome Measures: body mass index (BMI), waist circumference (WC), hip circumference (HC), insulin, glucose, cholesterol, triglycerides, high (HDL) and low density lipoproteins cholesterol (LDL) and triglycerides (TG).

Results: In the men who had received TU for 66 weeks there was a progressive improvement of BMI, WC, HC, insulin, and glucose, with no progressive improvement of cholesterol, LDL, HDL and TG after 30 weeks. In the men who had received placebo for 30 weeks and then shifted to TU for 33 weeks all study variables improved and caught up with the other group over these 33 weeks.

Conclusions: The beneficial effects of normalization of T in hypogonadal men with the metabolic syndrome on BMI, WC, HC, insulin, and glucose are progressive over at least 66 weeks, while maximal effects on cholesterol, LDL, HDL and TG have been reached after 30 weeks.

Sources of Research Support: Partly funded by Bayer Schering Pharma, Berlin, Germany.

Disclosures: SYK: Speaker, Bayer Schering Pharma, Pfizer Russia, Eli Lilly Russia, Solvay Russia. YAT: Speaker, Bayer Schering Pharma. LJGG: Speaker, Bayer Schering Pharma. FS: Employee, Bayer Schering Pharma.

Nothing to Disclose: GJM, EJG

P1-396

Blood Inflammatory Proteins and Psychological Symptoms during Testosterone Supplementation in Hypogonadal Men with the Metabolic Syndrome.

EJ Giltay MD, PhD¹, YA Tishova MD, PhD², GJ Mskhalaya MD³, LJG Gooren MD, PhD⁴, F Saad DVM, PhD^{5,6} and SY Kalinchenko MD, PhD².

¹Leiden Univ Med Ctr Leiden, Netherlands ; ²People's Friendship Univ of Russia Moscow, Russian Federation ; ³Scientific Ctr for Endocrinology Moscow, Russian Federation ; ⁴VU Med Ctr Amsterdam, Netherlands ; ⁵Bayer Schering Pharma Berlin, Germany and ⁶Gulf Med Univ Sch of Med Ajman, United Arab Emirates.

Introduction: Low testosterone levels and the metabolic syndrome (MetS) in men are associated with high levels of blood inflammatory proteins. There may be a direct link between inflammatory activation and low mood states.

Aim: To assess whether the beneficial effects of testosterone administration on subjective symptoms are determined by blood inflammatory protein levels.

Patients and Methods: In a randomized, placebo-controlled, double-blind trial 184 men suffering from both the metabolic syndrome and hypogonadism were included. At baseline C-reactive protein, interleukin 1b (IL-1b), interleukin-6 (IL-6) , interleukin-10 (IL-10), and tumor necrosis factor- α (TNF α) were measured. In the present analysis, 67 men treated with parenteral testosterone undecanoate (TU; 1000 mg IM testosterone undecanoate; Nebido®) were included, with measurements at baseline, and after 18 (n=66), 30 (n=65), 42 (n=58), 54 (n=55), and 66 (n=51) weeks.

Main Outcome Measures: The Beck Depression Inventory (BDI), Aging Males' Symptoms (AMS) scale, and International Index of Erectile Function 5-item (IIEF-5) scale were analysed according to baseline inflammatory proteins using multilevel analysis.

Results: The 67 men were aged mean 52.3 years old (SD 10.0; range 35-69), with a mean body mass index of 35.8 kg/m² (SD 4.5; range 24-54). Total testosterone level was 8.2 nmol/L (SD 4.4) at baseline, and normalized upon treatment. IL-1b, TNF α , and C-reactive protein decreased, while IL-6 and IL-10 did not change significantly. No associations were found with IL-1b, TNF α , and C-reactive protein. High baseline IL-6 and IL-10 levels were associated with higher BDI, AMS and lower IIEF scores over time.

Conclusions: High levels of IL-6 and the anti-inflammatory cytokine IL-10 were associated with more psychological discomfort in hypogonadal patients with the MetS during long-term TU administration.

Sources of Research Support: Partially funded by Bayer Schering Pharma, Berlin, Germany.

Disclosures: YAT: Speaker, Bayer Schering Pharma. LJGG: Speaker, Bayer Schering Pharma. FS: Employee, Bayer Schering Pharma. SYK: Speaker, Bayer Schering Pharma, Pfizer Russia, Eli Lilly Russia, Solvay Russia.

Nothing to Disclose: EJG, GJM

P1-397

The Effect of Testosterone Undecanoate on Cardiovascular Risk Factors in Men with Hypogonadism in Clinical Practice.

V Muraleedharan MBBS, MD, MRCP^{1,2}, N Ranjan MBBS¹, C Rolfe Dip. Nursing¹ and T H Jones BSc, MD, FRCP^{1,2}.

¹Barnsley Hosp NHS Foundation Trust Barnsley, UK and ²Univ Of Sheffield Sheffield, UK.

Background: Epidemiological studies have shown a close link between low testosterone and metabolic syndrome. Evidence suggests that testosterone replacement therapy (TRT) improves cardio-metabolic risk factors in men with hypogonadism.

Aim: This is a retrospective audit of cardio-metabolic parameters of hypogonadal men treated with testosterone undecanoate (Nebido ®) in routine clinical practice.

Methods: Patients with hypogonadism on testosterone undecanoate injections from 2005 to 2009 were identified from hospital data base. Weight, blood pressure, non-fasting lipid profile and HbA1c (diabetic men n=42) were collected at 3, 6 and 12 months. As different patient groups had 3, 6 or 12 months data, the base line value for each group was taken at each step and compared.

Results: Of the 120 patients 99 (82%) had previous TRT with other preparations. Mean age was 48±16 years. After excluding patients with changes in lipid lowering medications (n= 3) over the treatment period, the total cholesterol (TC) and calculated LDL- cholesterol (cLDL) showed significant improvement in one year. TC at three months was 3.8±1.4 (compared with 4.6mmol/L±1.4 at baseline p= 0.006 n=47), at 6 months 4.2±1.3 (vs. 4.5 ± 1.2;p=0.03, n= 36) and at 1 year 4.1±1.1 (vs. 4.6±1.4;p=0.005, n=41). cLDL at 3 months was 1.9mmol/L±1 (vs. 2.3 ±1.1 at baseline; p= 0.006, n=43) at 6 months 2.1±1 (vs. 2.3±1.2;p=0.13, n=38) and at 12 months 2.1±1 (vs. 2.4±1.1;p= 0.046, n=33). HDL- cholesterol (HDL) did not show any significant change at 3 months 1.1mmol/L±0.5(vs. 1.1±0.3; p=1, n=45), 6 months 1.05±0.3 (vs. 1.08±0.3;p=0.33, n=44) and 1.04 ± 0.28 (vs. 1.12±0.34; p=0.08, n=35) at 12 months. At 12 months no significant changes in weight (98 vs. 99kg; p=0.09, n=67) or blood pressure (SBP 135 vs. 137 p=0.3 DBP 78 vs. 80 p=0.2 n=69) were noted.

In diabetic patients HbA1c (after excluding the patients with diabetic medication increase and one with obesity surgery) fell by 0.4% at 6 months which did not reach significance (HbA1c was 7.1±1.3 vs. 7.5±1%;p=0.07, n=23) or 1 year (7.5 ±1 vs. 7.5±1.3%;p=1, n=23).

Discussion: Testosterone undecanoate therapy in routine clinical practise had beneficial effects on total cholesterol and cLDL, but no significant effect on HDL. No beneficial or adverse effects were found on weight and blood pressure.

Nothing to Disclose: VM, NR, CR, THJ

P1-398

Upon Continued Testosterone Administration to Hypogonadal Men, Improvements in Features of the Metabolic Syndrome Are Largest over the First 12-24 Months but Benefits Are Maintained.

F Saad DVM, PhD^{1,2}, A Haider MD, PhD³, AA Yassin MD, PhD^{2,4} and LG Gooren MD, PhD⁵.

¹Bayer Schering Pharma Berlin, Germany ; ²Gulf Med Univ Sch of Med Ajman, United Arab Emirates ; ³Private Urology Practice Bremerhaven, Germany ; ⁴Segeberger Kliniken Norderstedt, Germany and ⁵VU Med Ctr Amsterdam, Netherlands.

Objectives: Elderly men often show a concurrence of a decline of testosterone with features of the metabolic syndrome. This study tested the effects of normalization of testosterone over a period of 42 months.

Materials and methods: 122 men aged 34 - 69 years (mean \pm SD = 59.5 \pm 6.0), with baseline testosterone 5.9 - 12.1 nmol/L were treated with parenteral testosterone undecanoate for 42 months as the sole intervention.

Results: Plasma levels of testosterone rose from 9.3 \pm 1.7 to 18.7 \pm 2.1 nmol/L reaching their maximum at 9 months and remaining stable over the next 33 months. There was a remarkable progressive decline of body weight and waist circumference over the full study period, most outspoken over the first 24 months. Plasma glucose, cholesterol, triglyceride, and LDL-cholesterol decreased significantly over the 24 month study period and then stabilized. Plasma HDL increased significantly over the first 24 months and then declined. There was a significant decrease of levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) over the first 24 months, then values leveled off. Changes in variables were largely correlated with changes in testosterone levels. At baseline 47/122 met the criteria of the metabolic syndrome as defined by the National Cholesterol Education Program (2001); after two years of testosterone treatment this number had declined to 11/122.

Conclusion: With testosterone treatment over 42 months, the most significant improvement of the metabolic syndrome was noted over the first 12 months with further improvement over the following 12 months. Body weight and waist circumference declined further but glucose and lipids and liver functions did not further improve but were stable with continued testosterone treatment.

Disclosures: FS: Employee, Bayer Schering Pharma. AAY: Speaker, Bayer Schering Pharma, Berlin, Germany, Pfizer Germany, Ferring Pharmaceuticals. LGG: Speaker, Bayer Schering Pharma.

Nothing to Disclose: AH

P1-399

A High Prevalence of the Metabolic Syndrome (MetSyn) among Hypogonadal Males in the TRiUS Registry.

RK Bhattacharya MD¹, G Blick MD², MM Miner MD³, M Khera MD⁴, H Kushner PhD⁵ and D Nguyen PharmD⁵.

¹Univ of Kansas Med Ctr Kansas City, KS ; ²Circle Med Norwalk, CT ; ³Warren Alpert Sch of Med, Brown Univ Providence, RI ; ⁴Baylor Coll of Med Houston, TX and ⁵Auxilium Pharm Malvern, PA.

Background/Introduction:

A recent report suggests that hypogonadism is highly prevalent in men presenting with hypertension. We questioned whether the converse was true, if high rates of hypertension, dyslipidemia, central obesity and the metabolic syndrome (MetSyn) were present in hypogonadal men. TRiUS (Testim Registry in US) with 72 sites was started to collect scientific data on hypogonadal men initiated on testosterone replacement therapy with TESTIM in a variety of practice settings.

Results:

To date, 849 patients are enrolled in TRiUS of whom 553 (65%) have primary (including enzymatic defects N=115, HIV infection N=80, testicular damage from alcohol, drugs and heavy metals N=45, other N=325) and 296 (35%) have secondary hypogonadism based on clinician's response on the clinical report form. Definitive data to establish the presence or absence of MetSyn were available for 462 enrollees. Using the ATP III criteria 288/462 men (62%) had MetSyn. Mean serum testosterone (T) level in this group at baseline was 299±173 ng/dL. The presence of MetSyn was strongly associated with low testosterone levels at baseline (Relative risk=1.24, 95% CI=1.1-1.4, P=0.005). In addition, the cohort of 462 men had high rates of hypertension (51.4%), central obesity (48.6%), high fasting plasma glucose (40.5%), hypertriglyceridemia (49.8%), and low HDL levels (74.9%). The men were treated with TESTIM 1% dosed at 5 mg (91%) or 10 mg (8%) for a duration of 163±39 days; 341 (74%) completed 6 months of treatment. At 6 months, mean T levels increased significantly in both the MetSyn and non-MetSyn groups. In the MetSyn group, mean T levels increased from 264±121 to 430±291 (P<0.001) with statistically significant reductions in blood pressure (SBP mmHg: -3.5±17.9, P=0.01; DBP: -2.1±11.3, P=0.02; MBP: -2.6±12.0, P=0.007) and waist circumference (-3.1±10.0 cm, P<0.001). Body weight, glucose and triglycerides also declined compared to baseline, but not significantly. Among the MetSyn subjects who completed the six month visit and had an HDL measurement, HDL increased 1.4±9.8, P=0.27. LDL decreased -5.1 which was also not statistically significant.

Conclusions:

Low testosterone levels among males, may be an emerging marker associated with major cardiovascular risk factors of hypertension, dyslipidemia and central obesity. There may be measurable impact in treating male hypogonadism. Full analysis of the data will be available.

Sources of Research Support: Auxilium Pharmaceuticals.

Disclosures: RKB: Consultant, Auxilium. GB: Consultant, Auxilium. MMM: Consultant, Auxilium. MK: Consultant, Auxilium. HK: Employee, Auxilium. DN: Employee, Auxilium.

P1-400

Higher Testosterone Concentrations in Type 2 Diabetic Men Treated with Metformin.

Ajay Varanasi¹, Saurabh Bharadwaj¹, Ajaz Banka¹, Sandeep Dhindsa¹ and Paresh Dandona¹.

¹SUNY at Buffalo Buffalo, NY.

One third of men with type 2 diabetes have subnormal testosterone concentrations which are inversely related to plasma C- reactive protein (CRP) concentrations. We have now investigated whether drugs used in the treatment of type 2 diabetes affect testosterone concentrations in clinical practice. A review of 297 males with type 2 diabetes was carried out using the following indices: total testosterone (TT), calculated free testosterone (cFT), calculated bioavailable testosterone (cBT), sex hormone binding globulin (SHBG) and CRP. Mean hormone and CRP concentrations were adjusted for age, BMI, HbA1c, co-morbidities (such as congestive heart failure, coronary artery disease, chronic kidney disease using Charlson index) and medications. The concentrations of these indices were compared between patients on treatment with metformin, insulin, exenatide, sitagliptin, sulphonylureas and thiazolidinediones (TZD). Patients on metformin (M) had significantly higher TT, cFT and cBT concentrations and lower prevalence of subnormal testosterone concentrations when compared to non metformin users (NM) (Table 1). TZD treated patients had higher, but not statistically significant, TT, cFT and cBT concentrations. Patients treated with a combination of metformin and TZD also had significantly higher TT ($P=0.01$), cBT ($P=0.021$) and cFT ($P=0.022$) concentrations and lower prevalence of subnormal testosterone concentrations ($P=0.01$). Metformin and possibly TZD may have a beneficial effect on testosterone concentrations.

| Table 1 | Metformin NonUsers | Metformin users | P-Value |
|----------------------|--------------------|-----------------|---------|
| Number of Patients | 96 | 201 | |
| Age | 60.302 ± 2.2 | 57.07 ± 1.8 | 0.03* |
| BMI | 35.32 ± 1.4 | 34.1 ± 1.2 | 0.20 |
| cFT(ng/dl) | 6.95 ± .32 | 8.29 ± .44 | 0.01* |
| TT(ng/dl) | 314.5 ± 12.4 | 381.9 ± 8.8 | 0.001* |
| cBT(ng/dl) | 159.2 ± 4.2 | 189.5 ± 4.6 | 0.001* |
| SHBG(ng/dl) | 29.4 ± 2.1 | 29.6 ± 2.2 | 0.97 |
| CRP(mg/l) | 5.2 ± .26 | 3.5 ± .6 | 0.126 |
| % with subnormal cFT | 40 | 26 | 0.01* |

Disclosures: PD: Speaker, Sanofi-Aventis, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals; Research Funding, Sanofi-Aventis, GlaxoSmithKline, Amylin Pharmaceuticals.

Nothing to Disclose: AV, SB, AB, SD

P1-401

Red Cell Distribution Width (RDW): An Index of Abnormal Erythropoiesis and a Novel Biomarker in Cardiovascular Disease - Is Testosterone Deficiency Behind?.

EA Jankowska MD PhD^{1,2,3}, E Kalicinska MD^{1,2}, W Banasiak MD PhD² and P Ponikowski MD PhD^{1,2}.

¹Wroclaw Med Univ Wroclaw, Poland ; ²Military Hosp Wroclaw, Poland and ³Polish Academy of Scis Wroclaw, Poland.

Background: Red cell distribution width (RDW) is a measure of anisocytosis, reflecting deranged erythropoiesis. So far, this simple, cheap and easy obtainable index has been neglected. Recently, high RDW has been linked to poor outcome in patients with cardiovascular disease, including chronic heart failure (CHF). Pathophysiological background of high RDW in CHF remains unclear.

Methods: We examined 200 men with stable systolic CHF (age: 60±11 years, left ventricular ejection fraction [LVEF]: 30±8%, NYHA class reflecting the severity of CHF I/II/III/IV: 26/92/70/12, ischaemic aetiology: 66%).

Results: In men with CHF, mean RDW was 14.1±1.8% and high RDW was related to reduced haemoglobin ($r=-0.27$, $p<0.001$) and high serum soluble transferrin receptor reflecting the presence of iron deficiency ($r=0.40$, $p<0.01$). In univariate models, high RDW was associated with advanced NYHA class, reduced LVEF, high N-terminal pro-B type natriuretic peptide (NT-proBNP), high high-sensitivity C-reactive protein (hsCRP), high erythrocyte sedimentation rate, low sodium, impaired glomerular filtration rate, low total cholesterol, increased bilirubin, and reduced serum estimated free testosterone (eFT) (all $p<0.05$), but was related neither to age, BMI, CHF aetiology nor the presence of diabetes. In a multivariable model, we have established 3 major determinants of high RDW in men with CHF: high plasma NT-proBNP ($r=0.47$, $p<0.001$), reduced serum eFT ($r=-0.26$, $p<0.001$) and high serum hsCRP ($r=0.17$, $p<0.01$). In men with systolic CHF, independently of the other clinical parameters and androgen status, high RDW was related to impaired exercise capacity (reduced peak oxygen consumption - $r=-0.26$, $p=0.002$, high VE-VCO₂ slope - $r=0.41$, $p<0.001$, reduced distance during a 6-minute walk test - $r=-0.31$, $p<0.001$), impaired quadriceps muscle strength ($r=-0.31$, $p<0.001$), intensive depressive symptoms ($r=0.27$, $p=0.002$) and poor quality of life ($r=0.22$, $p=0.006$). One-year all-cause mortality rates increased along with the increasing quartiles of RDW (4%, 10%, 16%, 24% - respectively for Q1≤13.0%, Q2>13.0% and ≤13.6%, Q3>13.6% and ≤14.8%, Q4>14.8%, $p<0.01$).

Conclusions: In men with stable systolic CHF, high RDW is related to exercise intolerance, poor quality of life and increased mortality. In this group of patients, increased RDW reflects the severity of CHF, the presence of both low-grade inflammation and deficiency in circulating testosterone.

Nothing to Disclose: EAJ, EK, WB, PP

P1-402**Reduced Circulating Levels of Testosterone and Insulin-Like Growth Factor Predict the Prevalence of Hyperuricaemia, the Major Cardiovascular Risk Factor, in Men with Systolic Chronic Heart Failure.**

EA Jankowska MD PhD^{1,2,3}, P Rozentryt MD PhD⁴, B Ponikowska MD PhD⁵, J Nowak MD PhD⁴, L Borodulin-Nadziejka MD PhD⁵, L Polonski MD PHD⁴, W Banasiak MD PhD² and P Ponikowski MD PhD^{1,2}.

¹Dept of Heart Diseases Wroclaw, Poland ; ²Military Hosp Wroclaw, Poland ; ³Polish Academy of Scis Wroclaw, Poland ; ⁴ Silesian Ctr for Heart Diseases Zabrze, Poland and ⁵Wroclaw Med Univ Wroclaw, Poland.

Background: Hyperuricaemia (HU) is a marker of oxidative stress and endothelial dysfunction, and is also accepted as cardiovascular risk factor. HU constitutes an important element of pathophysiology of chronic heart failure (CHF) and predicts increased mortality in these patients. Multiple deficiencies in anabolic hormones (testosterone, dehydroepiandrosterone sulphate [DHEAS], insulin-like growth factor 1 [IGF1]) seen in elderly men predispose to metabolic and oxidative derangements. Although anabolic depletion is common in men with CHF and unfavourably affects outcome, it has not been studied as a factor promoting HU.

Methods: We examined 487 men with stable systolic CHF (mean age: 58±11 years [mean±SD], NYHA class I/II/III/IV: 55/227/170/35, LVEF: 29±8%, 71% of ischaemic aetiology).

Results: In a multiple regression model, serum uric acid (UA) was independently determined by serum total testosterone (TT) ($r=-0.19$, $p<0.0001$), serum IGF1 ($r=-0.18$, $p<0.0001$), age ($r=0.29$, $p<0.0001$), glomerular filtration rate estimated from the Cockcroft-Gault formula (eGFR, $r=-0.30$, $p<0.0001$) and NYHA class reflecting CHF severity ($r=0.14$, $p=0.002$). In men with CHF, mean values of UA, TT, IGF1 and eGFR were 7.0±2.0 mg/dL, 3.75±1.73 ng/mL, 141.6±57.8 ng/mL and 63.7±21.9 mL/min/1.73m², respectively. HU (serum UA≥9.5 mg/dL) was found in 13% of men with CHF. Receiver operating characteristics analysis revealed the cut-off values of established factors for predicting HU in men with CHF: TT ≤3.7 ng/mL, IGF1 ≤93.5 ng/mL, eGFR ≤56.8 mL/min/1.73m² and age ≥64 years. In a multiple logistic model, each factor was independently related to an increased prevalence of HU in men with CHF (TT ≤ vs. >3.7 ng/mL: OR=3.02, 95%CI: 1.56-5.88, $p=0.001$; IGF1 ≤ vs. >93.5 ng/mL: OR=2.01, 95%CI: 1.02-3.95, $p=0.04$; eGFR ≤ vs. >56.8 mL/min/1.73m²: OR=6.81, 95%CI: 3.57-12.99, $p<0.0001$; age ≥ vs. <64 years: OR: 8.22, 95%CI: 3.50-19.29, $p<0.0001$; NYHA class III-IV vs. I-II: OR=2.18, 95%CI: 1.18-4.01, $p=0.01$). HU was found in 0%, 6%, 17% and 24% of men with CHF with the presence of respectively 0, 1, 2-3 and 4-5 established risk factors ($p<0.0001$).

Conclusions: Testosterone and IGF1 deficiencies are accompanied by hyperuricaemia in men with CHF. Whether a supplementation of deficient anabolic hormones would improve oxidative metabolism and ameliorate HU in men with CHF, needs to be further studied.

Nothing to Disclose: EAJ, PR, BP, JN, LB-N, LP, WB, PP

P1-403

Risk of Venous Thromboembolism (VTE) in Estrogen-Treated Male-to-Female Transsexuals. a Review of Literature and Observations from 9 European Centers for Gender Dysphoria.

H Asscheman MD¹, G T'Sjoen MD², A Lemaire MD³, M Mas MD⁴, MC Meriggiola MD⁵, A Mueller MD⁶, J Buffat MD⁷, A Kuhn MD⁸, C Dhejne MD⁹, N Morel-Journel MD¹⁰ and LJ Gooren MD¹¹.

¹HAJAP Amsterdam, Netherlands ; ²Univ Hosp Ghent Ghent, Belgium ; ³Endocrinology Lille, France ; ⁴Univ of La Laguna Tenerife, Spain ; ⁵Univ of Bologna Bologna, Italy ; ⁶Univ Hosp Erlangen, Germany ; ⁷Psychiatrist Geneva, Switzerland ; ⁸ Univ Hosp Bern, Switzerland ; ⁹Karolinska Univ Stockholm, Sweden ; ¹⁰Univ de Lyon-Sud Lyon, France and ¹¹AndroConsult ChangMai, Thailand.

Objective: To evaluate the risk of venous thrombosis/embolism (VTE) in male-to-female transsexuals (MtF) treated with estrogens (+ anti-androgens) in order to recommend best practice.

Design: A review of publications on VTE in hormone-treated MtF transsexuals and a questionnaire on observed cases of VTE and type of hormone treatment.

Setting: 9 European (university) centers for gender dysphoria.

Main outcomes: Number and incidence of VTE/10,000 user-years and prescribed hormones.

Results: Published incidence of VTE varied greatly, from 0 to 148/10,000 user-years, highest in 1989 with ethinyl estradiol (EE) 0.1mg/day. Later a much lower incidence, but patient numbers and follow up duration were small, hence large 95% confidence intervals (0-540/10,000 user-years). Observational data with estimated numbers of treated patients and longer follow up, showed a much lower incidence of VTE 4.8 - 35.5/10,000 user-years in recent years, still 2-10 fold higher than in women with no hormone treatment (3/10,000) and 2-5 fold higher than in women on oral contraceptives (OC) or HRT. Remarkably, four out of 21 VTE's occurred in those MtF on OC containing EE + cyproterone acetate (CPA) though standard estrogen prescription is 17 β -estradiol transdermal (preferred) or oral 2-4 mg/day with CPA. Four cases of VTE with oral estradiol (two on 6 mg/day, one on 2 mg and 4 mg/day, respectively) and one on Premarin were reported and five in transdermal estrogen users, currently most prescribed E₂.

One surgical center reported a low but significant incidence of postoperative VTE (n=3, one fatal) with LMWH thrombosis prophylaxis and stopping of hormones 3-4 weeks before the operation. One center did not stop hormones before operation and observed no postoperative VTE in > 100 operated patients with only LMWH prophylaxis. No VTE was observed in the other centers which all stopped hormones and used LMWH.

Conclusion: The incidence of VTE in estrogen-treated MtF has much decreased in recent years, probably due lower estrogen dose and avoiding EE. However, the risk of VTE appears still increased compared to women, even to estrogen-using women (OC and HRT). Transdermal estradiol seems to have the lowest risk, and EE (including OC) the highest risk. Postoperative VTE incidence is low but significant, even in subjects who stopped estrogen before surgery. Peri-operative prophylaxis with LMWH is mandatory. Stopping estrogens before surgery seems prudent but we have insufficient data to recommend it.

Nothing to Disclose: HA, GT, AL, MM, MCM, AM, JB, AK, CD, NM-J, LJG

P1-404

Epigenetic Regulation of Adipogenesis in Collagen-Rich Microenvironment.

K Sato-Kusubata¹ and TH Chun¹.

¹Univ of Michigan Ann Arbor, MI.

Adipogenesis is dictated by both transcriptional network and posttranslational modification of chromatin proteins. While adipogenesis *in vivo* proceeds in collagen-rich environment, the impact of the ECM protein and its modifying enzymes in the epigenetic regulation of adipogenesis has been unknown. We aimed to define the role of fibrillar collagen and its modifying enzymes in regulating adipogenic chromatin signature and gene expression. **METHODS** 3T3L1 preadipocytes were cultured atop plastic, fibrillar or denatured type I collagen gel; adipogenesis was induced by insulin, IBMX, and dexamethasone. Posttranslational modification of histone H3 protein was assessed with WB and IF staining. Matrix metalloproteinases (MMPs) were knocked down with siRNA oligos. cDNA microarray, qPCR, and ChIP-PCR were used to define the impact on adipogenic gene regulation. **RESULTS** Adipogenic cocktail induced robust, 3-fold increase in H3K9 acetylation (H3K9ac) within 24h. While pro-transcriptional H3K4me3 level is moderately increased by 1.3-fold, H3K9me3 level displays no change. When cultured atop fibrillar type I collagen gel, adipogenic H3K9ac was still observed but reduced by 40%. This suppressive effect of type I collagen was concentration-dependent. The effect completely disappear once type I collagen is denatured, suggesting the critical role played by its fibrillar structure. By probing collagenolytic potential with a series of inhibitors, MMP family members are found to be most operative. Consistently, MMP inhibitor almost completely blocked the adipogenic induction of H3K9ac (n=4). When individual MMPs expressed are targeted, membrane-type MMPs (*MMP14* and *MMP15*) were found to be responsible for adipogenic collagenolysis. The targeting of *MMP14* most significantly suppressed adipogenic collagenolysis in tandem with H3K9ac (~50%, n=4). When adipogenic gene expression was interrogated with cDNA microarray, a selective set of genes (~4% of total transcripts) was found to be differentially (>1.5-fold) regulated by MMP. Pathway analysis points to the gene enrichment in SAPK/JNK (p=0.009), IL-4 signaling (p=0.025), and p53 signaling (p=0.026); ChIP-PCR confirmed that the promoter occupancy of the candidate genes, including *Ifi44*, by H3K9ac, is altered. **CONCLUSION** MMP-dependent collagenolysis plays a central role in regulating adipogenic chromatin signature by releasing the epigenetic constrains imposed by fibrillar collagen.

Sources of Research Support: Program for Improvement of Research Environment for Young Researchers (MEXT, Japan); American Society for Clinical Investigation, NIH DK020572 to MDRTC.

Nothing to Disclose: KS-K, THC

P1-405

Cell Stress Leads to Enhanced Constitutive Shedding of the Leptin Receptor Ectodomain Mediated by ADAM10 and ADAM17.

M Schaab¹, J Klammt², U Anderegg³, M Nowicki⁴, S Rose-John⁵, W Kiess², J Thiery¹ and J Kratzsch¹.

¹Inst of Lab Med, Clin Chemistry and Molecular Diagnostics Leipzig, Germany ; ²Hosp for Children and Adolescents, Univ of Leipzig, Germany ; ³Dept of Dermatology, Venerology and Allergology, Leipzig Univ Med Ctr Leipzig, Germany ; ⁴Inst of Anatomy, Fac of Med, Univ of Leipzig Leipzig, Germany and ⁵Inst of Biochem, Med Fac Christian-Albrechts-Univ Kiel Kiel, Germany.

Objective: The soluble leptin receptor (sOb-R) is generated through ectodomain shedding of membrane Ob-R by a so far unknown mechanism and may modulate leptin action in metabolic disorders. High sOb-R concentrations, until a 100-fold molar excess of sOb-R over leptin, can be found in type 1 Diabetes mellitus (T1DM) and hence, may suppress leptin action. The objective of our study was to investigate if cellular stress, going along with Diabetes and other metabolic disorders, could be responsible for generation of sOb-R in an in-vitro model.

Methods: Ob-R transfected HEK cells were subjected to stress inducers like glucose withdrawal, phorbol 12-myristate 13-acetate (PMA) and staurosporine. Inhibitors and siRNAs for ADAM10/17 were used for investigating the nature of the sheddase. Ob-R and ADAM gene expression were determined by real-time quantitative RT-PCR. sOb-R levels in cell supernatants were measured by immunoassay.

Results: Complete glucose deprivation and PMA stimulation ($p < 0.01$) were associated with increased sOb-R concentrations in the supernatant of the cells. Moreover, stimulation with staurosporine lead to a significant increase ($p < 0.01$) of sOb-R levels in the cell supernatant after 48 h and was accompanied by an activation of apoptosis as demonstrated by the induction of caspase 3. Pre-incubation with the pan-caspase inhibitor Z-VAD(OMe)-FMK or leptin significantly ($p < 0.01$) diminished the staurosporine-mediated increase of sOb-R. Specific ADAM10/17 inhibitors and siRNA knockdown resulted in significantly decreased sheddase activity that goes along with decreased levels of sOb-R.

Conclusion: The induction of cellular stress leads to increased shedding of the leptin receptor ectodomain as shown for other cytokine receptors like IL-6R, L-selectin, CD46 etc., before. The shedding mechanism appears to be associated with apoptotic processes as shown by the inducing effect of staurosporine and the anti-apoptotic effects of pancaspase-3 inhibitor or leptin. A reduced shedding caused by RNAi and inhibitors for ADAM 10 and or ADAM 17 suggests the involvement of both enzymes in the release of sOb-R. Therefore, increased sOb-R in peripheral blood may indicate cellular stress and, assuming a decreased Ob-R expression in parallel, a reduced peripheral leptin sensitivity. Hence specific inhibition of Ob-R shedding may restore sensitivity to the ligand.

Sources of Research Support: Stiftung fuer Pathobiochemie und Molekulare Diagnostik der Deutschen Vereinten Gesellschaft fuer Klinische Chemie und Laboratoriumsmedizin e.V.

Nothing to Disclose: MS, JK, UA, MN, SR-J, WK, JT, JK

P1-406

Regulation of the Human Growth Hormone Receptor (GHR) Expression in Obesity.

A Erman^{1,2} and CG Goodyer^{1,2}.

¹McGill Univ Montreal, Canada and ²Montreal Childrens' Hosp Res Inst Montreal, Canada.

Objectives: Growth Hormone (GH) treatments significantly reduce the fat mass of individuals with obesity associated with certain endocrinopathies. However, similar treatments of persons with idiopathic obesity have minimal effects on body composition (1). In our previous analysis of GH receptor (GHR) levels in a cohort of 55 women ranging in body mass index from lean to obese we found significant reductions in GHR mRNA expression in the omental and subcutaneous fat depots with increasing obesity (2). These data suggest that idiopathic obesity is associated with GH resistance at the level of the adipocyte due to reduced GHR expression. To understand the mechanisms behind this down-regulation we performed an *in silico* analysis of the GHR promoter regions which revealed putative response elements for a number of obesity adipose-associated factors, including glucocorticoids, Hypoxia-Inducible Factor 1-alpha (HIF-1 α) and Tumor Necrosis Factor-alpha (TNF α). In the present study we characterized the dose-dependent effects of dexamethasone (Dex), HIF-1 α and TNF α on GHR expression using qRT-PCR, luciferase-reporter assays and site-directed mutagenesis.

Results: Dex had biphasic effects on both GHR mRNA and promoter activity in HEK293 cells. There was a significant increase in GHR mRNA levels at 10⁻¹⁰M (p=0.007 vs. control) and in promoter activity at doses of 10⁻¹⁰ and 10⁻⁸M (p=0.019, p=0.023 vs. control) while a significant decline in mRNA levels occurred at 10⁻⁶ (p=0.002 vs. 10⁻¹⁰M) and in promoter activity at 10⁻⁶ (p \leq 0.003 vs. 10⁻¹²-10⁻⁸M). Transient transfection with 100 and 200ng of HIF-1 α expression vector led to a significant increase in GHR promoter activity (p=0.046, p=0.038 vs. control) and in mRNA levels at 100ng (p=0.012). Site-directed mutagenesis of a putative GRE and HRE site on the GHR promoter resulted in the loss of these effects. TNF α treatments significantly reduced GHR mRNA levels at doses of 10 and 20ng/ml (p=0.009, p=0.003 vs. control) and GHR promoter activity at 20ng/ml (p=0.000).

Conclusion: The results of this study suggest that the increased activity of specific factors (glucocorticoids, HIF-1 α , TNF α) in obese adipose tissues can regulate GHR transcription by acting through specific response elements and that glucocorticoids and TNF α maybe involved in the development of GH resistance in the obese.

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Sources of Research Support: Canadian Institutes of Health Research (CIHR).

Nothing to Disclose: AE, CGG

P1-407

Adipose Tissue Expandability and Metabolic Health in Severe Obesity.

J O'Connell¹, L Lynch¹, A Hogan¹, TJ Cawood² and D O'Shea¹.

¹St Vincent's Univ Hosp Dublin, Ireland and ²Christchurch Hosp Christchurch, New Zealand.

Up to 30% of severely obese individuals are metabolically healthy, and the factors protecting this subgroup remain unclear. Fat cells are thought to become more 'unhealthy' as they increase in size, influencing general metabolic health by promoting chronic inflammation. We proposed that metabolically healthy obese (MHO) individuals have greater adipose tissue expandability, thereby preventing ectopic fat deposition and subsequent metabolic dysfunction. We studied adipose tissue preadipocytes; stromovascular cells capable of becoming adipocytes. The adipogenic potential of these cells may play a key role in healthy adipose tissue expansion.

Twelve MHO, and 17 age-, BMI-, and gender-matched metabolically unhealthy obese (MUO) individuals underwent bariatric surgery. Omental and subcutaneous adipose tissue were fixed and paraffin-mounted for determination of mean adipocyte diameter and immunohistochemical staining (pref-1, CD14, MCP-1, and G-CSF antibodies).

Mean age was 41±8years, median BMI was 48 (40-71) kg/m², and 72% of the group were female. The MHO group had a higher number of preadipocytes (per 1000 adipocytes) in both subcutaneous [40(29-46) versus 20(8-24); p<0.05] and omental adipose tissue [69(23-106) versus 15(6-26); p<0.0005]. Increasing preadipocyte number was inversely associated with adipocyte size (r= -0.80, p<0.0001) and macrophage number (r= -0.59, p<0.005). Omental preadipocyte numbers also negatively correlated with metabolic parameters such as the degree of hepatic steatosis (r= -0.51, p<0.05), fasting glucose (r= -0.37, p<0.05), and fasting triglycerides (r= -0.63, p<0.005), as well as proinflammatory cytokines MCP-1 (r= -0.56, p<0.05) and G-CSF (r= -0.63, p<0.05).

Adipose tissue in MHO subjects had higher numbers of preadipocytes, when compared with MUO. This may permit 'healthier', hyperplastic fat cell expansion, demonstrated by smaller adipocyte size and fewer markers of inflammation. These factors may be key to protecting this subgroup of obese individuals from the adverse metabolic profile associated with excess adiposity.

Sources of Research Support: The Irish Health Research Board and the Medical Research Charities Group and Diabetes Federation of Ireland.

Nothing to Disclose: JO, LL, AH, TJC, DO

P1-408

ECM Modifiers Acting Downstream of Hypoxia Inducible Factor during Adipogenesis.

M Inoue MD, PhD¹ and TH Chun MD, PhD¹.

¹Univ of Michigan Ann Arbor, MI.

Adipogenesis is the biological process central to obesity development. Low oxygen tension (hypoxia) negatively regulates adipogenesis via hypoxia inducible factor-1 alpha (*HIF1A*). The molecular mechanism by which *HIF1A* regulates adipogenesis, however, remains undefined. We aimed to identify a set of genes that act downstream of *HIF1A* regulating adipogenesis. **METHODS.** Genome-wide cDNA microarray analysis, coupled with qPCR, was used to identify genes that are differentially expressed in 3T3L1 preadipocytes and adipocytes. *HIF1A* was knocked down with siRNA oligos. The role in adipogenesis for respective *HIF1A* downstream genes was assessed with *in vitro* adipogenesis, lipogenesis and adipogenic gene expression. **RESULTS.** *HIF1A* knockdown in preadipocytes increased lipid accumulation and adipogenic gene expression as previously reported. However, this anti-adipogenic effect of *HIF1A* disappears almost completely when preadipocytes were cultured atop fibrillar type I collagen gels, suggesting a role for ECM context mediating *HIF1A* function. When *HIF1A*-dependent genes in adipocytes were probed with genome-wide expression analysis, the gene expressions of ECM modifiers, e.g., *MMP9*, *Thbs1*, *Col3*, and *Fat4*, were found to be suppressed with *HIF1A* knockdown by more than 50%. Subsequent assessment has demonstrated that these genes were significantly down-regulated during adipogenesis (80~90%), pointing to their potentially anti-adipogenic roles. Indeed, the knockdown of *Fat4*, a cadherin family member, significantly enhanced adipocyte differentiation (~50%). When preadipocytes were cultured atop type I collagen fibers, the suppressive effect of *HIF1A* knockdown on these gene expressions disappear almost completely in tandem with the loss of pro-adipogenic effect. **CONCLUSION.** *HIF1A* regulates the gene expression of ECM modifiers, which play a coordinated role in regulating ECM remodeling and adipogenesis.

Sources of Research Support: American Heart Association; NIH DK020572 to MDRTC.

Nothing to Disclose: MI, THC

P1-409

Subcutaneous Adipose Tissue Displays Major Phenotypic and Proteomic Differences from Intra-Abdominal Fat Depots.

L Sackmann Sala¹, RD Munn¹, ER Lubbers¹, DE Berryman PhD, RD, LD¹ and JJ Kopchick PhD¹.

¹Ohio Univ Athens, OH.

White adipose tissue (WAT) plays a role in metabolism, immunity, glucocorticoid and steroid hormone synthesis as well as other functions (1). However, WAT is not a homogeneous organ, and individual WAT depots differ in their contributions to each of these functions (2). Even though several reports have described depot-specific variations in the response to hormones, lipolytic rates, and specific mRNA and protein levels, further characterization of the protein profiles of different WAT depots is necessary to help unravel their distinctive features. Given that proteomic techniques provide a more thorough view of protein expression than classical protein assays, we used two-dimensional gel electrophoresis followed by mass spectrometry to analyze and compare the proteomes of four WAT depots in C57BL6/J mice (12 month-old males, n=6). Samples of subcutaneous (inguinal) and intra-abdominal (mesenteric, epididymal and retroperitoneal) WAT were obtained, and their weights, protein contents and adipocyte sizes were determined. Additional data included fasting plasma levels of insulin (3.16 ± 0.58 ng/ml, mean \pm SE), leptin (31.4 ± 5.1 ng/ml), total adiponectin (29.1 ± 2.0 μ g/ml), and HMW-adiponectin (10.6 ± 1.4 μ g/ml). The epididymal WAT depot was the largest in mass, and the mesenteric fat pad had the highest protein content of the WAT depots analyzed. Mean adipocyte size was highest in epididymal fat and lowest in mesenteric and inguinal WAT depots. Proteomic results showed major differences in protein expression in inguinal WAT as compared to mesenteric and epididymal WAT depots. Inguinal fat displayed lower expression of proteins involved in energy metabolism, fatty acid re-esterification, glycolysis/glyceroneogenesis and fatty acid synthesis, and decreased levels of antioxidant proteins. These results are consistent with lower triglyceride turnover (3) and consequently lower production of reactive oxygen species in subcutaneous WAT as compared to mesenteric and epididymal WAT depots. Together, this information adds to the understanding of WAT physiology and the role of different WAT depots in metabolism and homeostasis. Also, our results provide a basis for the search for diagnostic/therapeutic targets of obesity-related human diseases, including the metabolic syndrome, type 2 diabetes, cardiovascular disease, and cancer.

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Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll; by NIH Grants DK075436-01, AG019899-06, and 1P01AG031736-01A1; by the Diabetes Research Initiative at Ohio University; the BioMolecular Innovation and Technology Partnership at Ohio University; and by AMVETS.

Nothing to Disclose: LSS, RDM, ERL, DEB, JJK

P1-410

Growth Hormone Stimulated Macrophage Infiltration and Inflammation in Adipose Tissue.

J Wright-Piekarski¹, EO List Ph.D.¹, JD Blischak¹, A Zacharias¹, KR Funk M.S.¹, D Ivins¹, G Andrews¹, E Walcutt¹, N Anderson¹, R Leone¹, DE Berryman Ph.D.¹ and JJ Kopchick Ph.D.¹.

¹Ohio Univ Athens, OH.

Obesity is characterized by a constant state of low-grade inflammation accompanied by elevated levels of macrophages in the adipose tissue itself. This phenomenon appears to be dependent on the anatomical location of the adipose depot. Previously our laboratory has demonstrated that growth hormone (GH) has the ability to decrease fat mass in a depot specific manner; however, its role in adipose tissue inflammation or macrophage infiltration has not been evaluated. Using a diet-induced obese mouse model, the purpose of this study was to determine the effects of GH or insulin-like growth factor-1 (IGF-1) administration on gene expression of macrophage and inflammation markers in different depots of adipose tissue. Eighty-four C57BL/6J mice were placed on a high fat diet for 16 weeks to induce obesity. Mice were then separated into 12 groups (n=7) based on 4 experimental treatments (control, GH, IGF-1 or diet reversal) and 3 lengths of treatment (2, 7 or 21 days). The control group and the diet reversal group received twice daily injections of phosphate buffered saline (PBS), and the experimental groups received twice daily injections of either GH (5.0 µg/g body weight) or IGF-1 (2.5 µg/g body weight). At the end of each time period, the mice were sacrificed and dissected. The fat pads collected and analyzed included subcutaneous, retroperitoneal, mesenteric, and epididymal white adipose depots. There was no difference in depot mass among the experimental groups following 2 days of treatment. After 7 days of treatment, significant differences were present among the diet reversal group and either the control or IGF-1 treated groups within the retroperitoneal adipose depots. Finally, 21 days of treatment resulted in significantly lower fat masses in the GH and diet reversal treatments as compared to the control and IGF-1 treated groups for all white adipose depots. Expression levels of several macrophage and inflammation markers (F4/80, cd68, TNFα) as determined by real-time PCR among depots and treatment groups over time will be presented. Collectively, these data will help determine whether the GH/IGF-1 axis, which has been reported to impact adipose depot-dependent mass, influences the depots by altering the degree of macrophage infiltration and inflammation.

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by grants from the NIDDK (DK075436) and NIA (AG031736 and AG019899), by a grant from the AMVETS organization, by a grants from the Diabetes Research Initiative and the VP for Research at Ohio University. The bGH used in this study was a gift from the Monsanto Company. The IGF-1 used in this study was a gift from Tercica.

Nothing to Disclose: JW-P, EOL, JDB, AZ, KRF, DI, GA, EW, NA, RL, DEB, JJK

P1-411

Beyond the Adipose Tissue - Granulocytes as an Additional Source of Circulating NAMPT in Obesity.

D. Friebe¹, K. Dittrich¹, A. Garten¹, S. Petzold MD¹, W. Kiess MD¹ and A. Korner MD¹.

¹Univ of Leipzig Leipzig, Germany.

NAMPT has been proposed as an adipocytokine that is involved in the regulation of glucose homeostasis. We recently found that NAMPT was independently associated with leukocyte number. Therefore, we analysed in this study NAMPT production among several tissues and leukocyte subpopulations and assessed whether NAMPT is enzymatically active in cell lysates and supernatants.

First, we examined NAMPT mRNA expression in 12 different tissues (adipose tissue, aorta, brain, heart, hypothalamus, kidney, liver, pancreas, peripheral blood leukocytes, skeletal muscle, smooth muscle, spleen). The expression of NAMPT was more than 5-fold higher in peripheral blood leukocytes ($P < 0.0001$) than in all other tissues. We then determined NAMPT serum concentration, NAMPT mRNA and protein expression in and protein secretion from granulocytes, monocytes and lymphocytes isolated from 12 children (age: 11.7 ± 2.7 years, BMI SDS: 2.14 ± 1.12). Moreover, complete blood count was performed in 6 of these subjects to assess a potential association of NAMPT serum concentration with counts of leukocyte subpopulations. Serum concentrations of NAMPT were highly correlated to leukocyte count in particular to monocyte ($P < 0.01$, $r = 0.942$) and neutrophil granulocytes count ($P < 0.01$, $r = 0.922$) but not to lymphocyte count. The mRNA expression was more than 6-fold higher in monocytes and granulocytes ($P < 0.0001$) compared to lymphocytes. Consistent with this, we detected a higher amount of NAMPT protein in cell lysates of monocytes ($P < 0.05$) and granulocytes ($P < 0.05$) in comparison to lymphocytes after normalization to total protein. Granulocytes secreted more than 22-fold higher amounts of NAMPT protein into cell culture supernatants ($P < 0.0001$) compared to monocytes and lymphocytes when normalized to total protein. We next addressed the question whether NAMPT protein is enzymatically active in cell lysates and supernatants. We found a similar enzymatic activity in cell lysates of monocytes, lymphocytes and granulocytes after normalization to NAMPT protein amount. Contrary, supernatants of granulocytes exhibited significantly lower enzymatic activity ($P < 0.001$) despite high NAMPT concentration compared to supernatants of monocytes and lymphocytes.

Conclusions: Obesity is characterized by an increase in adipose tissue mass and number of leukocytes. Granulocytes may represent a previously unrecognized source of circulating NAMPT in obesity in addition to the adipose tissue.

Nothing to Disclose: DF, KD, AG, SP, WK, AK

P1-412

Simultaneous Measurement of Mouse Acute Phase Proteins with Novel Multiplex Assays Using Luminex xMAP Technology.

MK Sinha Ph.D.¹, JF McDonald Ph.D.¹, X Qiang Ph.D.¹, IW Ingrid Ph.D.², JS Mistry Ph.D.¹ and PE Scherer Ph.D.².

¹Millipore Corporation St Charles, MO and ²Univ of Texas Southwestern Med Ctr Dallas, TX.

Low grade inflammation has been implicated in various metabolic diseases. Acute Phase Proteins, such as C-Reactive Protein (CRP), Serum Amyloid A (SAA) and α -1 Acid Glycoprotein (AGP), have been used as inflammatory biomarkers, but some of the recently recognized secretory proteins particularly from adipocytes are not effectively evaluated. Therefore we developed multiplex immunoassay(s) capable of simultaneously measuring several Acute Phase Proteins using Luminex xMAP technology. Based on sample dilution and basal levels in mouse serum and plasma, Acute Phase Proteins were plexed in two separate multiplex panels. Assay panel 1 includes Pentraxin-3 (PTX3), Lipocalin-2 (Lcn2), Adipsin and Serum Amyloid A-3 (SAA3) and utilizes 25 μ l of a 1/40 diluted sample. Sensitivity for PTX3, Lcn2, Adipsin and SAA3 were 0.01, 0.1, 2 and 5 ng/ml respectively. Assay panel 2 includes Haptoglobin (HPTGN), α -2 Macroglobulin (A2M), AGP, Serum Amyloid Protein/Pentraxin-2 (SAP) and CRP, and utilizes 25 μ l of a 1/50000 diluted sample. In these two multiplex panels, no significant cross-reactivity was observed among different analytes. For most of the Acute Phase Proteins, the recovery of exogenously spiked analytes and the linearity of dilutions of either basal or spiked samples were within 70-130% of the expected levels. The biological significance of these two multiplex Acute Phase Proteins assays was evaluated by determining the effect of subcutaneous LPS (0.6 μ g/g body weight) administration in mice at 6 hr (n=5) and 30 hr (n=5) in comparison to control mice (n=5). Significant ($p < 0.05$ or less) and time-dependent increases of different Acute Phase Proteins following the LPS challenge were observed: PTX3 (86- and 25-fold up regulation at 6 and 30 hours, respectively), Lcn2 (444- and 246-fold up regulation), SAA3 (3994- and 3924-fold up regulation), Adipsin (2- and 2-fold across the board), HPTGN (8- and 44-fold up regulation), AGP (2- and 45-fold up regulation), SAP (5- and 16-fold up regulation) and CRP (3- and 2-fold up regulation). LPS challenge did not alter A2M levels in mice. Feasibility of measuring 9 different mouse acute phase proteins in multiplex format provides a useful tool to study the role of sub-clinical or low grade inflammation in the etiology and patho-physiology of many metabolic diseases including obesity and diabetes, in addition to other inflammatory states.

Nothing to Disclose: MKS, JFM, XQ, IWI, JSM, PES

P1-413

Incretins Modulate Adipokine Gene Expression in Cultured 3T3-L1 Adipocytes.

TJ Hadden PhD^{1,2} and RE Miller MD^{1,2}.

¹John D Dingell VA Med Ctr Detroit, MI and ²Wayne State Univ Detroit, MI.

Obesity is an important risk factor for the development of cardiovascular disorders, some cancers, insulin resistance, type 2 diabetes and its complications. Adipocytes have the capacity to synthesize and secrete a variety of adipokines that act locally or distally, impacting processes and conditions such as feeding and satiety, insulin sensitivity and inflammatory state. Adipocytes respond to differences in nutrient status, not only by accumulating or mobilizing triglycerides, as conditions require, but also by altering the types and quantities of adipokines synthesized and secreted. New targets for therapeutic intervention in diabetes are the incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), mediators of the enteroinsular axis. Previously, we investigated potential roles of the incretins in modulation of adipocyte differentiation of cultured 3T3-L1 cells. We found that cells incubated with GLP-1 or GIP during differentiation accumulated more lipid than control cells. Additionally, incubation of differentiating cells with either incretin increased abundance of mRNAs encoding the adipogenic transcription factors PPAR-gamma and C/EBP-alpha, aP2 and adiponectin, while decreasing that of Pref-1, a preadipocyte-specific factor. We have extended these studies to evaluate the effects of incretins on adipokine expression in developing 3T3-L1 cells. We find that the presence of GLP-1 during differentiation has little, if any effect on PAI-1 mRNA abundance, while that of resistin increases and IL-6 mRNA abundance decreases. By contrast, incubation of differentiating cells with GIP increases abundance of PAI-1 and IL-6 mRNAs; and does not affect resistin mRNA abundance. These results suggest that the incretins act via distinct mechanisms to alter adipokine gene expression in 3T3-L1 adipocytes.

Sources of Research Support: Department of Veterans Affairs Medical Research Service.

Nothing to Disclose: TJH, REM

P1-414

Selective Inactivation of c-Jun NH2 Terminal Kinase (JNK) in the Adipose Tissue Is Sufficient To Protect Against Diet-Induced-Obesity and Its Associated Metabolic Disorders in Mice.

KSL Lam MD¹, X Zhang PhD¹, RLC Wong¹ and A Xu¹.

¹Univ of Hong Kong Hong Kong, China.

Introduction: Obesity caused by feeding a high fat diet is associated with increased activation of c-Jun NH2-terminal kinase (JNK), which has been implicated in the development of obesity-related insulin resistance and type 2 diabetes. However, the relative tissue-specific contribution and the underlying mechanisms remain to be defined.

Method: In this study, we generated a transgenic mouse model with adipose tissue-specific over-expression of dominant negative (DN) JNK. Their phenotypic changes on a high fat diet were comprehensively characterized. Adipose tissues, liver, muscle and serum were collected for further biochemical and morphological analysis.

Results: On the standard chow diet, the transgenic mice showed no significant difference in body weight gain, insulin sensitivity, glucose or lipid profiles, from their wild-type littermates. However, on a high fat diet, the DN-JNK transgenic mice were protected against diet-induced obesity, with reduced weight gain, fat mass and size of adipocytes in the adipose tissues. Significantly, the DN-JNK transgenic mice were resistant to the deleterious impact of high-fat diet on systemic insulin sensitivity and glucose tolerance. They also demonstrated a lower level of hepatic gluconeogenesis in vivo, and greater insulin-induced glucose uptake in skeletal muscles ex vivo. These metabolic changes were accompanied by a markedly decreased macrophage infiltration in the adipose tissue, reduced production of pro-inflammatory adipokines, increased expression of adiponectin and reduced circulating levels of adipocyte fatty acid binding protein. As a secondary effect, the DN-JNK transgenic mice also exhibited a resistance to the hepatosteatosis induced by high fat diet. The DN-JNK mice, when on a high fat diet, had significant increases in 24-hour oxygen consumption and reductions in respiration exchange rates, compared with their wild-type littermates.

Conclusion: Selective suppression of JNK activation in the adipose tissue alone was sufficient to counteract high fat diet-induced obesity and its associated metabolic dysregulations in mice, in part through an increase in energy expenditure and a decrease in systemic inflammation.

Acknowledgment: Hong Kong Research Council GRF (RGC 767208M).

Nothing to Disclose: KSLL, XZ, RLCW, AX

P1-415

Metabolic Response of White Adipose Tissue to High-Fat Diets Rich in n-3 and n-6 Fatty Acids in Mice with Genetic Predispositions for Either Leanness or Obesity.

K Huber Prof¹, H Bergmann¹, U Renne Dr², M Langhammer Dr², D Dannenberger Dr², BH Breier Prof³ and K Nurnberg Dr².

¹Univ of Veterinary Med Hannover, Germany ; ²FBN Dummerstorf Dummerstorf, Germany and ³Massey Univ Auckland, New Zealand.

High-fat diets rich in n-3 and n-6 polyunsaturated fatty acids (PUFA) are believed to improve metabolic health in contrast to the adverse effects of diets rich in saturated fatty acids. It has been proposed that health benefits of diets high in PUFA are mediated by improving insulin sensitivity and lipid metabolism in liver, muscle and adipose tissue (1). The aim of the present study was to determine the metabolic response of white adipose tissue to diets rich in n-3 and n-6 PUFA in mice of known predisposition for leanness or obesity (2,3). Blood plasma and abdominal adipose tissue samples were investigated from mice of two genetically different lines: DU6 mice, selected for high body weight gain develop obesity; and DUhTP mice, selected for running performance, stay lean throughout their lifecycle. Mice were fed with control diet (7.2 % fat), or a diet high in n-3 PUFA levels (fish oil, 27.7 % fat) or a diet high in n-6 PUFA levels (sunflower oil, 27.7 % fat). Food intake, body and adipose tissue weight were measured. Plasma insulin was determined by RIA, leptin by ELISA and glucose and free fatty acids were measured by an automated analyzer. Adipose tissue homogenates were used for Western Blotting. Expression of metabolic markers was quantified using Quantity One software (BioRad). Data were statistically analysed by Two Way ANOVA.

Food intake was increased in DU6 mice causing higher body weight in all nutrition groups of this genetic line. Interestingly, both PUFA diets significantly decrease the food intake in DU6 mice in comparison to standard chow. This physiological response to diets of high caloric density was less pronounced in DUhTP mice, indicating that high-fat diets may trigger altered energy regulatory systems in this genetic line. Body weights were highest in DU6 mice fed PUFA-diets, mainly due to larger abdominal fat depots. However, in DUhTP mice, PUFA-diets had only small effects on body weight and size of abdominal adipose tissue. While plasma leptin concentrations were lower in DUhTP independently of the different diets, leptin levels increased in DU6 mice in parallel with larger adipose tissue depots. A diet rich in n-3 PUFA was able to increase the capacity to oxidise fatty acids and to improve insulin sensitivity only in DUhTP mice, suggesting that selection for high running performance led to improved handling of high-fat diets.

(1) Simopoulos AP, Forum of Nutrition 2003; 56:67

(2) Renne U et al., J Exp Anim Sci 2003 ; 42:218

(3) Falkenberg H et al., Arch Tierz Dummerstorf 2000; 43:513

Nothing to Disclose: KH, HB, UR, ML, DD, BHB, KN

P1-416

Enhanced Adipocyte Glycogen Metabolism Impedes Non-Esterified Fatty Acid Release and Weight Loss.

KR Markan BA¹, MJ Jurczak PhD¹, MB Allison BS¹, HH Ye PhD¹ and MJ Brady PhD¹.

¹Univ of Chicago Chicago, IL.

Adipose tissue is instrumental in storing and mobilizing energy to meet the body's demand in response to various hormonal and nutritional stimuli. It has been reported that feeding an animal following a prolonged fast produces a dramatic yet transient spike in adipose glycogen levels. We have previously generated a transgenic mouse model over-expressing the glycogen targeting subunit PTG (aP2-PTG) in which adipocyte glycogen levels were stably elevated 100-400 fold but were mobilized upon fasting. We have utilized this aP2-PTG model to further address how increased adipose glycogen turn-over impacts carbohydrate and lipid metabolism under changing nutritional states. In vitro lactate production from the transgenic adipose tissue was elevated basally and following isoproterenol stimulation suggesting the mobilization of glycogen-derived G1P into glycolysis. Furthermore, in vitro NEFA levels from transgenic adipose tissue under basal and isoproterenol treated conditions were decreased as were circulating NEFA levels relative to wild-type in fed and fasted states. Concomitant with altered NEFA levels, the expected fasting up-regulation of adipocyte PEPCK-C was blunted in the transgenic model. Collectively, a decrease in adipocyte released NEFAs in concert with elevated glycogenolysis support the notion that glycogen release into the glycolytic pathway is providing a carbon source for the re-esterification of free fatty acids thus serving as a nutritional cue impacting PEPCK expression. Indirect calorimetry with these animals revealed no significant changes in global metabolism despite the dramatically elevated glycogen turn-over in fed or fasted states. However, a significant change in physiology was detected in the aP2-PTG mice under a weight loss protocol. Following 5 weeks of high fat feeding both wild-type and transgenic animals were returned to standard chow. Upon this transition in diet composition wild-type animals lost 15% of their weight. Interestingly, the transgenic animals did not lose any of the weight gained during the high fat feeding time course. Collectively, these results demonstrate a vital coordination between glycogen and lipid metabolism in the adipocyte offering support for a basally low 'set-point' for glycogen metabolism within adipose tissue.

Sources of Research Support: NIH Grant R01 DK-064772; Pre-doctoral Fellowship Cardiovascular and Biochemistry Training Program NIH T32 HL-007237; University of Chicago Diabetes Research and Training Center P60 DK-20595; and a kind gift from the Kovler Family Foundation.

Nothing to Disclose: KRM, MJJ, MBA, HHY, MJB

P1-417

Low Birth Weight Leading to Obesity and Metabolic Changes Associated with Transgenic Marshmallow Mice.

A.D. Patel DO¹, S.A. Demauro MD¹, W. Philbrick PhD¹, V.T. Samuel MD, PhD¹, G.I. Shulman MD, PhD¹, S. Diano PhD¹, E. Gyengesi PhD¹ and J.J. Wysolmerski MD¹.

¹Yale Sch of Med New Haven, CT.

Intrauterine growth retardation (IUGR) and low birth weight (LBW) are associated with adult onset obesity and related metabolic complications. These patients are thought to have abnormalities in their HPA axis that contribute to the metabolic syndrome. Serendipitously, we produced a mouse line with LBW but late-onset obesity and metabolic syndrome, which we have named Marshmallow (Marsh).

Objective: To define the metabolic and genetic abnormalities leading to the progressive obesity in Marsh mice.

Design/Methods: We examined weight gain and body composition in male Marsh and WT mice. Metabolic cage studies and normoglycemic, hyperinsulinemic clamp were done. ACTH and corticosterone (Cort) levels were measured in response to exogenous dexamethasone. Cohorts of 4-5-week-old Marsh and WT mice had adrenalectomy (Adx) and given replacement Cort via pellets for 8 weeks, after which we studied their metabolism.

Results: Marsh mice weigh less than their WT littermates at birth, but gain weight faster and become progressively obese after 4 months. However, body fat content is elevated two-fold in Marsh mice at weaning when total weight is not elevated. Weight gain is the result of decreased basal energy expenditure and activity levels. Marsh mice have elevated leptin and Cort levels. Cort levels were elevated in Marsh mice at baseline and had an exaggerated response to stress.

However, Cort levels were appropriately suppressed in response to high dose dexamethasone. Marsh mice were not frankly hyperglycemic, but were insulin resistant and had mild glucose intolerance. Adx partially corrected the alterations in glucose metabolism, but not body composition and weight gain. Initial genetic studies suggest that integration of the transgene led to a small non-reciprocal translocation between chromosomes 3 and 6 that disrupted a previously uncharacterized gene encoding a GPCR-like receptor.

Conclusions: Marsh mice appear to be a new genetic model of IUGR/LBW leading to progressive obesity, insulin resistance and the metabolic syndrome. A hyper-responsive HPA axis causing chronic elevations in corticosterone contributes to the obesity and metabolic abnormalities. Further characterization of the genetic lesions in these mice may provide a window into previously unrecognized mechanisms linking the regulation of ACTH secretion, appetite and body composition.

Nothing to Disclose: ADP, SAD, WP, VTS, GIS, SD, EG, JJW

P1-418

Adipose-Specific Overexpression of ChREBP Plays a Protective Role Against Obesity.

AM Nuotio-Antar¹, N Pongvarin¹, MV Li¹ and L Chan¹.

¹Baylor Coll of Med Houston, TX.

The global obesity epidemic is associated with increased prevalence of cardiovascular disease, dyslipidemia, non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes. Western-type diet, which is thought to contribute to this epidemic, is characterized as being not only high in dietary fat, but also high in simple carbohydrates. Carbohydrate response element binding protein (ChREBP) is a glucose-responsive transcription factor that regulates intracellular glucose and lipid homeostasis, and thus may contribute to risk for obesity, dyslipidemia, and cardiovascular disease, by directly modulating the expression of glycolytic and lipogenic genes in key metabolic tissues. Recent studies indicate that ChREBP may play a role in adipocyte metabolism. It has been previously reported that white adipose tissue (WAT) and brown adipose tissue (BAT) depots highly express ChREBP. However, there is a paucity of knowledge regarding effects of adipocyte-specific ChREBP activation, which genes may be modulated by differential ChREBP activity in these cells, and the extent to which ChREBP activity in adipocytes impacts adipose tissue or whole-body metabolism. We have found that in both gonadal and subcutaneous WAT depots of wild type C57BL/6J mice, expression of ChREBP, ChREBP-binding partner Mlx, and lipogenic ChREBP target genes ACC1 and FASN are markedly increased after 48h fasting/24h refeeding with a high sucrose, no fat diet, but not affected by 48h fasting, alone, in comparison to ad lib fed controls. Interestingly, expression of ChREBP paralog MondoA is only increased in gonadal WAT after 48 h fasting, suggesting a different role for MondoA in adipose tissue metabolism. Adipose tissue-specific overexpression of constitutively active ChREBP (caChREBP) in C57BL/6J mice (ap2-Cre;eGFP^{flox}-caChREBP mice) results in decreased percent body fat when compared with littermate controls, and this difference is exacerbated when mice are placed on a Western-type diet. Histological examination reveals the appearance of large pockets of BAT-like cells in gonadal WAT of ap2-Cre;eGFP^{flox}-caChREBP mice, and ap2-Cre;eGFP^{flox}-caChREBP mice exhibit increased fed-state energy expenditure and altered thermogenic capacity when compared with littermate controls. These findings indicate that ChREBP activity in adipose tissue plays an important role in modulating adipocyte metabolism and risk for obesity.

Sources of Research Support: BCM-NIDDK Pediatric Gastroenterology Postdoctoral Training Grant T32 DK007664 (2007-2009) and CNRC-NICHD Postdoctoral Training Grant T32 HD007445 (2009-2010) awarded to AMN-A; NIH Grant R01 HL051586 awarded to LC.

Nothing to Disclose: AMN-A, NP, MVL, LC

P1-419

Angiotensin 1-7 Prevents the Metabolic Syndrome, Hepatosteatosi s and Adipose Tissue Inflammation (Adipositi s) in the Fructose-Fed Rat.

Y Marcus Dr.¹, Y Shechte Prof.², F Kohen Dr.², R Limor Dr.¹, K Sasson Dr.², I Biton Dr.², M Fridkin Prof.² and N Stern Prof.¹.

¹Tel Aviv-Sourasky Med Ctr and Sackler Fac of Med Tel Aviv, Israel and ²Weizmann Inst of Sci Rehovot, Israel.

The metabolic syndrome (MetSyn) usually evolves in the context of visceral adiposity which fosters insulin resistance, hypertension, dyslipidemia, fatty liver and white adipose tissue (WAT) inflammation induced by adipocyte enlargement and macrophage infiltration. We have previously shown that the receptor for angiotensin 1-7 (Ang 1-7), Mas, is expressed in the adipose tissue. Since Mas knockout mice were shown to feature MetSyn, we reasoned that treatment with Ang 1-7 may prevent the development of MetSyn. Two groups of rats were fed on high-fructose/low magnesium diet over 24 wks: one (n= 6) received a continuous infusion of Ang 1-7 (576 µg/kg/day, s.c., via an Aldzet pump (6 months) and the other served as a control group (n=9). By the end of the study, Ang 1-7 -treated animals had lower final body weight (457±8.9 vs 483±7.8g; p=0.03), lower fat mass (detected by MRI, p<0.05) and lower serum triglycerides (97.1±16.3 vs 227.5±21.7 mg/dl; p<0.001). Additionally, Ang 1-7 treatment markedly lowered serum aldosterone levels (11.1±2.2 vs 19.1±2.1 ng/dl; p<0.01). Ang 1-7 treatment did not induce changes in basal serum glucose or insulin, but did attenuate the increase in serum glucose (P<0.05) in response to acute intraperitoneal glucose challenge (2 grams/kg). Histological examination of the liver revealed that fructose-fed rats developed hepatosteatosi s which was nearly absent in the fructose fed, Ang1-7-treated rats. Mean adipocyte size in epididymal fat was significantly larger in untreated than in Ang1-7 treated, fructose fed rats (4133±729 vs 8370±3934 µm², respectively; p=0.008). Additionally, macrophage infiltration was present in white adipose tissue (WAT) from untreated, but not from Ang1-7 treated rats. This was associated with reduced epididymal fat tissue pP65 protein expression (p<0.05), suggesting lower activation of the NFκB pathway. Finally, based on lucigenin-enhanced chemiluminescence, WAT from Ang1-7 treated rats showed reduced NADPH stimulated superoxide production. In conclusion, Ang 1-7 had an ameliorating effect on insulin resistance, hypertriglyceridemia, fatty liver, obesity and adipositi s in the high fructose fed rats. These beneficial effects could be related, at least partially, to the anti-oxidative and anti-inflammatory influence in adipose tissue and to the prevention of hepatosteatosi s by Ang1-7.

Nothing to Disclose: YM, YS, FK, RL, KS, IB, MF, NS

P1-420

Angiotensin II-AT2 Receptor Stimulation with the Selective Nonpeptide Agonist M24 (C21) Prevents Diet-Induced Insulin Resistance in Rats and Modulates Several Aspects of Adipocyte Physiology.

M Shum MSc¹, S Menard PhD¹, A Emond MSc¹, T Grenier-Larouche MSc¹, C Roberge MSc¹, M-F Langlois MD¹, J-P Baillargeon MD², A Hallberg PhD¹, A Carpentier MD¹ and N Gallo-Payet PhD¹.

¹Univ of Sherbrooke Sherbrooke, Canada and ²Univ of Uppsala Uppsala, Sweden.

Several studies have shown that angiotensin (Ang II) stimulates growth and differentiation of adipocytes as well as lipogenic and lipolytic activities. Essentially all previous studies used Ang II type 1 receptor (AT1R) blockers (ARBs) or PD123319 as tools to study AT2R in adipocyte physiology and insulin resistance. We have characterized a nonpeptide and highly selective AT2R agonist, M24 (formerly called Compound C21) (*Wan et al. J Med Chem, 47:5995, 2004*). M24 was used to test our hypothesis that activation of AT2R increases insulin sensitivity by enhancing preadipocyte differentiation and functionality. *In vivo* studies were conducted with Wistar rats submitted to a 6-week high-fat/high-fructose diet and treated (using infusion with osmotic mini-pumps i.p.) with saline, M24 (0.1mg/kg/day), losartan (1mg/kg/day) or M24 + losartan during the 6-week diet. At the end of the treatment, 2 h of euglycemic-hyperinsulinemic clamps with constant insulin infusion and whole-blood glucose level (maintained using a variable 20% dextrose intravenous infusion according to the glucose level), was performed to measure insulin sensitivity. Results indicate that treatment with M24, losartan and M24+losartan prevented insulin resistance induced by the diet in control group. To better understand the role of AT2R in adipocyte physiology, primary cultures of subcutaneous and retroperitoneal preadipocytes were performed. AT1R and AT2R receptors are expressed in both cell types, with AT1R being the predominant type. Cells were stimulated as above for 12 to 15 days. Cells stimulated through AT1R contained several small lipid droplets, whereas cells stimulated through AT2R specifically had lipid droplets of larger diameter. Furthermore, when infected with shRNA in order to abolish the expression of AT2R, cells exhibited a spindle-like morphology without any evidence of adipocyte differentiation. Under these conditions, AT2R was non detectable along with a very low expression level of expression of aP2 and PPAR γ 2, suggesting a crucial role of AT2R in adipocyte differentiation. These results support our hypotheses that AT2R may improve insulin resistance and may be involved in adipocyte differentiation. These studies will help establish whether AT2R agonists may be a new therapeutic venue to prevent insulin resistance in humans and may potentially be involved in adipocyte differentiation.

Sources of Research Support: Grants from The Centre de recherche clinique Etienne-LeBel, Sherbrooke.

Nothing to Disclose: MS, SM, AE, TG-L, CR, M-FL, J-PB, AH, AC, NG-P

P1-421

Effects of Transgenic Human CIDE-A Expression on Body Composition and Lipid Accumulation in Mice.

H Tanda¹, B Kelder Ph.D.¹, D Berryman Ph.D.¹, S Okada Ph.D.¹, M Tarnowski¹, EO List Ph.D.¹ and JJ Kopchick Ph.D.¹.

¹Ohio Univ Athens, OH.

CIDE-A (cell-death-inducing DFF45-like effector A) is a member of a family of proapoptotic proteins that includes CIDE-B and DFF40. CIDE-A's major function is believed to be in the regulation of lipid metabolism. CIDE-A gene disrupted mice exhibit a lean phenotype and are resistant to diet-induced obesity (1). We have previously demonstrated that CIDE-A expression is increased in the liver of obese/type2 diabetic mice and this increased expression correlates with liver steatosis (2). We have also found that there is a concomitant decrease in CIDE-A expression in subcutaneous adipose tissue of these mice (3). To further delineate CIDE-A's role in the regulation of lipid metabolism, we have generated transgenic mice that express the human CIDE-A cDNA under the control of the mouse metallothionein-I transcriptional regulatory element. We have characterized these mice with respect to body weight, fasting blood glucose and plasma insulin levels, glucose tolerance and by determining whole body composition (fat, body fluid and lean tissue) by NMR. At 1 year of age, female CIDE-A transgenic mice have a significantly lower body weight, lower fasting blood glucose and plasma insulin levels, and increased glucose tolerance compared to their nontransgenic littermates. Whole body composition analysis indicates that these mice also have significantly less fat compared to control mice. Female CIDE-A transgenic mice and their nontransgenic littermates were sacrificed at 1.5 years of age. Tissues (several adipose depots, liver, kidney, heart, and skeletal muscle) were dissected and weighed. We found that the difference in fat mass between transgenic and control mice was not a result of decreased fat in any one particular depot. Rather, the CIDE-A mice have a decreased amount of fat in all adipose depots tested (subcutaneous, perigonadal, retroperitoneal, mesenteric and brown adipose). Recently, we have initiated studies to characterize CIDE-A transgene expression levels in all adipose as well as selected other tissues. We will determine the effect of transgene expression on endogenous mouse CIDE-A RNA and protein levels. We also are analyzing the levels of triacylglycerol content in each tissue to determine the effect of CIDE-A expression on lipid accumulation. Together, these results may help to elucidate the mechanism(s) by which CIDE-A affects lipid metabolism and accumulation.

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Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll; by funds from the NIH (grant DK075436-01 and AG019899-06 for JJK); support from the Diabetes Research Initiative at Ohio University; by funds from the Provost's Undergraduate Research Fund at Ohio University, and a generous gift from AMVETS.

Nothing to Disclose: HT, BK, DB, SO, MT, EOL, JJK

P1-422

***Pnpla3*/Adiponutrin-Deficient Mice Maintain Normal Adipose Development and Liver Lipid Homeostasis.**

W Chen Ph. D¹, BH Chang Ph. D¹, L Li MD¹ and L Chan MD¹.

¹Baylor Coll of Med Houston, TX.

PNPLA3 (Adiponutrin), a novel patatin-like phospholipase domain containing (PNPLA) enzyme, is predominantly expressed in adipose tissue. Its expression appears to be regulated by changes in energy balance. Genetic studies in human have recently identified polymorphisms in PNPLA3 linked to obesity and insulin sensitivity, and are strongly associated with human non-alcoholic fatty liver disease (NAFLD), suggesting that it may play an important role in regulating body weight, adiposity and whole body lipid homeostasis. We have generated *Pnpla3*-inactivated mice by gene targeting to examine its function. We found that *Pnpla3* inactivation has no effect on body fat composition and adipose tissue development whether the mice were fed a regular chow or high-fat diet. Mice with or without *Pnpla3* maintained normal glucose homeostasis and showed no difference in insulin sensitivity. Loss of *Pnpla3* had no effect on liver triglyceride (TG) content or plasma AST or ALT levels under normal or different fatty liver-inducing diets. However, the mRNA expression of *Pnpla5*, another patatin-like family protein, was found to be upregulated in the adipose tissue, though not the liver, of *Pnpla3*-deficient mice. *In vivo* and *in vitro* molecular studies demonstrated that *Pnpla3* and *Pnpla5* shared similar but slightly differential regulations by different diets as well as insulin and glucose activated lipogenic transcription factors such as Sterol response element binding protein (Srebp) 1c and carbohydrate response element binding protein (Chrebp). Both genes in white adipose tissue and liver were also highly regulated by thyroid hormones, a key regulator of adipocyte differentiation and metabolic homeostasis. In conclusion, *Pnpla3* appears dispensable for normal adipose tissue development and liver TG metabolism in mice. The upregulation of adipose tissue *Pnpla5* may partly compensate for the loss of *Pnpla3* in this tissue, suggesting redundant/overlapping functions of these two paralogous enzymes.

Sources of Research Support: NIH grant HL-51586 (to L.C.); AHA South Central Affiliate Postdoctoral Fellowship No. 0825134F (to W. Chen).

Nothing to Disclose: WC, BHC, LL, LC

P1-423

c-Ski Transgenic Mice Are Resistant to Diet-Induced Obesity.

M Diaz BSc¹, Nick Martel BSc¹, R Fitzsimmons BSc¹, M Pearen BSc¹, G.E. Muscat PhD¹ and G. M. Leong MB BS, PhD^{1,2}.

¹The Univ of Queensland St Lucia, Australia and ²Mater Children's Hosp South Brisbane, Australia.

Introduction

Transgenic mice over-expressing chicken Ski (c-Ski) develop marked muscle hypertrophy, and decrease in body fat (1). The underlying mechanisms for the decreased fat mass in the c-Ski mice are unclear.

Previous studies in our laboratory suggest that the skeletal muscle expression of the master lipogenic regulator SREBP1c and the nuclear receptor, LXR α are suppressed in the Ski mice (2). Based on these and other findings, we hypothesized that c-Ski mice are resistant to diet-induced obesity and glucose intolerance.

Methods: Wild type (WT) and Ski male mice were challenged on a high fat (HF) diet for 12 weeks from 6 weeks old, weighed regularly, and glucose tolerance tests performed at 12-14 weeks old, with body composition analysed by tissue weighing and dual energy x-ray absorptiometry (DXA). In addition, food intake, energy expenditure and physical activity over 24 hours within metabolic cages was measured using the Continuous Live Animal Monitoring Systems (CLAMS), Columbus Instruments.

Results: At 18-20 weeks of age, HF-fed WT mice compared to chow-fed WT mice were significantly heavier by 25%. By contrast, body weights of HF- and chow-fed Ski mice were not significantly different, despite Ski mice having a lower rate of physical activity and the same amount of food consumption than WT mice. Moreover, in HF-fed Ski mice, the fat pads were significantly smaller and the muscle mass larger than in HF-fed WT littermate controls. Glucose tolerance tests revealed HF-fed Ski mice had unaltered glucose tolerance compared to chow-fed Ski mice, whilst as expected, WT mice on HF-diet compared to chow-fed WT mice had significantly worse glucose tolerance. The underlying mechanisms for these observations in the Ski mice remain uncertain, but is the focus of further study in our laboratory.

In conclusion, these studies suggest that c-Ski transgenic mice are resistant to diet-induced obesity and glucose-intolerance. These results suggest Ski regulates body composition and risk for obesity and metabolic disease.

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Sources of Research Support: Queensland Smart State PhD Scholarship to MD; National Health and Medical Research Council Project Grant and Career Development Award to GML and Fellowship to GEM; The Endocrine Society Bridge Grant to GML ;Mater Children's Hospital Golden Casket Medical Research Fund.

Nothing to Disclose: MD, NM, RF, MP, GEM, GML

P1-424

Dietary Fat, but Not Sugar, in Early Life Promotes Visceral Adiposity and Insulin Resistance in Maturing Piglets - Pointers to a Role of IGFBP2 in the Nutritional Regulation of Insulin Sensitivity.

MA Sabin MRCPCH(UK) FRACP PhD^{1,2,3}, SW Yau BSc(Hons)^{1,2}, JZY Chau BSc(Hons)^{1,2}, IJ Clarke PhD³, VC Russo PhD^{1,2}, FR Dunshea PhD², M Cox² and G Werther MD FRACP^{1,2}.

¹Murdoch Childrens Res Inst and Royal Children's Hosp Melbourne, Australia ; ²Univ of Melbourne Melbourne, Australia and ³Monash Univ Melbourne, Australia.

Background. Childhood obesity predisposes to Type 2 diabetes, but factors other than level of adiposity also modify risk (1). Nutrition in early life may independently influence insulin sensitivity (IS) (2) by impacting upon insulin/Insulin-like Growth Factor (IGF) function. Recent evidence also demonstrates a link between IGF binding protein 2 (IGFBP2) and adiposity/IS (3).

Aims. 1. To investigate (using a juvenile porcine model) the effect of continuous dietary fat/sugar supplementation in early life on body composition, IS and IGFBP2 expression. 2. To explore (using *in-vitro* cultures of pre-adipocytes (3T3-L1) and myoblasts (C2C12)) changes in IGFBP2 expression during differentiation, and in response to fatty acids \pm insulin.

Methods. Piglets (n=50) received a control diet or one supplemented with 18% saturated fat (SF), 18% mono-unsaturated fat (MUF), 18% mixed fat (MF), or 50% sucrose (SUC) from 7 days of age. Weight was recorded over 16 weeks. End of study measures included a) body composition by DEXA, b) fasting insulin, c) Whole Body IS (WBIS) by hyperinsulinaemic-euglycaemic clamps and d) mRNA expression in skeletal muscle (SM) and subcutaneous adipose tissue (SAT). *In-vitro* experiments assessed IGFBP2 mRNA and protein in differentiating pre-adipocytes and myoblasts, and the effect of fatty acids \pm insulin on IGFBP2 expression in myotubes.

Results. SUC-fed piglets gained most weight due to a larger overall intake ($p < 0.01$), while SFA, MUF and MF piglets gained more weight per unit energy intake ($p < 0.001$). Total percentage fat ($p = 0.03$) and visceral fat ($p = 0.04$) was highest in those fed MUF. WBIS was reduced in fat-fed piglets ($n = 26$; $p = 0.04$), although only those fed MUF had raised fasting insulin ($p = 0.03$). SM IGFBP2 mRNA was also increased in MUF-fed piglets ($p = 0.009$). Overall, IGFBP2 mRNA in SM showed more association with measures of fat mass and IS, than IGFBP2 mRNA in SAT. *In-vitro* studies confirmed that myoblasts, but not pre-adipocytes, express IGFBP2 throughout differentiation and that exposure of myotubes to fatty acids and insulin increases IGFBP2 mRNA levels ($p < 0.05$).

Conclusions. Dietary composition in early life impacts upon later fat mass accrual and IS. Dietary fat, rather than sugar, promotes adiposity and insulin resistance *in-vivo*, and *in-vitro* studies confirm that fatty acids regulate IGFBP2 expression in muscle. These studies point to potential mechanisms whereby IGFBP2 may mediate effects of early nutrition on insulin sensitivity.

(1) Rosenbloom, et al. *Pediatr Diabetes* 2008; 9:512

(2) Demmelmair et al *Early Hum Dev* 2006; 82:567

(3) Wheatcroft, S.B., et al. *Diabetes* 2007; 56:285

Nothing to Disclose: MAS, SWY, JZYC, IJC, VCR, FRD, MC, GW

P1-425

The Corepressor SMRT Regulates Adipose Tissue Accumulation and Adipocyte Insulin Sensitivity In Vivo.

MM Sutanto BA¹, KK Ferguson BA¹, MJ Brady PhD¹ and RN Cohen MD¹.

¹Univ of Chicago Chicago, IL.

The silencing mediator of retinoid and thyroid hormone receptors (SMRT) serves as a corepressor for nuclear receptors and other factors. Recent evidence suggests that SMRT is an important regulator of metabolism, but its role in adipocyte function in vivo remains unclear. We generated heterozygous SMRT knock-out (SMRT +/-) mice to investigate the function of SMRT in the adipocyte and the regulation of adipocyte insulin sensitivity. We show that SMRT +/- mice are normal weight on a regular diet, but develop increased adiposity on a high-fat diet (HFD). The mechanisms underlying this phenotype are complex, but appear to be due to a combination of increased adipogenesis, resulting in a greater number of smaller subcutaneous adipocytes, and decreased leptin expression, resulting in greater caloric intake. Interestingly, adipocyte insulin sensitivity, as measured by insulin-induced Akt phosphorylation and insulin-mediated suppression of lipolysis, was enhanced in SMRT +/- adipocytes. These findings suggest that SMRT regulates leptin expression and limits the ability of fat mass to expand with increased caloric intake, but that SMRT also negatively regulates adipocyte insulin sensitivity.

Sources of Research Support: NIH grant R01DK078125 awarded to RNC.

Nothing to Disclose: MMS, KKF, MJB, RNC

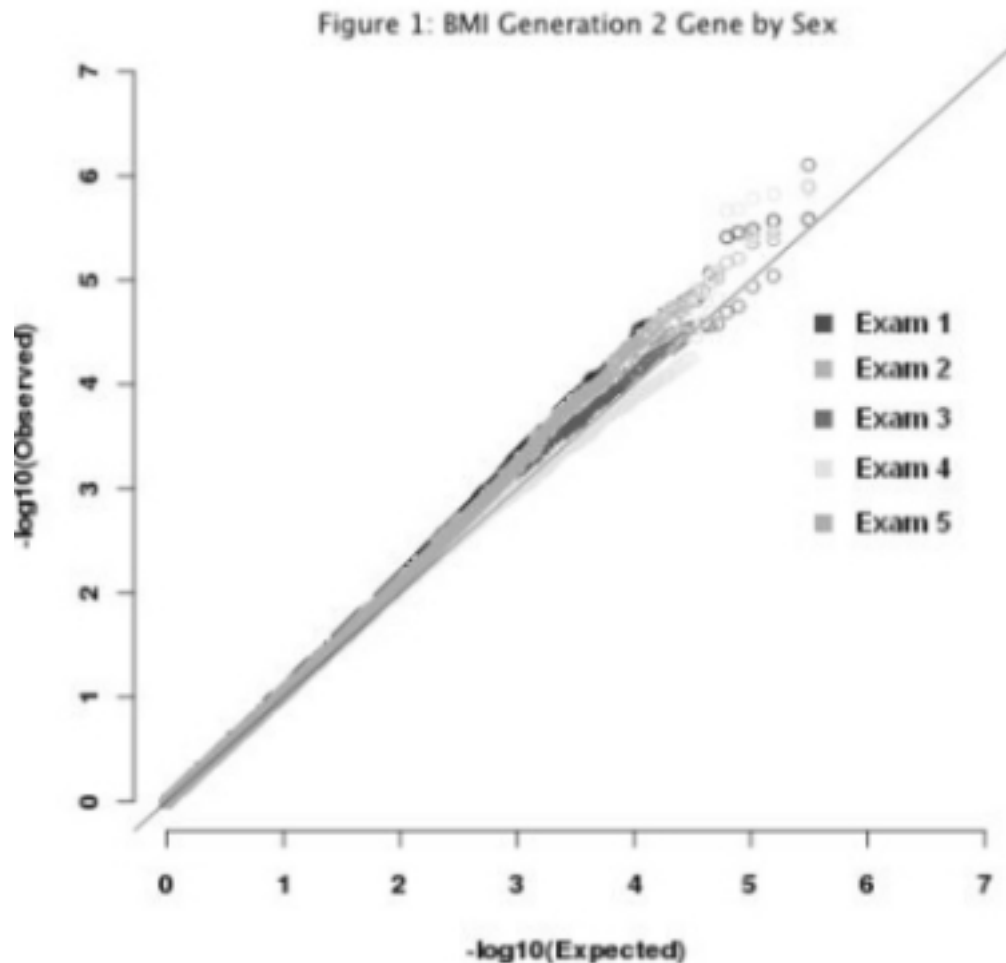
P1-426

Gene by Sex Interaction for Body Mass Index in the Framingham Heart Study.

A Benjamin¹, S Suchindran¹, K Pearce¹, J Rowell MD¹, LF Lien MD¹, JR Guyton MD¹ and JJ McCarthy MPH, PhD¹.

¹Duke Univ Med Ctr Durham, NC.

Obesity is an increasingly prevalent and severe health concern with a substantial heritable component. Obesity phenotypes, such as body mass index (BMI), also show marked sex differences. We sought to determine if the effect of genetic variants on BMI also differed by sex. We performed a genome-wide association study modeling the effect of genotype by sex interaction on BMI, using open-access genotype data obtained from the Affymetrix 550K gene array in the population-based Framingham Heart Study. Linear mixed models were run, accounting for relatedness between individuals, controlling for age, and restricting to adults <50 years of age to enhance potential estrogen-related associations. Data from five different exams were used to run five individual genome-wide association studies and the consensus of the top 1000 hits for each was evaluated. The theoretical vs. observed quantiles plots for all five exams are shown in Figure 1.



No variants showed genome-wide significant evidence of sex differences in their effect on BMI in any of the individual exams. However, five polymorphisms displayed a consistent gene-by sex BMI association across all five exams. The p-values of the five polymorphisms across the five exams ranged from 0.0014 to 1.39e-6. These variants were clustered in a region near the gene LYPLAL1, which encodes a monoacylglycerol lipase. Variants at this locus resulted in increased BMI in men, but decreased BMI in women. This gene has also been found to show increased mRNA expression in the subcutaneous adipose tissue of obese, postmenopausal women (1). Our data lend support to a prior study that identified this locus as influencing measures of obesity in a sex-specific fashion (2).

(1) Gregory R. Steinberg, Bruce E. Kemp and Matthew J. Watt
Am J Physiol Endocrinol Metab 293:958-964, 2007.

(2) Cecilia M. Lindgren et al., PLoS Genet. 2009 June; 5(6): e1000508.

Sources of Research Support: In part by grant RO1 HL085191-04 from the National Heart, Lung and Blood Institute (NHLBI), National Institutes of Health (P.I. McCarthy). A. Benjamin is supported by National Institutes of Health Training Grant T32 GM071340-06. The Framingham Heart Study is conducted and supported by the NHLBI in collaboration with Boston University. This manuscript was not prepared in collaboration with investigators of the Framingham Heart Study

and does not necessarily reflect the opinions or views of the Framingham Heart Study, Boston University, or the NHLBI.

Nothing to Disclose: AB, SS, KP, JR, LFL, JRG, JJM

P1-427

Brown Adipose Tissue in Humans: Prevalence, Anthropometric and Metabolic Predictors.

P Lee^{1,2}, JR Greenfield^{1,2}, MJ Fulham³ and KKY Ho^{1,2}.

¹St Vincent's Hosp Sydney, Australia ; ²Garvan Inst of Med Res Sydney, Australia and ³Royal Prince Alfred Hosp Sydney, Australia.

Brown adipose tissue (BAT) plays a major role in energy homeostasis in animals. The detection of adipose tissue with high glucose utilisation, using Positron Emission Tomography (PET)-CT in humans, has challenged the view that BAT disappears after infancy.

The aim of our study is to determine the prevalence of BAT and factors associated with its presence in adult humans. We undertook a retrospective evaluation of 4834 PET-CT scans, using 18F-fluodeoxyglucose (FDG), in 2935 oncology patients between 2003-2008. BAT was identified as areas of increased FDG uptake within fat that was delineated on CT. Age, sex, disease activity, body mass index (BMI) and fasting blood glucose concentration were assessed for association with presence of BAT.

Positive scans were identified in 250 patients, yielding a prevalence of 8.5%. Among 747 patients who were scanned more than once, at least one positive scan was identified in 145 patients, yielding a higher prevalence of 19.4%. BAT was detected most commonly in the upper torso in the cervico-thoracic region. BAT was more commonly present in women, in younger (33 ± 10 vs 52 ± 22 years, $p<0.001$) and less overweight (20.1 ± 2.3 vs 24.9 ± 4.4 kg/m², $p<0.01$) individuals. Mean blood fasting glucose was lower in patients with PET-CT positive for BAT (4.8 ± 0.6 vs 5.5 ± 0.3 mmol/L, $p<0.01$). Among patients scanned more than once (mean scanning interval: 7.2 ± 1.1 months), BAT was detected when body weight and fasting glucose were significantly lower (54.9 ± 0.5 vs 58.2 ± 0.8 kg, $p<0.001$ and 4.9 ± 0.3 vs 5.5 ± 0.3 mmol/L, $p=0.03$).

In summary, BAT is present in up to 1 in 5 patients and correlates negatively with BMI and glucose concentration between and within subjects.

In conclusion, BAT is present in a proportion of adult humans and may play a significant role in the regulation of energy metabolism in adult life.

Sources of Research Support: NHMRC Australia.

Nothing to Disclose: PL, JRG, MJF, KKYH

P1-428

Increased Epicardial Fat Volume in HIV-Infected Men and Relationships to Body Composition and Metabolic Parameters.

J Lo MD, MMSc.¹, S Abbara MD¹, JA Rocha-Filho MD¹, L Shturman MD¹, J Wei¹ and SK Grinspoon MD¹.

¹Massachusetts Gen Hosp Boston, MA.

Background: Patients with HIV infection can develop body fat distribution changes and associated metabolic abnormalities that might predispose to earlier development of cardiovascular disease (CVD).

Methods: Seventy-eight HIV-infected men (age 46.5 ± 6.5 yrs and mean duration of HIV infection 13.5 ± 6.1 yrs, CD4+ T-lymphocytes 523 ± 282 ; 81% undetectable HIV viral load) and 32 HIV-seronegative men (age 45.4 ± 7.2 yrs) with similar BMI, total body fat mass and CAD risk factors, without history or symptoms of CVD, were prospectively recruited. Epicardial fat volume was obtained using 64-slice cardiac multidetector row computed tomography (MDCT).

Results: HIV-infected men demonstrated higher epicardial fat volume than non HIV-infected men (112.3 (81.9, 158.7)[median (interquartile range)] cm^3 vs. 84.6 (57.7, 130.7) cm^3 ; $p=0.04$). Among HIV-infected patients, epicardial fat volume was most strongly associated with abdominal visceral adipose tissue area (VAT) (Spearman $\rho=0.76$, $p<0.0001$) and this association remained highly significant ($\beta=0.30\text{cm}^3/\text{cm}^2$, $p<0.0001$) in a multivariate model controlling for multiple other body composition parameters including BMI, abdominal subcutaneous adipose tissue area (SAT), and total fat mass. In addition, among the HIV-infected patients, epicardial fat was related to fasting glucose ($\rho=0.41$, $p=0.001$) and insulin ($\rho=0.44$, $p=0.0003$) and these relationships remained significant in multivariate modeling controlling for numerous other factors known to be related to glucose homeostasis in this population, including age, race, BMI, adiponectin, VAT, and use of antiretroviral therapy ($\beta=0.10\text{cm}^3/\text{mg/dL}$, $p=0.02$ for glucose and $\beta=0.07\text{cm}^3/\mu\text{U/mL}$, $p<0.03$ for insulin).

Conclusions: This was the first study to investigate epicardial fat volumetrically in HIV-infected patients and we demonstrated that HIV-infected men without symptoms of cardiovascular disease had higher epicardial fat volume compared to non HIV-infected patients with similar BMI, total body fat mass and traditional cardiovascular risk factors. Epicardial fat was significantly related to visceral fat and independently related to glucose and insulin among HIV patients.

Sources of Research Support: Bristol Myers Squibb, Inc.(Dr. Grinspoon), NIH K23 HL092792 (Dr. Lo), K24 DK064545 (Dr. Grinspoon), T32 HL076136 (Dr. Shturman) and M01 RR01066-25S1. Funding sources had no role in the design of the study, data analysis or the writing of the manuscript.

Nothing to Disclose: JL, SA, JAR-F, LS, JW, SKG

P1-429

Contributions of Adiposity, Leptin, Adiponectin, and Muscle Mass to Insulin Resistance in the Baltimore Longitudinal Study of Aging (BLSA).

R Ramachandran MD¹, KS Gravenstein BS¹, DL Melvin RN², OD Carlson PhD¹, JM Egan MD¹, L Ferrucci MD, PhD¹ and CW Chia MD¹.

¹Natl Inst on Aging, Natl Inst of Hlth Baltimore, MD ; ²Natl Inst on Aging, Natl Inst of Hlth Baltimore, MD and ³Medstar Res Inst Baltimore, MD.

Insulin resistance(IR) is a key metabolic feature of obesity and contributor to the development of T2DM. Adipose tissue (especially visceral fat) and skeletal muscle have been associated with higher and lower IR respectively. A recent study showed older adults with T2DM are at increased risk of accelerated loss in muscle mass[1]. It has never been conclusively demonstrated that the effect of fat on IR is mediated by adipokines. We examined the contributions of muscle mass(MM), adiposity and adipokines to IR.

Seven hundred and twenty-six BLSA subjects had data on fasting glucose, insulin, leptin, adiponectin, anthropometrics, MM (by CT mid-thigh muscle cross-sectional area), and trunk fat mass (TFM, by DXA).Independent associations of age, MM, TFM, leptin and adiponectin with HOMA-IR, a marker of IR, were tested in linear models. Biochemical markers were log-transformed for analyses.

Characteristics

| | Women | Men |
|-----------------------|-----------------|---------------|
| N | 365 | 361 |
| Age,yrs | 66.8±0.7 | 69.4±0.7 |
| BMI,kg/m ² | 26.5±0.3 | 27.4±0.2 |
| Leptin,ng/mL¶ | 21.2(11.0-39.1) | 8.6(4.7-15.9) |
| Adiponectin,ng/mL¶ | 14.0(8.9 -23.1) | 9.8(5.5-17.7) |
| HOMA-IR | 1.6(1.0-2.7) | 1.7(1.1-2.6) |
| *MM,cm ² ¶ | 100.2±1.0 | 121.6±1.4 |
| *TFM,kg¶ | 15.2±0.4 | 14.1±0.3 |

Data expressed as mean±SE or median (interquartile range);*adjusted for height(ht)= (area or mass)/(ht²)X(mean ht)²; ¶P<0.05

MM was positively correlated with HOMA-IR after adjusting for age in both genders. This association was substantially attenuated after adjusting for TFM. In the final model, leptin, adiponectin and TFM were each independently associated with HOMA-IR while MM was not.

Linear Models Estimating HOMA-IR

| Model | | Women | | Men | |
|-------|-------------|--------|----------------|--------|----------------|
| | | β | R ² | β | R ² |
| 1 | MM | 0.20‡ | | 0.23‡ | |
| | Age | 0.21‡ | 0.04 | 0.18† | 0.03 |
| 2 | MM | -0.03 | | 0.09 | |
| | Age | 0.09 | | 0.12† | |
| | TFM | 0.50‡ | 0.24 | 0.50‡ | 0.27 |
| 3 | MM | -0.04 | | 0.07 | |
| | Age | 0.09 | | 0.14† | |
| | TFM | 0.23‡ | | 0.34‡ | |
| | Leptin | 0.33‡ | | 0.19‡ | |
| | Adiponectin | -0.20‡ | 0.34 | -0.15‡ | 0.30 |

‡P<0.001, †P< 0.05

The lack of association between HOMA-IR and MM may signify that adiposity and adipokines, but not MM, are the predominant contributors to IR. An alternative explanation is that the morphological measure of MM by CT may not capture specific qualitative characteristics relevant to IR. The interactions among adipokines, adipocytes and muscle need to be explored further using better measures of muscle mass.

(1)Park SW et al., Diabetes Care 2009; 32:1993-1997

Sources of Research Support: In part by the Intramural Research Program of the National Institutes of Health, National Institute on Aging. A portion of that support was through a R&D contract with MedStar Research Institute.

Nothing to Disclose: RR, KSG, DLM, ODC, JME, LF, CWC

P1-430

A Retrospective Study on the Association between Increasing BMI and Adverse Pregnancy Outcomes in Black Women.

N Ugokwe MD¹, O Butt MD¹, C Bowers MD², P Flom PhD¹ and O Muneyyirci-Delale MD^{1,2}.

¹SUNY Downstate Med Ctr Brooklyn, NY and ²Kings County Hosp Ctr Brooklyn, NY.

The aim of this study is to assess the affects of increasing BMI and associated risks of adverse pregnancy outcomes in a population of black women.

Study Design

This was a retrospective study with chart review from March 2008 to June 2008. The study consisted of 501 black females with singleton pregnancies, seen and delivered at Kings County Hospital. Patients' age, parity, pre-pregnancy medical and surgical conditions, weight, height, BMI, race, pregnancy, and mode of delivery were recorded from medical charts. Jonckheere-Terpstra test as well as logistic regression were used for data assessment with a confidence interval was of 95% with $p < .05$.

Results

Those in the overweight, class 1, class 2, and class 3 categories of BMI, according to WHO and NIH, were associated with significant increase in gestational diabetes mellitus (GDM) and hypertension as BMI increased compared to those in the normal weight category. 44% were normal weight (BMI >18.5 to 24.9 kg/m²). 21% were overweight (>25.0 to 29.9 kg/m²) with an OR of 2.30 (1.23-4.32) for hypertension and an OR of 1.39 (0.57-3.93) for GDM. 20% were in class 1 (>30.0-34.9 kg/m²) with an OR of 1.91 (1.00-3.63) for hypertension and an OR of 2.24 (0.99-5.12) for GDM. 10.5% were in class 2 with an OR of 2.48 (1.11-5.55) for hypertension and an OR of 3.36 (1.33-8.47) for GDM. 4.9% were in class 3 with an OR of 9.12 (3.18-26.2) for hypertension and an OR 6.02 (1.98-18.31) for GDM.

Conclusion

There is a statistically significant increase in gestational diabetes mellitus and hypertension associated with increasing BMI during pregnancy. Preconceptional and during pregnancy nutritional counseling particularly in this population of women is imperative in preventing unfavorable pregnancy outcomes.

Nothing to Disclose: NU, OB, CB, PF, OM-D

P1-431

Relationship between Maternal Obesity and Birth Weight in Pregnancies Complicated by Gestational Diabetes.

A St-Yves R.D.¹, AS Morisset R.D., M.Sc.^{1,2}, MC Dube Ph.D.², A Tchernof Ph.D.^{1,2}, SJ Weisnagel M.D.² and J Robitaille R.D., Ph.D.¹.

¹Laval Univ Quebec, Canada ; ²Laval Univ Quebec, Canada ; ³Laval Univ Med Res Ctr Quebec, Canada and ⁴Laval Univ Med Res Ctr Quebec, Canada.

Gestational diabetes and overweight or obesity are both associated with poor neonatal outcomes. The aim of this study was to investigate the relationship between anthropometric measures at birth and maternal obesity among women with gestational diabetes. **Methods:** Medical records of women with gestational diabetes who gave birth at the Laval University Hospital (Quebec, Canada) were retrospectively reviewed. The study group included 163 women and their newborn. Women were diagnosed and treated by a multidisciplinary team according to the Canadian Diabetes Association Guidelines. Body mass index (BMI) was calculated from self-reported prepregnancy weight in kilograms and measured height in meters. Anthropometric measures at birth, including infant birth weight, birth length, abdominal circumference and head circumference, were obtained from the review of infants' medical records. **Results:** The proportion of women with a prepregnancy BMI value greater or equal to 25 kg/m² was 61.5%, whereas 38.5% of women with gestational diabetes were considered obese (BMI ≥ 30 kg/m²). Infant birth weight was higher among women with a prepregnancy BMI greater or equal to 25 kg/m² compared to normal weight women (3351 ± 515 g vs. 3041 ± 709 g, p < 0.01). Obese women with gestational diabetes also delivered an infant with a higher birth weight compared to normal weight women with gestational diabetes (3344 ± 506 g vs. 3041 ± 709 g, p < 0.05). Results were similar after adjustments for gestational age and smoking status. Infants of women with a prepregnancy BMI greater or equal to 25 kg/m² had a higher head circumference than infants of normal weight women (34.3 ± 1.7 cm vs. 33.3 ± 2.2 cm, p < 0.01). The head circumference was also higher among infants of obese women compared to infants of normal weight women (34.4 ± 1.7 cm vs. 33.3 ± 2.2 cm, p < 0.05). Birth length and abdominal circumference were similar between infants of normal weight women, overweight women and obese women. Prepregnancy BMI was significantly correlated with birth weight and head circumference among women with gestational diabetes (r = 0.20 and r = 0.24, p < 0.05). **Conclusion:** A large proportion of women with gestational diabetes are overweight. Diabetic women with maternal overweight/obesity delivered larger babies than diabetic women with normal weight. These results suggest that even among women who are treated for gestational diabetes, overweight and obesity are associated with higher infant birth weight and head circumference.

Sources of Research Support: Fonds de la recherche en sante du Quebec (FRSQ); Canadian Institutes of Health Research (CIHR).

Nothing to Disclose: AS-Y, ASM, MCD, AT, SJW, JR

P1-432

Cutoff Points of Abdominal Obesity Indices in Screening for Non-Alcoholic Fatty Liver Disease.

SJ Yang MD¹, HY Choi MD¹, HJ Yoo MD¹, YJ Kim MD¹, CR Eun MD¹, JH Kim MD¹, HY Kim MD¹, JA Seo MD¹, SG Kim MD¹, NH Kim MD¹, KM Choi MD¹, SH Baik MD¹ and DS Choi MD¹.

¹Korea Univ Hosp Seoul, Korea.

Objective: Abdominal obesity is associated with metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). Although there have been many studies to determine the optimal cutoff points of waist circumference or visceral fat area in screening for metabolic syndrome, there have been no reports to establish adequate cutoff points of abdominal obesity indices in screening for NAFLD. Therefore, we examined the appropriate cutoff points of abdominal obesity indices associated with NAFLD in Korean men and women using receiver operating characteristic (ROC) curve analysis. Furthermore, we compared the usefulness of various abdominal obesity indices measured using computed tomography (CT), dual-energy X-ray absorptiometry (DXA) and waist circumference for detecting NAFLD.

Design and methods: We analyzed the baseline data of an ongoing prospective, observational cohort study, including a total of 456 healthy subjects 20-88 years of age. NAFLD was diagnosed by un-enhanced CT using liver attenuation index.

Results: All ROC curves of waist circumference, DXA-measured trunk fat mass and CT-measured visceral fat area were significantly above the diagonal line. There were no significant differences in the area under the curve values among these abdominal obesity indices when compared with waist circumference in both genders. In men, the optimal cutoff point of waist circumference in screening for NAFLD was 89 cm, which had 75% sensitivity and 64% specificity. In women, the optimal cutoff value of waist circumference was 84 cm with 84% sensitivity and 62% specificity. Both cutoff values for men and women had high negative predictive values (88% and 97%, respectively). The optimal cutoff points of the CT-measured visceral fat area for detecting NAFLD were 132 cm² for men and 119 cm² for women. The optimal cutoff points of DXA-measured trunk fat mass for the prediction of NAFLD were 6.5 kg for men and 7.8 kg for women. Furthermore, DXA-measured trunk fat mass (Odds ratio: 2.87 [1.74~4.75]) had the greatest increase of risk for NAFLD in men, whereas CT-measured visceral fat area (Odds ratio: 4.25 [2.62~6.90]) had the most prominent risk increment in women suggesting gender dimorphism in the risk profiles for NAFLD.

Conclusions: The waist circumference measurement is as useful as DXA and CT for predicting NAFLD in Korean adults. The appropriate waist circumference cutoff value in screening for NAFLD is 89 cm for men and 84 cm for women.

Nothing to Disclose: SJY, HYC, HJY, YJK, CRE, JHK, HYK, JAS, SGK, NHK, KMC, SHB, DSC

P1-433

Catechol O-Methyltransferase Val158Met Polymorphism Is Related to the Increased BMI, Visceral Fat and High Blood Pressure in Japanese Obese Patients.

Toshihide Yoshida MD,PhD¹, Yasuto Takakura MD,PhD², Akinori Kogure MD,PhD³, Keiji Yoshioka MD,PhD⁴, Takuya Harayama MD¹ and Naoto Nakamura MD,PhD³.

¹Kyoto City Hosp Kyoto, Japan ; ²Kyoto 2nd Red Cross Hosp Kyoto, Japan ; ³Kyoto Prefecture Univ of Med Kyoto, Japan and ⁴Matsushita Memorial Hosp Moriguchi, Japan.

[Background of study]

The gene coding for catechol O-methyltransferase (COMT) contains Val158Met polymorphism that causes methylation activity in Met allele reduced 60 to 75%. Recently this polymorphism was reported to associate with abdominal obesity and high blood pressure in Swedish men.

[Objective]

The aim of this study is to research whether this functional polymorphism induces abdominal obesity and elevation of blood pressure in Japanese as the same as Caucasians.

[Methods]

161 obese Japanese subjects (age 50.0 ± 14.4 yrs, BMI 33.3 ± 5.9 kg/m²) were enrolled in this study. The COMT genotypes were measured by a fluorescent allele-specific DNA primer assay system. We studied how this polymorphism affects the clinical characteristics and blood chemistry data.

[Results]

The genotype frequency of COMT Val158Met polymorphism in obese subjects was similar to the lean control subjects (obese subjects: Val/Val 46.6%, Val/Met 45.3%, Met/Met 8.1%, the frequency of Met allele was 0.31, lean subjects: Val/Val 51.5%, Val/Met 42.3%, Met/Met 6.2%, the frequency of Met allele was 0.27). We could not find significant differences on body weight and waist to hip ratio, but BMI (Val/Val 32.2 ± 4.7 kg/m², Val/Met 33.8 ± 6.5 kg/m², Met/Met 37.2 ± 6.5 kg/m², $p < 0.05$), waist circumference (Val/Val 105.9 ± 12.0 cm vs Met allele 110.5 ± 13.2 cm, $p < 0.05$), visceral fat (Val/Val 132.5 ± 55.1 cm² vs Met allele 154.5 ± 67.2 cm², $p < 0.05$) was significantly increased in subjects with Met allele. The significant differences in systolic (Val/Val 139 ± 18 mmHg vs Met allele 150 ± 13 mmHg, $p < 0.01$) and diastolic (Val/Val 81 ± 11 vs Met allele 89 ± 13 , $p < 0.01$) blood pressure was demonstrated. FBG (Val/Val 100.0 ± 9.3 mg/dl, Val/Met 100.2 ± 9.3 mg/dl, Met/Met 104.9 ± 12.1 mg/dl), IRI (Val/Val 10.8 ± 7.5 μU/ml, Val/Met 9.3 ± 5.5 μU/ml, Met/Met 11.5 ± 5.9 μU/ml), and HOMA-R did not show significant differences among three genotypes. We could not find significant relation on frequency of IGT patients and this polymorphism (NGT: Val/Val 48.4%, Val/Met 48.8%, Met/Met 2.4%, frequency of Met allele was 0.27, IGT: Val/Val 52.0%, Val/Met 36.0%, Met/Met 12.0%, frequency of Met allele was 0.30).

[Conclusion]

The Met allele of COMT Val158Met polymorphism had significant influence on BMI, waist circumference, visceral fat and blood pressure in Japanese obese subjects. These results were similar to the data showed in Caucasians.

Nothing to Disclose: TY, YT, AK, KY, TH, NN

P1-434

Cardiometabolic Risk and Sleep Duration in Severely Obese Women.

JA Brown MD¹, K Hames MS¹, F Amati PhD,MD¹, JP DeLany PhD¹ and BH Goodpaster PhD¹.

¹Univ of Pittsburgh Sch of Med Pittsburgh, PA.

PURPOSE: Sleep duration less than 6 hours is associated with a higher risk for obesity and greater cardiometabolic risk. It is not known, however, whether sleep duration is associated with greater cardiometabolic risk within the severely obese. The first aim of this study was to evaluate sleep duration in severely obese women compared to controls using wearable monitors. The second aim was to determine whether or not sleep duration is associated with cardiometabolic risk factors within severely obese women.

METHODS: 92 Class II and Class III obese women (BMI >40 kg/m²) and 23 age matched (30-55 years) control women ranging from normal weight to Class I obese (mean BMI 25.86±4.71 kg/m²) completed assessments for body composition, total daily energy expenditure and physical activity energy expenditure and nightly sleep duration measured over the course of 5-7 days by wearable activity monitors, blood pressure (BP) and fasting biochemical profiles in blood.

RESULTS: The sleep duration in the severely obese (374 min±8 min/night) was significantly lower compared to controls (450 min±16 min/night) ($p = <0.0001$). As expected, severely obese women had a higher BP, waist circumference, fasting insulin and a lower physical activity energy expenditure compared to controls (all<0.01). Within severely obese women, however, these selected cardiometabolic risk factors were not different in those who slept less than 6 hr/night vs those who slept >6hr/night, nor was cardiometabolic risk associated with mean sleep duration in severely obese. In conclusion, in conjunction with their higher cardiometabolic risk, severely obese women sleep significantly less than non-severely obese women. However, sleep duration alone does not appear to account for cardiometabolic risk within the severely obese.

Nothing to Disclose: JAB, KH, FA, JPD, BHG

P1-435

The Proper Value of Abdominal Circumference for Prediction of Atherosclerosis in Type 2 Diabetic Patients in Korea.

Jeong Seon Yoo MD¹, Eun Hae Lee MD¹, Woo Ho Shim MD¹, Sun Hee Beom MD¹, Ji Sun Nam MD¹, Kyung Rea Lee MD¹, Soon Ae Kim MD¹, Minho Cho MD¹, Jong Suk Park MD¹, Chul Woo Ahn MD¹, Bong Soo Cha MD¹, Eun Jig Lee MD¹, Sung Kil Lim MD¹, Hyun Chul Lee MD¹ and Eun Sook Kim MD¹.

¹YUMC Seoul, Korea.

Objectives: We investigated to decide the proper value of waist which could predict subclinical atherosclerosis in type 2 diabetic patients.

Methods: We supposed that increased carotid IMT was equivalent to subclinical atherosclerosis and abdominal obesity was judged by four well-known criteria. Mean IMT was measured at both carotid arteries by high resolution B-mode US in 771 type 2 diabetic patients. We calculated the relative risk of increased IMT when patients' waist was over the value of each criterion.

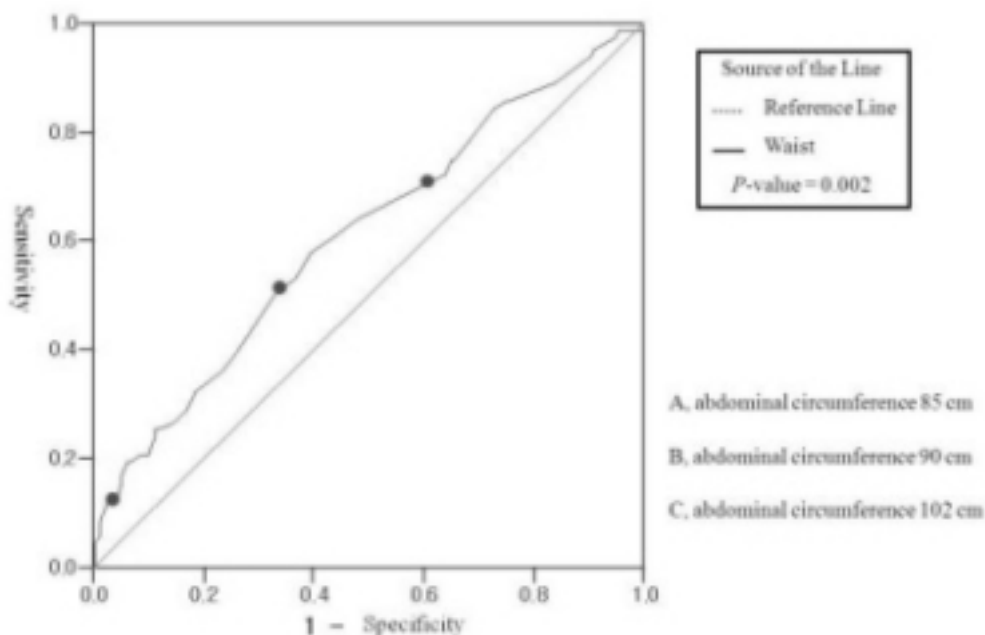
Results: The risk of subclinical atherosclerosis was increased in men with abdominal obesity according to three criteria except JASSO. The relative risk of increased IMT was 1.682 (95% CI = 1.147, 2.466) in whose waist was over 90 cm compared with less than 90 cm. But in women, the risk was not increased at all criteria.

Univariate and Multiple Logistic Regression Analysis of Atherosclerosis Related Factors

| | Mean ± SD | Univariate Correlation Coefficient | P-value | Multivariate Logistic Regression Analysis-Beta | Multivariate Logistic Regression Analysis-P-value |
|--------------------------|--------------|------------------------------------|---------|--|---|
| Age (years) | 61.0 ± 6.8 | 0.287 | <0.0001 | 0.009 | <0.0001 |
| BMI (kg/m ²) | 24.1 ± 2.7 | 0.088 | 0.076 | | |
| Waist (cm) | 168.9 ± 7.4 | 0.276 | <0.0001 | 0.006 | <0.0001 |
| Hypertension | 26.4%* | | | | |
| T.cholesterol (mg/dl) | 26.4% | -0.053 | 0.296 | | 0.294 |
| LDL-C (mg/dl) | 97.5 ± 32.8 | -0.088 | 0.098 | | |
| HDL-C (mg/dl) | 45.0 ± 12.8 | -0.151 | 0.004 | -0.001 | 0.193 |
| HbA1c (%) | 8.1 ± 1.9 | 0.053 | 0.209 | | |
| Fasting glucose (mg/dl) | 151.1 ± 65.7 | 0.033 | 0.517 | | |

SD, standard deviation; BMI, Body Mass Index; T.cholesterol, Total Cholesterol; HDL-C, high density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol. * percent of patients who have medical history of hypertension

Conclusion: The abdominal circumference has practical value as the marker of subclinical atherosclerosis in type 2 diabetic males in Korea, and the proper cut-off value of abdominal circumference as abdominal obesity is 90 cm.



Nothing to Disclose: JSY, EHL, WHS, SHB, JSN, KRL, SAK, MC, JSP, CWA, BSC, EYL, SKL, HCL, ESK

P1-436

High Rate of Attention Deficit Hyperactivity Disorder (ADHD) in Obese Adult Patients.

MF Docet MD¹, RV Garcia-Mayor MD¹, A Larranaga MD¹, M Carnero MD¹ and LF Perez MD¹.

¹Univ Hosp of Vigo Vigo, Spain.

BACKGROUND: The obesity prevalence and incidence has increased in almost the whole world becoming a public health problem. It is possible that a high frequent comorbid Attention Deficit Hyperactivity Disorder (ADHD) and obesity exists. It could be that the distinctive impulsivity and no-attentional behavior of ADHD patients may be a risk factor for the development of abnormal eating behaviors which may contribute to the arise of obesity. Then, ADHD may be the reason of the difficult treatment in obese patients.

OBJECTIVE: Determine the rate of ADHD in obese adult patients (cases) and in normal weight adult patients (controls), compare both rates and correlate them with subjects' gender, age and obesity level.

HYPOTHESIS: High rate of ADHD in obese adult patients of both sexes.

SETTING: University Hospital.

DESIGN: A prospective case-control study.

SUBJECTS: The study includes **128** cases which are obese patients defined by BMI ≥ 30 (**38,10 \pm 5,58**) consecutively attended at the Nutrition Section of the University Hospital of Vigo, aged **50,97 \pm 13,34**, range **18-79, 98** female and **30** male; and **50** controls which are normal weight patients defined by a BMI between **20-24,9**, aged **44,96 \pm 12,89**, range **19-72, 33** female and **17** male. Being the exclusion criteria: patients with cognitive disorders and unable to perform the symptoms screening questionnaire.

ETHICAL ASPECTS: All patients were invited to participate in it and asked to give their informed consent. This study has been approved by the local Ethical Committee.

METHODS: The rate of ADHD was determined by the Adult ADHD Self-Report Scale-V1.1 (ASRS-V1.1). All patients were weighted, measured and categorized by obesity level (BMI).

RESULTS: **Twenty eight out of one hundred and twenty eight (21,87%) obese adult patients** and **four out of fifty (8%) normal weight patients** had four or more checkmarks which indicate that their symptoms may be consistent with Adult ADHD. **OR=3,22.**

CONCLUSIONS: A high rate of ADHD was observed in our obese adult patients, almost it trebles the rate found in normal weight patients. Our results point to an association between ADHD and development of obesity. The presence of this disorder, and the distinctive impulsive and no-attentional behavior, may contribute to develop impulsive eating behaviors and, as a consequence, the development of obesity. Also, the ADHD may be the cause of the unsuccessful treatment of these subjects.

Nothing to Disclose: MFD, RVG-M, AL, MC, LFP

P1-437

The Correlation of Adiponectin and Kidney Function in South Korea.

SR Kim M.D.,PhD.,¹, SS Lee M.D.,¹, SJ Yoo M.D.,PhD.,¹, SM Kim M.D.,PhD.,², OK Hong PhD.,¹, MW Lee PhD.,², CB Lee M.D.,PhD.,³, SJ Oh M.D.,PhD.,⁴, HY Lee PhD.,⁵, SH Jee PhD.,⁵ and SK Kang M.D.,PhD.,¹.

¹Catholic Univ, Med Coll Bucheon, Republic of Korea ; ²Coll of Med, Korea Univ Seoul, Republic of Korea ; ³Hanyang Univ Sch of Med Seoul, Republic of Korea ; ⁴Kyung Hee Univ Sch of Med Seoul, Republic of Korea and ⁵Yonsei Univ Seoul, Republic of Korea.

PURPOSE: we investigated the association with the adiponectin level and glomerular filtration rate (GFR) in South Korean population.

METHODS: We reviewed the cross sectional data from 1,588 men and 1,371 women. We measured the Modification of Diet in Renal Disease (MDRD), insulin, adiponectin, HOMA-IR. The chronic kidney disease(CKD) are defined less than 60 ml/min/1.73 m² or more than 60 ml/min/1.73 m² and microalbuminuria

RESULTS: The patients' mean GFR was 87.8±36.6 (mean ± SD) ml/min/1.73 m² in male and 90.2 ±25.9 ml/min/1.73 m² in female. Adiponectin levels were 6.6± 4.2(mean± SD) ug/ml in male and 9.9± 6.9 (mean ±SD) ug/ml in female. The healthy persons of normal kidney function were 2752 (1455 male/1261 female). The patients of CKD were 245 (133 male/110 female). The adiponectin levels were 7.9± 5.2 (mean±SD) ug/ml in healthy persons and 10.6±10.5 (mean±SD) ug/ml in CKD patients. The persons of normal kidney function were divided into four by GFR(MDRD). The adiponectin level was not correlated with GFR (p=0.823), but adiponectin showed a trend toward increment as GFR increased in person of normal kidney function .

CONCLUSIONS: In the person of normal kidney function, serum adiponectin increases when renal function increases . But the significance is not. Also, the patient of chronic kidney disease has more increment of adiponectin level than the person of normal kidney function has. This discrepancy may be result form adiponectin resistance.

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. J Clin Invest 2006; 116(7): 1784-1792
Saraheimo et al, Diabetes Care. 2008 Jun;31(6):1165-9.

Sources of Research Support: Seoul City Research and Business Development Program, Republic of Korea (10526).

Nothing to Disclose: SRK, SSL, SJY, SMK, OKH, MWL, CBL, SJO, HYL, SHJ, SKK

P1-438

Vitamin D Status and Metabolic Risk Factors in Elderly Koreans; Ansan Geriatric Study.

JA Seo MD¹, H Cho¹, JH Kim MD¹, SJ Yang MD¹, HJ Yoo MD¹, HY Kim MD¹, SG Kim MD¹, KM Choi MD¹, SH Baik MD¹, DS Choi MD¹, MH Park MD¹, C Han MD¹ and NH Kim MD¹.

¹Korea Univ Med Ctr Seoul, Korea.

The association between vitamin D level and metabolic risk is not known in elderly Koreans. We examined whether vitamin D levels have an impact on body composition and the metabolic risk factors. 25-hydroxyvitamin D [25(OH)D] levels were measured in the cross-sectional sample of 218 men and 273 women aged ≥ 65 years from an prospective, population-based study. Visceral fat area (VFA) and liver minus spleen attenuation (LSA) which has inverse relationship with hepatic fat accumulation were measured by abdominal CT scanning. Body composition was measured by dual energy X-ray absorptiometry.

About 69.3% of men and 86.8% of women had vitamin D deficiency [25(OH)D level < 20 ng/ml]. Low 25(OH)D levels were strongly associated with high VFA and percent body fat and low LSA in men, and only with low LSA in women (p for trend 0.0007, 0.0157, 0.0003, and 0.018, respectively). Fasting plasma glucose (FPG) and triglycerides (TG) levels in men were inversely associated with 25(OH)D (p for trend 0.056, 0.043 respectively) after adjusting for age, body mass index, exercise, alcohol, smoking and socioeconomic status. In men, odds ratio (95% confidence interval) for those in the lowest quartile compared with the highest quartile of 25(OH)D was 3.748 (1.399-10.042) for hypertriglyceridemia [TG ≥ 150 mg/dL]; 2.634 (1.095-6.338) for hyperglycemia [FPG ≥ 100 mg/dL or 2hr oral glucose tolerance test glucose ≥ 140 mg/dL]; 4.326 (1.437-13.019) for visceral obesity [VFA ≥ 100 cm²]; 5.39 (1.316, 22.075) for fatty liver [LSA < 0] after multiple adjustment. In women, odds ratio for fatty liver was 4.788 (1.246-18.398).

Vitamin D deficiency is common and highly correlated with visceral adiposity in elderly Koreans. Low vitamin D levels are associated with visceral obesity, fasting hyperglycemia and hypertriglyceridemia only in men.

Sources of Research Support: Korea National Institute of Health Intramural Research Grant (091-4800-4845-300-210 and -260).

Nothing to Disclose: JAS, HC, JHK, SJY, HJY, HYK, SGK, KMC, SHB, DSC, MHP, CH, NHK

P1-439

Increased Expression of Alzheimer's Disease Related Genes in Obesity.

Islam Mohamed¹, Husam Ghanim¹ and Paresh Dandona¹.

¹SUNY at Buffalo Buffalo, NY.

Many epidemiological studies have demonstrated that obesity is associated with an increased incidence and prevalence of Alzheimer's disease (AD) which is characterized by plaques of β - amyloid ($A\beta$) formed from its precursor, amyloid precursor protein (APP) and neurofibrillary tangles made up of hyperphosphorylated tau protein. We have now investigated the expression of APP and tau as well as the proteins/enzymes involved in the formation and destruction of these proteins in the adipose tissue of obese subjects. Obese subjects ($n=24$, age= 36.8 ± 1.12 , BMI= 33.8 ± 0.446) had higher mRNA expression levels of the two key proteins, APP by 22.4% ($P=0.018$) and TAU by 34% ($P=0.023$) when compared to lean control subjects ($n=19$, age= 33.2 ± 1.577 , BMI= 22.5 ± 0.579). The mRNA expression of β -amyloid converting enzyme (BACE1), the main enzyme involved in the formation of $A\beta$ was increased as was the mRNA expression of the enzymes involved in the preclusion and degradation of $A\beta$ peptides: ADAM-9, ADAM-17 and IDE by 22.3% ($P=0.016$), 53.8% ($P\leq 0.001$), 32.6% ($P=0.002$) and 19% ($P=0.024$) respectively in the obese group. In addition, there was an increase in the mRNA expression of the major pro-inflammatory mediators: MIF by 26.8% ($P=0.007$), MCP-1 by 195% ($P=0.004$) and MMP-9 by 79.6% ($P=0.037$) in the obese group. BMI correlated significantly with BACE1, ADAM-9, ADAM-17, IDE, TAU, GSK3 β , MIF and MCP-1 mRNA expression and MIF and MCP-1 mRNA expression in turn correlated significantly with that of BACE1, ADAM 9, 17 and IDE. The state of obesity is, therefore, associated with an increased expression of APP, TAU and BACE1 with a concomitant increase in $A\beta$ degrading enzymes, ADAM-9, ADAM-17 and IDE in parallel with an increase in pro-inflammatory genes. The expression of GSK-3 β , which hyperphosphorylates TAU, was significantly related to that of TAU, ADAM-17 and RAGE. Whether this up-regulation of both the synthetic and degrading pathways in adipose tissue reflects a similar activity in the brain and whether this increase is dependent upon inflammatory mechanisms needs further investigation.

Disclosures: PD: Speaker, Sanofi-Aventis, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals; Research Funding, Sanofi-Aventis, GlaxoSmithKline, Amylin Pharmaceuticals.

Nothing to Disclose: IM, HG

P1-440

Osteopontin and Atherosclerosis in Obese and Non-Obese, Hypertensive and Normotensive Female Patients.

G Gursoy¹, S Alagoz¹, Y Acar¹, B Demirbas¹, H Cetiner¹ and Z Kilic¹.

¹Ministry of Hlth Ankara Education and Res Hosp Ankara, Turkey.

Atherosclerosis is one of the major reason of mortality as causing ischemic diseases such as coronary artery disease (CAD), stroke and peripheric arterial disease. It was accepted that inflammation takes part in all stages and complications of atherosclerosis. Osteopontin (OPN) having matricellular protein properties, is demonstrated that it is synthesized in atherosclerotic plaques and is in positive relation with the severity of atherosclerosis. This study evaluated the relation of OPN with atherosclerosis in female patients who have risk factors for atherosclerosis such as hypertension and obesity. 23 obese-hypertensive, 22 obese-normotensive, 20 nonobese-hypertensive patients and 22 nonobese-normotensive female person were evaluated. Body mass index, systolic and diastolic blood pressure, fasting plasma glucose, post-prandal plasma glucose, lipid profile, lipoprotein-a, uric acid, c-reactive protein(CRP), OPN levels were obtained in all female persons. Obese-hypertensive group had significantly higher plasma OPN levels than obese-normotensive, nonobese-hypertensive and nonobese-normotensive groups ($p < 0,015$, $p < 0,001$, $p < 0,001$, respectively). Plasma OPN levels of obese-normotensive group was significantly higher than nonobese-hypertensive and nonobese-normotensive groups ($p < 0,021$, $p < 0,001$, respectively). Plasma OPN levels of nonobese-hypertensive group was also found statistically higher than nonobese-normotensive group ($p < 0,005$). When the parameters were investigated altogether positive correlation was obtained in OPN and body mass index, waist hip ratio, systolic blood pressure, diastolic blood pressure, CRP ($p < 0,001$, $p < 0,022$, $p < 0,001$, $p < 0,001$, $p < 0,01$, respectively). In conclusion, our data points toward a specific role of OPN in obesity and hypertension, as well as atherosclerosis. Therefore, we think that studies about blocking of biosynthesis of OPN in prevention of atherosclerosis and using OPN as a marker in asymptomatic atherosclerosis will be noteworthy.

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Nothing to Disclose: GG, SA, YA, BD, HC, ZK

P1-441**Osteopontin a New Marker for Atherosclerosis in Obese Women?.**S Alagoz¹, G Gursoy¹, Y Acar¹, B Demirbas¹, H Cetiner¹ and Z Kilic¹.¹Ministry of Health Ankara Education and Research Hospital Ankara, Turkey.

Obesity is associated with a state of chronic, low-grade inflammation characterized by abnormal cytokine production and macrophage infiltration into adipose tissue, which may contribute to the development of insulin resistance and type 2 diabetes. It was emphasized that inflammation takes part in all stages and complications of atherosclerosis. Osteopontin (OPN) is a multifunctional protein involved in various inflammatory processes, cell migration, and tissue remodeling. This study evaluated the role of OPN in female patients who have at least one risk factor for atherosclerosis such as obesity. The study included 45 morbidly obese normotensive female patients and 22 age and sex-matched control subjects. As well as making physical and anthropometric examinations, fasting plasma glucose and insulin, post prandial plasma glucose and insulin, lipid profile, C-reactive protein, OPN levels were obtained in all female person. Our obese group had significantly higher levels in glucose and lipid parameters as well as atherosclerotic markers and anthropometric measures. Obese group also had significantly higher plasma OPN and C-reactive protein levels than the control group ($p < 0.05$). We also performed the correlation analysis of the groups and find positive correlations in OPN levels and body mass index, C-reactive protein, fasting insulin. In conclusion, our data may point toward a specific role of OPN in atherosclerosis and obesity.

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Nothing to Disclose: SA, GG, YA, BD, HC, ZK

P1-442

Increased Energy Expenditure Predicts Natural Mortality in Humans.

Reiner Jumpertz MD¹, Robert G Nelson MD PhD¹, Robert L Hanson MD¹ and Jonathan Krakoff MD¹.

¹Natl Inst of Diabetes and Digestive and Kidney Diseases Phoenix, AZ.

Objective: Lower energy turnover may delay aging. In support of this, studies in animals report marked increases in lifespan after caloric restriction. Recent intervention studies in humans also demonstrated favorable changes in markers of longevity after caloric restriction with corresponding reduction in metabolic rate. Therefore, it can be hypothesized that increased metabolic rates are associated with a higher risk for natural mortality. **Aim:** To test this hypothesis, we investigated whether increased energy expenditure (EE) predicted natural mortality in humans. **Methods:** EE was measured in non-diabetic Pima Indians from the Gila River Indian Community by whole room calorimeter over 24 hours (24EE: n=512, 57% men, BMI=33.9±8.1 kg/m², age=28.6±7.1 years) or by measurement of resting metabolic rate (RMR) with an open-circuit hood system performed on a separate day (n=331: 58% men, BMI=33.5±7.5 kg/m², age=25.5±5.7 years). Vital status was ascertained separately on all community members with complete follow-up through the end of 2006. Cause of death was determined by systematic review of available clinical records. Mean follow-up after measurement was 13.1±6.3 years in individuals with 24EE and 15.4±6.3 years in individuals with RMR. Glucose regulation status at the time of EE measurements was assessed by a 75g oral glucose tolerance test. Cox regression models with age, sex and body weight as covariates were used to test EE as a predictor of natural mortality. Bootstrap analyses using 1000 replicates of the datasets were applied to test the validity of the predictive outcome. **Results:** In each study group 27 deaths from natural causes occurred during follow-up. 24EE predicted mortality with a hazard rate ratio (HRR) of 1.31 (95% CI 1.03-1.76, p=0.03) per 100 kcal/d increase. RMR also predicted mortality with a HRR: 1.25 (1.01-1.54, p=0.04) per 100 kcal/d increase. Bootstrapping results were consistent with those using standard statistics. **Conclusion:** Higher metabolic rates adjusted for body size predict increased natural mortality in humans, which is consistent with the hypothesis that increased energy turnover accelerates aging. Chronic latent oxidative damage to the cell upon accumulation of free radicals which occur during metabolic processes could represent a possible underlying mechanism as proposed by the free radical theory of aging.

Nothing to Disclose: RJ, RGN, RLH, JK

P1-443

Higher BMI and Dental Decay May Be Linked.

KE Bethin MD, PhD¹, L Rafalson PhD¹, PR Creighton DDS², R Borowski BS¹, H Ghanim PhD¹, P Dandona MBBS, DPhil¹, AT Stone DDS² and T Quattrin MD¹.

¹Univ at Buffalo Sch of Med Buffalo, NY and ²Univ at Buffalo Sch of Dental Med Buffalo, NY.

BACKGROUND: Obesity in youth has been a growing problem and dental caries remain the most common chronic disease of childhood. Poor nutritional habits possibly link both of these conditions. The AIM of this study was to obtain preliminary data on the body mass index (BMI, kg/m²), energy intake and metabolic profiles in young children with dental decay. **METHODS:** 2-5 year old children (n=65, 56.9% females) requiring anesthesia due to severity of disease or lack of cooperation were included. On the day of dental surgery, after obtaining consent, height and weight were measured with standardized protocol. After induction with nitrous oxide, a blood sample was drawn, spun within 30' and frozen at -70 until assayed. Reported herein are the results of lipid profile, insulin and blood glucose (BG) performed at Kaleida Health and the Diabetes/Endocrinology Research Laboratory at the University at Buffalo, respectively. Nutritional data were collected using Block Kids questionnaires. **RESULTS:** The data are expressed as mean \pm SD or percentage. Statistical comparisons were performed using t-tests or chi-square test as applicable. The demographic characteristics and laboratory results of children with BMI \geq 85th percentile were similar to the children with BMI <85th percentile with the exception of cholesterol which was significantly higher in the overweight/obese group (p=0.04). All BGs were below 126 except for a single reading of 137.

| Characteristic | BMI <85th percentile (n=47) | BMI \geq 85th percentile (n=18) | p value |
|---------------------------|-----------------------------|-----------------------------------|---------|
| Age (years) | 4.1 \pm 1.1 | 4.1 \pm 1.4 | 0.98 |
| BG \geq 100 mg/dL (n,%) | 4 (8.5%) | 3 (16.7%) | 0.34 |
| BG (mg/dL) | 84 \pm 13 | 89 \pm 15 | 0.18 |
| Insulin (uU/mL) | 1.8 \pm 1.4 | 2.0 \pm 1.4 | 0.65 |
| Cholesterol (mg/dL) | 142 \pm 25 | 160 \pm 44 | 0.04 |
| Kcal/day | 1568 \pm 617 | 1444 \pm 526 | 0.45 |

CONCLUSIONS: The prevalence of BMI \geq 85th percentile was 27.7% (n=18), more than 5% higher than the 2007-8 NHANES estimates (21.2%). Due to the fact that these children were fasting (8-12 hours), the prevalence of BMI \geq 85th percentile may be underestimated. The stress of induction may account for the impaired fasting BG observed in 7 subjects without elevated insulin levels. Daily caloric intake was abnormally high with intake exceeding 1200 calories in 71.4% of these children. Further analyses are needed to explore if juice and sweet consumption accounts for the excessive caloric intake and links high BMI and dental decay.

Sources of Research Support: NYS Department of Health Diabetes Center of Excellence.

Disclosures: PD: Speaker, Sanofi-Aventis, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals; Research Funding, GlaxoSmithKline, Sanofi-Aventis, Amylin Pharmaceuticals.

Nothing to Disclose: KEB, LR, PRC, RB, HG, ATS, TQ

P1-444

Visceral Hypercortisolism Observed in Central Obesity and Metabolic Syndrome Is Associated with Insulin Resistance and Beta Cell Dysfunction.

R Baudrand MD¹, C Campino BS¹, CA Carvajal MS¹, O Olivieri MD², G Guidi MD², G Faccini MD², F Pasini MD², J Sateler MD¹, J Cornejo³, B San Martin³, JM Dominguez MD¹, C Tabilo MD¹, LM Mosso MD¹, G Owen PhD¹, AM Kalergis MD, PhD¹ and C Fardella MD, PhD¹.

¹Pontificia Univ Católica de Chile Santiago, Chile ; ²Univ of Verona Verona, Italy and ³Univ de Chile Santiago, Chile.

Introduction: The exposure to glucocorticoid excess leads to increased adiposity, insulin resistance and type 2 diabetes. It has been postulated that central obesity and metabolic syndrome (MetS) could have splanchnic hypercortisolism despite normal systemic cortisol. The enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which generates cortisol (F) from inactive cortisone (E) has been implicated in the pathogenesis. 11 β -HSD1 is present mainly in adipose tissue and liver, but also within pancreatic α cells decreasing insulin secretion from β cells. Portal cortisol is metabolized by liver reductases to inactive tetrahydrometabolites (THM). **Objectives:** 1.-To asses THM excretion and their association with: 1) Biochemical parameters of insulin resistance and β cell function. 2) Anthropometric measures and presence of MetS. **Patients and Methods:** We recruited 218 subjects [age: 52.4 \pm 9.5 years, 77% female, BMI: 29.3 \pm 4.4 kg/m²; metS 58.7 % by ATPIII criteria]. We evaluated, in plasma: F, E, glucose, insulin (RIA) and lipid profile; in urine (24 h): free F and E by HPLC-MS/MS and metabolites THE, THF, α THF, Cortolone, β Cortolone, Cortol and β Cortol (THM) by GC/MS. We evaluated β cell function from values of β cell secretion (% B) and insulin sensitivity (% S) by HOMA Oxford University Calculator 2.2[®]. **Results:** There was a positive correlation between THM concentration with glycemia ($r=0.16$, $p=0.02$), insulin ($r=0.22$, $p=0.001$), HOMA ($r=0.23$, $p=0.001$), and an inverse correlation with β cell function (ID) ($r=-0.21$, $p=0.003$), not observed with F and E in urine. Waist circumference was positively associated with THM ($r=0.41$, $p<0.001$) and HOMA ($r=0.4$, $p<0.001$), negatively associated with %S ($r=-0.39$, $p<0.001$) and β cell function ($r=-0.28$, $p<0.001$), but not with F and E in plasma or urine. Patients with MetS showed higher concentrations of THM ($p<0.001$), HOMA ($p<0.001$) and %B ($p=0.003$), and lower %S ($p<0.001$) and β cell function ($p<0.001$), but with no differences in F and E plasma or urine levels. **Conclusions:** Our results confirm that THM excretion is associated with decreased peripheral insulin sensitivity and lower pancreatic β cell function. Visceral but not systemic hypercortisolism in central obesity and MetS could have a key role in the pathogenesis of insulin resistance by 11 β -HSD1 in splanchnic circulation. Hepatic metabolism of cortisol could explain normal plasma and urinary cortisol in obesity, unlike Cushing's syndrome.

Sources of Research Support: Fondecyt n°1070876, Fondecyt n°1100356, Fondef n°D0811087 and Millenium on Immunology and Immunotherapy Grant P04/030F.

Nothing to Disclose: RB, CC, CAC, OO, GG, GF, FP, JS, JC, BSM, JMD, CT, LMM, GO, AMK, CF

P1-445

The Prevalence of Abnormal Cortisol or TSH Levels in Patients Presenting for Initial Consultation at a Weight Management Clinic.

N Bakhru M.D.¹, K Fujioka M.D.¹ and J McCallum M.D.¹.

¹Scripps Clin La Jolla, CA.

Objective: Patients often associate their weight gain with underlying hormonal abnormalities. Although it is well known that hypercortisolism and hypothyroidism can perpetuate obesity, it is questionable how often such metabolic derangements are present in patients with abnormal weight gain. This study was designed to evaluate such a population for aberrations in measured cortisol and thyroid function.

Study Design: We conducted a prospective study evaluating the prevalence of previously undetected abnormal cortisol levels and primary hypothyroidism in a group of overweight and obese individuals in our weight management clinic. We screened 92 patients with BMI > 25 kg/m² utilizing midnight salivary cortisol (MSC) and 3rd generation serum TSH measurement. In addition to its noninvasive nature, MSC has been shown in prior studies to have good reproducibility, and similar diagnostic performance compared to traditional 24hr urine cortisol measurement as a screening tool for hypercortisolism.

Results: Between July 2009 and January 2010, a total of 92 patients presenting for initial consultation at our weight management clinic were consecutively selected to participate in this prospective study. Mean age of patients was 48.6 +/- 14.7yrs, BMI 37.7 +/- 6.5 kg/m², with 71.7% of the patients being female. The majority of patients studied had a BMI > 30 kg/m² (27.1% with BMI 30.0-34.9 kg/m², 29.3% with BMI 35.0-39.9 kg/m², and 33.7% with BMI > 40 kg/m²). Only 51 patients (55.4%) were compliant with measuring MSC, whereas 77 patients (83.7%) presented for serum TSH measurement. Of patients who were compliant in measuring MSC, 12 (23.5%) had an abnormally elevated level. Only 2 patients (2.6%) had elevated TSH levels noted - both exhibiting subclinical hypothyroidism and a BMI > 40 kg/m².

Conclusion: Such data suggests that elevated cortisol (as measured by MSC) may be present in a higher than previously suspected subset of our obese population. This raises the question of the specificity of MSC in this population. Furthermore, it can be inferred that MSC may not be an effective screening tool for hypercortisolism due to the fact that almost half of patients fail to complete the test. The same does not hold true of TSH, as the majority of patients were compliant in being screened for primary hypothyroidism and did not exhibit evidence of abnormal thyroid function.

Nothing to Disclose: NB, KF, JM

P1-446

Relationship between Body Mass Index and Thyrotropin in Euthyroid Women: Differences by Smoking, Race and Menopause Status.

AM Souza PhD student¹ and R Sichieri PhD¹.

¹State Univ of Rio de Janeiro Rio de Janeiro, Brazil.

Background: Although, overt thyroid dysfunction is associated with weight changes, it is not known whether minor changes in thyroid function within normal serum thyrotropin (TSH) concentrations could affect body mass index (BMI). **Objective:** To evaluate the association between BMI and TSH by smoking, race and menopause status. **Methods:** A population-based study was carried out in the city of Rio de Janeiro, Brazil, in 2004-2005. Households (1500) were selected using three-stage probability sampling. A total of 1298 woman aged 35 years or older agreed to participate, and 1084 women without thyroid disease were investigated. **Results:** A high prevalence of overweight and obesity, 33.5% and 22.0%, respectively was found. Forty-four percent of the women were White, 16.8% were Black, the proportion of smokers was 23.0% and 52.0% of the women reported menopausal status. Overall, BMI was significantly associated with TSH ($\beta=0.90$; $p\text{-value}=0.02$). The analysis stratified by smoking status showed a not significant association between BMI and TSH among the never smokers ($\beta=0.56$; $p\text{-value}=0.21$) and former smokers ($\beta=0.58$; $p\text{-value}=0.32$).

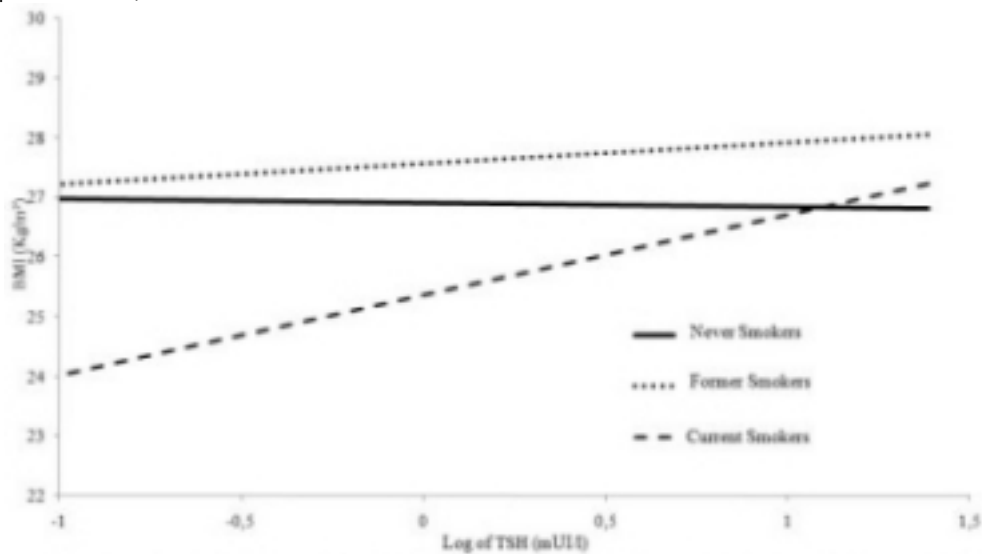


Figure 1. Age-adjusted linear regression of body mass index (BMI) on thyrotropin (TSH) by smoking status. Women 35 years and older, Rio de Janeiro, Brazil.

Among smokers, BMI was strongly associated with serum TSH ($\beta=1.77$; $p\text{-value}=0.04$). Differences in the association of BMI and TSH were also observed according to race.

Table 1. Weighted regression coefficients (β)* of linear models with body mass index (BMI) as dependent variable by race. Women 35 years and older, Rio de Janeiro, Brazil.

| Independent variables | Black | | Non-Black | |
|-----------------------|---------|---------|-----------|---------|
| | β | p-value | β | p-value |
| Log of TSH | 1.39 | 0.14 | 0.79 | 0.03 |
| Smoking (Yes/No) | -1.69 | 0.16 | -1.16 | 0.02 |
| Menopause (Yes/No) | 0.54 | 0.67 | 1.41 | 0.02 |

*adjusted by age and age squared

The analysis stratified by menopause status showed a significant association between BMI and TSH only among premenopausal women ($\beta=1.04$; $p\text{-value}=0.04$). **Conclusion:** TSH appears to modulate body weight among women even in the normal range. Smoking, race and menopausal status are strong effect modifiers of the association between TSH and BMI and future studies should take these effect into account.

Sources of Research Support: Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES).

Nothing to Disclose: AMS, RS

P1-447

Visceral Fat to Thigh Muscle Thickness Ratio: New Index Using Ultrasound for Assessing the Disproportion between Body Fat and Muscle Masses and Carotid Atherosclerosis.

SK Kim MD¹, KS Park MD¹, SW Park MD, PhD¹ and YW Cho MD, PhD¹.

¹CHA Bundang Med Ctr, CHA Univ Seongnam, Republic of Korea.

Objective: The role of body fat and muscle masses for developing cardiometabolic disorders is totally opposed. Many epidemiologic studies on the disproportion between these two compartments using simple anthropometric indexes, such as waist or hip circumference, and waist-to-hip ratio (WHR), were available. However, such indexes have a commonly overlooked fault not able to measure fat and muscle masses completely separately. The aim of this study was to analyze whether visceral fat to thigh muscle thickness ratio (VMTR) measured by ultrasonography could be a simple index for assessing the disproportion between visceral fat and thigh muscle masses and whether VMTR is associated with a carotid artery intima-media thickness (C-IMT).

METHODS: This was an observational study performed on 15 healthy men and 68 men with newly diagnosed type 2 diabetes. Using ultrasonography, visceral fat thickness, mid-thigh muscle thickness, and C-IMT were determined. And abdominal visceral fat area, mid-thigh muscle area, liver attenuation, and mid-thigh muscle attenuation were measured by computed tomography. The mid-thigh muscle was divided into low- and normal-density muscle areas.

RESULTS: There were no differences in the proportion of diabetic subjects, hip and thigh circumference, and glucose control status among VMTR tertiles. However, age, body mass index (BMI), waist circumference, blood pressure, total cholesterol, triglyceride, and homeostasis model assessment of insulin resistance (HOMA-IR) increased across VMTR tertiles. Increasing VMTR was significantly associated with increasing visceral fat area, lipid rich muscle mass (greater low-density muscle area and decreased muscle attenuation), and C-IMT. With the exception of mid-thigh low density muscle area, these trends were significant after adjusting for age, BMI, waist, blood pressure, total cholesterol, triglyceride, and HOMA-IR. On the basis of multiple regression analyses, age and VMTR only were associated with C-IMT, independently of other traditional risk factors including waist circumference or WHR.

CONCLUSION: Subjects with a higher VMTR have an adverse metabolic phenotype. The measure of VMTR using ultrasonography might be a relevant index for identifying individuals at an increased risk of cardiovascular disease.

Nothing to Disclose: SKK, KSP, SWP, YWC

P1-448

C677T Mutation in the Methylenetetrahydrofolate Reductase Gene Increases Serum Uric Acid in Obese Subjects.

A Kogure MD. PhD^{1,4}, T Harayama MD.², Y Takakura MD. PhD¹, T Umekawa MD. PhD¹, K Yoshioka MD. PhD³ and T Yoshida MD. PhD².

¹Kyoto Prefectural Univ of Med Kyoto, Japan ; ²Kyoto City Hosp Kyoto, Japan ; ³Matsushita Memorial Hosp Moriguchi, Japan and ⁴Kyoto Prefectural Yosanoumi Hosp Yosa-gun, Japan.

Methylenetetrahydrofolate reductase (MTHFR) is a regulatory enzyme in the remethylation phase of homocysteine metabolism. A common mutation of MTHFR gene (C-to T transition at nucleotide position 677) has been reported as a genetic risk factor for hyperhomocysteinemia, cardiovascular disease. Recently Zuo et al. and Itou et al. reported that C677T mutation in the MTHFR gene contributed to higher uric acid levels. Furthermore, Lambrinoudaki et al. reported that the mutation has been associated with central adiposity in healthy postmenopausal women. Since obesity is a risk factor for hyperuricemia, determining whether obese subjects have C677T mutation of the MTHFR gene is important. To investigate whether the C677T MTHFR mutation is a risk factor for hyperuricemia in obese subjects, we performed MTHFR genotyping analysis and clinical laboratory determinations including serum uric acid in obese Japanese subjects. We studied 141 obese Japanese who consulted the outpatient clinic of our hospital for treatment of their condition. Obesity was diagnosed according to the criteria of the Japan Society for the Study of Obesity. The control group was comprised of 146 age-matched healthy people who consulted Sakazaki Clinic for a routine medical examination. Blood samples were drawn after an overnight fast. Serum uric acid, creatinine and total cholesterol were determined by standard enzymatic methods. Genomic DNA was extracted from peripheral blood leukocytes. The genotype of SNP 677 of the MTHFR was determined with a fluorescent allele-specific DNA primer assay system. Distribution of the MTHFR genotypes was CC, 38%; TC, 39%; TT, 23%; with a T-allele frequency 0.42 in obese subjects, and CC, 39%; TC, 52%; TT, 9%; with a T-allele frequency 0.36 in the controls. T-allele frequency was higher among obese subjects than among control subjects. There is a significant association between MTHFR polymorphism and levels of uric acid (CC 5.3±1.2mg/dl, CT/TT 5.9±1.2 mg/dl). There were no differences between MTHFR polymorphisms and creatinine, cholesterol and blood pressure. Subjects were classified into two groups based on uric acid levels; a low uric acid group (< 7.0 mg /dl) and a high uric acid group (≥ 7.0 mg/dl). T allele frequency was significantly greater in the high uric acid group than in the low uric acid group. In this study, the C677T MTHFR mutation is associated with hyperuricemia in obese subjects. People with this mutation should be careful to avoid a high-purine diet.

Nothing to Disclose: AK, TH, YT, TU, KY, TY

P1-449

Relationship of Serum Uric Acid Concentration to Metabolic Syndrome: Age and Gender Discrepancies.

WK Chiou PhD¹, MH Wang MID¹, DH Huang MID¹, HT Chiu MID¹, YJ Lee MRS¹ and JD Lin MD¹.

¹Chang Gung Univ Taoyuan, Taiwan and ²Chang Gung Memorial Hosp, Chang Gung Univ Taoyuan, Taiwan.

Background: Metabolic syndrome and hyperuricemia are recognized as important risk factors for cardiovascular disease. However, findings regarding the relationship between serum uric acid concentration and components of MetS have been inconsistent. This study was performed to explore the potential value of UA concentration as a marker of MetS among Chinese subjects of different age and gender.

Methods: A total of 5,896 subjects (2,960 females and 2,936 males) were recruited from the Department of Health Management at the Chang Gung Medical Center. Hyperuricemia was defined as serum UA values <7.0 mg/dL for males and <6.0 mg/dL for females. MetS was defined according to the criteria of the Adult Treatment Panel III as modified for Chinese subjects. Serum UA values were used to differentiate MetS and to calculate epidemiological indices through discriminate analysis and logistic regression.

Results: The sensitivity and specificity of serum UA concentration as a marker of MetS ranged 55.2% to 61.4% and from 61.9% to 68.4%, respectively. Subjects with high UA values had a higher risk of MetS, with odds ratios ranging from 1.23 to 1.82 ($p < 0.01$). A positive correlation between serum UA and MetS was observed for both females and males. Serum UA concentration and occurrence of MetS were both observed to increase with age in females; in males, however, UA values did not vary with age.

Conclusion: Serum UA concentration is more closely associated with MetS in females than in males. High UA values among middle-aged females may predict development of MetS.

Nothing to Disclose: WKC, MHW, DHH, HTC, YJL, JDL

P1-450

The Role of Leptin Resistance in Non-Alcoholic Fatty Liver Disease in the Patients with Metabolic Syndrome.

I.O. Kostitska PhD¹.

¹Ivano-Frankivsk Natl Med Univ Ivano-Frankivsk, Ukraine.

Background. Impairment of both insulin and leptin resistance has been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). One of the unconventional areas in which leptin resistance is now receiving great attention is metabolic steatosis. Several published studies indicate that circulating leptin is increased in patients with metabolic syndrome (MS), cirrhosis and non-alcoholic steatohepatitis (NASH)^{1,2}. Type 2 diabetes mellitus (DM) and obesity are associated with a chronic inflammatory response characterized by abnormal adipocytokines production³. Aim the present study aims to assess serum leptin levels and index of leptin resistance in patients with MS without HCV infection.

Methods. The present study included 53 patients with NAFLD divided into 2 groups. Group A: 27 patients with all clusters of MS (8 males and 19 females, mean age 42 ± 11.9 years). Group B (control): 26 patients with type 2 DM (11 males and 15 females, mean age 40.9 ± 13.1 years). Serum leptin, insulin levels were measured by radioimmunoassay. The clinical and biochemical characteristics of hepatosis, and particularly its association with diabetes, dyslipidemia and with the increase for levels of insulin and leptin.

Results. NAFLD in the patients with MS correlated with the body mass index and leptin levels. Serum leptin levels were found to be significantly higher in group A of patients with NAFLD, as compared with the controls group ($P < 0.001$). Higher levels of insulin and HOMA - IR in patients of group A than the patients of group B. Serum leptin correlated positively with serum insulin and leptin levels in both groups. The findings of the present study show that the adipocytokine leptin is involved in metabolic abnormalities that lead to steatosis in NAFLD patients.

Conclusions. Leptin resistance can be used as a non-invasive marker for the predication of steatosis and fibrosis in patients with NAFLD as clusters of MS.

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Nothing to Disclose: IOK

P1-451

Severe Osteomalacia Induced by Biliopancreatic Diversion.

RV Perez¹, D Iguchi¹, V Jorgetti³, RM Martin², PHS Correa², C Cercato¹, MC Mancini¹ and A Halpern¹.

¹Hosp das Clins, Sao Paulo Univ Sch of Med Sao Paulo, Brazil.

Introduction: Bariatric surgery (BS) is a well-established option for treatment of obesity. Biliopancreatic diversion (BD) consists of a partial gastrectomy, a gastroileostomy with a long segment of Roux limb and a short common channel resulting in weight loss. Although BD is an efficient procedure for obesity and comorbidities control, its use has been limited due to significant protein-calorie malabsorption, anemia, metabolic bone disease (MBD), deficiencies of fat-soluble vitamins and vitamin B12.

Clinical Case: A 66-year-old white woman who had an obesity past (BMI 44.5 kg/m²) underwent BD 5 years ago (78% excess weight lost). She complained about weakness, bone pain and myalgia 2.5 years after surgery. Following one year, she had a femoral neck fracture and a motor deficit of all muscular groups which preclude her of standing. Her diet calcium (Ca) intake was around 600 mg/d without any vitamin or mineral supplementation. Her laboratory findings disclosed: 25-OH vitamin D < 4.0 ng/mL (n > 30); PTH 789 pg/mL (n 16-87); total Ca 7.1 mg/dL (n 8.6 -10.2); albumin 3.6 g/dL (n 3.4-4.8); P 2.5 mg/dL (n 2.7-4.5); FGF23 < 10 pg/mL (n 10-50); alkaline phosphatase 432 U/L (n 35-104); 24h urinary Ca 6,60 mg/vol (n 100-320). X-ray analysis revealed hip and tibia pseudofractures, salt and pepper lesions on the skull and areas of subperiosteal resorption on hands. In addition, bone mineral density was reduced in all sites and her ⁹⁹Tc-MDP bone scan showed active MBD with marked activity at skull, ribs and pathological fracture sites. A transiliac crest bone biopsy was performed confirming the osteomalacia diagnosis. Initial treatment consisted of 3 g/d of Ca and ergocalciferol 600,000 IU IM 21/21d. After 1 month, she mentioned improvement in all symptoms.

Clinical Lessons: Duodenum and jejunum are responsible for 90% of Ca absorbed during adequate Ca intake. The main source of vitamin D (VD) comes from the production during sunlight exposure but it can be obtained from food and oral supplements that needs to be absorbed by the intestine. Because duodenum and jejunum are excluded from the intestinal transit, Ca and VD absorption may be impaired in BD patients leading to MBD at a long term. The clinical, laboratorial, imaging and hystomorphometric data of this patient shows a severe osteomalacia picture induced by BS. It highlights the importance of a close surveillance for calcium metabolism on BS patients.

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Nothing to Disclose: RVP, DI, VJ, RMM, PHSC, CC, MCM, AH

P1-452

Treatment of Presumed Bacterial Overgrowth Normalized Serum 25-Hydroxyvitamin D: A Case Series.

SE Williams MD, MS¹, CL Deal MD² and AA Licata MD, PhD².

¹Kettering Hlth Network - Greene Memorial Hosp Hlth Ctr Xenia, OH and ²Cleveland Clin Foundation Cleveland, OH.

Purpose: To report five cases in which treatment for presumed small intestinal bacterial overgrowth (SIBO) in bariatric surgery patients resulted in normalization of 25-hydroxyvitamin D (25OHD) that had become increasingly resistant to oral repletion.

Background: Bacterial overgrowth is a common finding after gastric bypass however; these patients often have anemia, steatorrhea, and rapid oral-cecal transit times that cause traditional indicators of SIBO to be unreliable. Absorption of dietary vitamin D is dependant upon intact bile acids and micelle formation yet among its many effects, SIBO causes bile acid deconjugation and hence decreased micelle formation.

Vitamin D deficiency in compliant gastric bypass patients has traditionally been thought of as a malabsorptive process due to bypassed sites of absorption. Oral repletion with vitamin D3 has proven effective in achieving normal serum 25OHD but maintaining normal levels in these patients can unexpectedly require enormous increases in D supplements. Although the mechanisms of vitamin D absorption and the metabolic effects of SIBO are well known, little has been published regarding SIBO and vitamin D deficiency in humans.

Methods: Compliant patients with a history of bariatric surgery and 25OHD levels increasingly resistant to repletion were identified. Half-dose antibiotic regimens were prescribed for a minimum of 14 days. Oral supplements and diet remained unchanged; sun exposure was determined not to be a factor. Lab indices were repeated four to six weeks after treatment.

Results: Following treatment, 25OHD improved by an average of 20 ± 11.76 points, and in all cases achieved normal levels. The PTH decreased by an average of 33.7 ± 23.64 points.

| | | Patient #1 | Patient #2 | Patient #3 | Patient #4 | Patient #5 |
|-------------------------|-------------------|--|---------------|---------------|---------------|----------------------------------|
| Daily Calcium (mg): | | 1200 | 1200 | 600 | 1200 | 1200 |
| Weekly Vitamin D3 (IU): | | 1,400,000 | 350,000 | 200,000 | 350,000 | 500,000 |
| Antibiotic: | | clarithromycin | metronidazole | metronidazole | metronidazole | metronidazole & amox/clavulanate |
| Serum Indices | Reference Range | Lab Indices Before / After Antibiotics | | | | |
| Calcium | 8.7 - 10.2 mg/dL | 8.5 / 8.5 | 9.1 / 9.0 | 8.0 / 9.0 | 8.5 / 8.7 | 8.2 / 8.5 |
| 25OHD | 32 - 80 ng/mL | 24 / 61 | 30 / 43 | 37 / 68 | 25 / 32 | 25 / 37 |
| PTH | 11.2 - 79.5 pg/mL | 137 / 70 | 56 / 37 | 101 / 89 | 82 / 69 | 180 / 122 |

Nothing to Disclose: SEW, CLD, AAL

P1-453

Severe Post-Prandial Hypoglycemia Following Gastric Bypass Surgery, Successfully Treated with Acarbose.

L Tabatabaeian MD¹ and T Donner MD¹.

¹Univ of Maryland Baltimore, MD.

Background

Postprandial hypoglycemia is a common complication of bariatric surgery, usually due to dumping syndrome and less commonly due to non-insulinoma pancreatogenous hypoglycemic syndrome (NIPHS) or insulinoma. Most reported cases of post gastric bypass hyperinsulinemic hypoglycemia have been managed successfully with pancreatectomy. We present a case of severe hyperinsulinemic hypoglycemia post gastric bypass treated successfully with acarbose.

Clinical Case

A 37-year-old lady presented with frequent episodes of 1-2 hour post-prandial neuroglycopenia two years after Reux-en-Y gastric bypass surgery. One episode of severe hypoglycemia resulted in seizure activity and a motor vehicle accident. The biochemical work up during one episode of hypoglycemia was consistent with hyperinsulinemic hypoglycemia including a blood glucose of 39 mg/dl, insulin 14 mcU/ml, proinsulin 13.9 mcU/ml, both high in the setting of hypoglycemia, C-peptide 5.7 ng/ml (n 0.8-3.9 ng/ml), insulin antibody 2.2 U/ml (n 0-5 U/ml) and a negative sulfonyleurea screen. An inpatient 72-hour fast did not induce hypoglycemia. When given high carbohydrate mixed meal, she demonstrated a peak plasma glucose of 390 mg/dl. An MRI of abdomen and an abdominal ultrasound did not reveal a pancreatic mass. The patient was started on acarbose 25 mg TID with the first bite of each meal. This dose was slowly titrated up to 100 mg TID. For the past 3 months on this dose, she has remained entirely free of any postprandial hypoglycemic symptoms and has had no blood glucose levels less than 70 mg/dl.

Conclusion

NIPHS is a rare complication of gastric bypass surgery with a poorly understood mechanism. Surgical treatment is effective for hypoglycemia management but leaves patients with insulin-requiring diabetes. There is one reported case of successful medical treatment with diazoxide, and another case of improved symptoms on combination of verapamil and acarbose. Our patient is the first reported to our knowledge with severe hypoglycemia who became symptom-free on acarbose monotherapy. This case demonstrates the importance of considering pharmacological treatment for such patients, given the complications associated with pancreatectomy.

Nothing to Disclose: LT, TD

P1-454

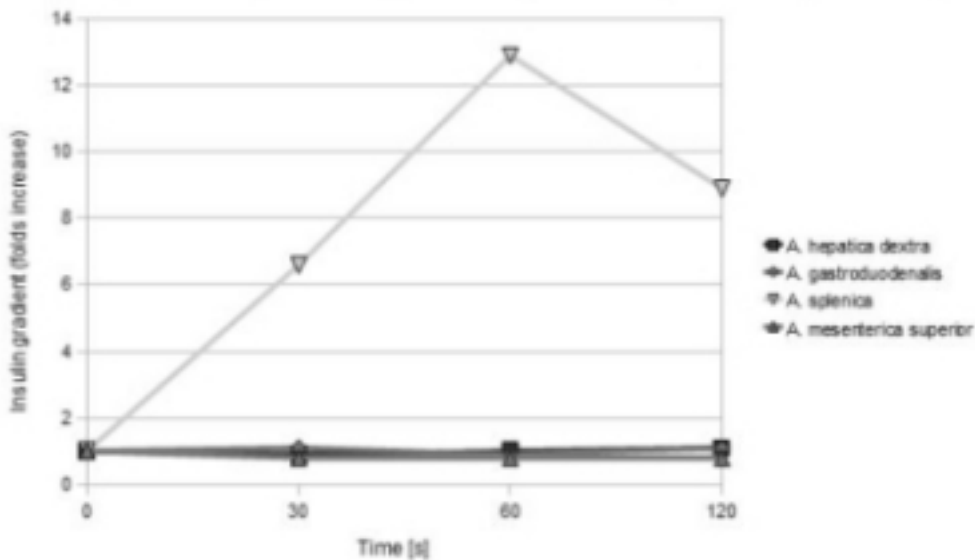
Gastric Bypass Surgery Discloses Insulinoma.

Christof Lipowsky¹, Stefan Bilz¹, Bernd Schultes², Thomas Clerici³ and Michael Braendle¹.

¹Div of Endocrinology and Diabetes St Gallen, Switzerland ; ²Interdisciplinary Obesity Ctr St Gallen, Switzerland and ³EBSQ Endocrine Surg St Gallen, Switzerland.

Objective: To describe a patient with postgastric bypass hyperinsulinemic hypoglycemia syndrome (PGBHS) due to a subsequently diagnosed insulinoma.

Case: We report on a severely obese 64-year-old woman with type 2 diabetes who started to regain weight 8 years after gastric banding and therefore underwent gastric bypass surgery (GBS). Symptomatic hypoglycemia (plasma glucose 2.5mmol/l) occurred on postoperative day three and was treated with intravenous dextrose. During the next few weeks recurrent hypoglycemic symptoms were noted despite resumption of normal food intake. 3 months after surgery the patient was admitted to the emergency room with neuroglycopenic symptoms and severe hypoglycemia (plasma glucose 1.5mmol/l). Insulinoma or noninsulinoma pancreatogenous hypoglycemia syndrome was suspected. Since plasma glucose concentrations remained within the normal range after a 75g oGTT a 72-hour fasting test was performed. Neuroglycopenia was noted after 6.5 hours at a glucose level of 1.5mmol/l and the concomitantly increased serum C-peptide and insulin concentrations confirmed the diagnosis of endogenous hyperinsulinemic hypoglycemia. The subsequent abdominal CT scan showed a 2cm hypervascular mass in the tail of the pancreas. Selective arterial calcium stimulation with hepatic venous sampling demonstrated a 13-fold rise in insulin levels after injection of calcium into the splenic artery, suggesting an insulinoma in the pancreatic tail.



Distal pancreatectomy was performed and revealed a neuroendocrine pancreatic tumor with strong immunostaining for insulin. Hypoglycemic symptoms disappeared completely after surgery and no hypoglycemic events recurred during the follow-up over three years.

Conclusion: Persistence of PGBHS, especially in the fasting state, should raise the suspicion for an underlying insulinoma. Our findings suggest that GBS may inappropriately increase insulin secretion from insulin-producing tumor cells, thereby disclosing insulinoma postoperatively.

Nothing to Disclose: CL, SB, BS, TC, MB

P1-455

Starvation Ketoacidosis Associated with Gastric Banding.

A Lulsegged BSc, MB.BS, MRCP¹, E Saeed MB.BS¹, C Duffield MB.BS¹, N Pareek MA, MRCP¹, E Langford MA, MD, FRCP¹ and S El-Hasani M.B.ChB, FRECS (Gen)¹.

¹South London NHS Trust Orpington, UK.

A 31 year old lady was admitted with breathlessness, pleuritic chest pain, nausea for 2 weeks and severe vomiting for one week. She had a gastric band fitted one year prior without complications and had lost approximately 50kg. On examination pulse 109 regular, BP 128/73mmHg, O₂ saturation 96% room air, respiratory rate 18/minute and afebrile. Chest & Abdominal examination was unremarkable. Chest X-ray was clear, CBC, amylase, troponin, renal and liver function were normal. Glucose 4.0mmol/L (72mg/dl). Arterial Blood Gases: pH 7.289, pCO₂ 2.75KPa (21mmHg), pO₂ 15.47KPa (116mmHg), HCO₃ 9.7mmol/L (9.7mEq/L), Base Excess -14.7, Anion gap 21.3mmol/L (21.3mEq/L) - normal, Lactate 0.57mmol/L (5.1mg/dl) - normal. Urine strongly positive for ketones.

CT abdomen demonstrated fluid level within the oesophagus with a pouch isolated by the gastric band, the band had herniated & there was no free fluid or evidence of perforation.

A diagnosis of starvation ketoacidosis associated with a tight gastric band, nausea & vomiting was made. She was treated with intravenous dextrose and insulin and the band deflated the following day. Contrast study post deflation confirmed no surgical problem. She was discharged from hospital 3 days later following normalisation of her pH. Cases of starvation ketoacidosis are rare. There have been reports of this in pregnancy owing to prolonged periods of vomiting, and fasting. However, the finding of starvation ketoacidosis due to gastric banding has only been reported once before. We present a case of starvation ketoacidosis in a young lady 1 year after gastric banding.

Diagnosis of starvation ketoacidosis is based on history of reduced calorific intake, low plasma glucose, presence of ketonaemia/ketonuria and high serum beta-hydroxybutyrate level. Other causes of a metabolic acidosis with a raised anion gap must also be excluded: lactic acidosis, diabetic & alcoholic ketoacidosis, methanol/ethylene glycol ingestion or salicylate toxicity.

This case demonstrates a complication of gastric banding, and could occur in other weight reducing surgery. This is important to recognise as obesity is worsening worldwide & more surgical procedures are being employed. We also describe a method of treating starvation ketoacidosis notably dextrose/insulin infusion and mechanically, relaxation of the band. Lastly, our aim is to emphasise the importance of a methodical approach to acid-base disturbances.

Nothing to Disclose: AL, ES, CD, NP, EL, SE-H

P1-456

Extreme Hypertriglyceridemia Responsive to Therapeutic Plasma Exchange.

J Kravarusic MD PhD¹, NJ Stone MD¹, A Zakarija MD¹ and A Dunaif MD¹.

¹Northwestern Univ Feinberg Sch of Med Chicago, IL.

INTRODUCTION: The patient is a 47 year old postmenopausal woman on no hormonal replacement with chronic myelogenous leukemia and type 2 diabetes admitted for extreme triglyceride (TG) elevation of 17393 mg/dL (desirable < 150 mg/dL).

CLINICAL CASE: She had graft versus host disease of the gut after a stem cell transplant, which was being treated with glucocorticoid and immunosuppressive therapy. The TG level was elevated at 343 mg/dL before total parenteral nutrition (TPN), and reached 7463 mg/dL a week before the admission even though TPN was discontinued one month previously. She denied confusion, vision loss and abdominal pain. The prednisone dose was maintained at 20 mg daily.

She had a remote history of ileocectomy for Crohn's disease and no 1st degree relatives with dyslipidemia. The skin had no eruptive xanthomas, the abdomen was not tender and without hepatosplenomegaly. Lipase and amylase were within the normal range, blood glucose was 243 mg/dL. CT scan of the abdomen excluded pancreatitis. Despite total fat restriction, insulin infusion, and fibrate therapy, the TG rose to 26250 mg/dL, therefore therapeutic plasma exchange (TPE) was performed twice with desired decrease in TG to 530 mg/dL. Discharge instructions included very low fat diet, unchanged insulin and prednisone dose, and new fenofibrate and omega-3-acid ethyl esters. In the next 3 months, the TG remained in the range of 195-530 mg/dL. We asked her to have family members obtain fasting lipid profiles. The etiology of this extreme TG elevation is likely genetic, exacerbated by immunosuppressive and glucocorticoid therapy.

CONCLUSION: Hypertriglyceridemia over 1000 mg/dL and fasting hyperchylomicronemia is a clinical challenge because, although the risk of pancreatitis is increased, there is no level above which it invariably occurs (1,2). This case shows that even extreme elevations of TG can be tolerated for brief periods and, although there is no single recommendation, complete fat restriction at the outset is critical (1-3). Insulin and/or heparin infusion have been used with success (3). TPE is usually reserved for resistant cases, but as noted here, reduces TG levels promptly. Biliopancreatic diversion has been performed for a familial case (4) lacking adherence to a medical regimen. We show that TPE effectively lowered TG where insulin infusion initially had no striking effect. Of clinical interest was that history of bowel resection did not prevent her severe TG elevation.

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(3) Tsuang W, Navaneethan U, Ruiz L, Palascak JB, Gelrud A. Hypertriglyceridemic pancreatitis: presentation and management. *Am J Gastroenterol* 104:984, 2009

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Nothing to Disclose: JK, NJS, AZ, AD

P1-457

Hypertriglyceridemia-Induced Pancreatitis: Establishing Treatment Guidelines.

DP Houser MD¹ and EA Christofides MD².

¹Mount Carmel Hlth Med Ctr Columbus, OH and ²Endocrinology Associates, Inc Columbus, OH.

Introduction: The National Cholesterol Education Program guidelines highlight the importance of treating dyslipidemia based on cardiovascular risk factors with a stated goal of improved outcomes. Although recent analyses suggest that hypertriglyceridemia is an independent cardiovascular risk factor, clinical directives are lacking regarding hypertriglyceridemia in the setting of acute pancreatitis. Treatment typically involves the use of a fibric acid derivative or even apheresis, but combination therapy is not delineated. The infrequency of hypertriglyceridemia-induced pancreatitis makes treatment guidelines difficult to establish.

Case Description: A twenty-one year old Caucasian male was admitted to the hospital with abdominal pain, was found to have significantly elevated triglycerides and was subsequently diagnosed with acute pancreatitis. On physical exam, the patient was morbidly obese and exhibited eruptive xanthomas all over his body. Family history was significant for diabetes mellitus type 2 and what he suggested as "typical high cholesterol." There was no family history of premature CAD or xanthomas/xanthelesmas. Social history was negligible for alcohol. His admission labs showed a total cholesterol of 1046mg/dl, a triglyceride level of 20,803mg/dl, amylase 28U/L, lipase 182U/L, TSH 1.07 μ IU/mL, and HbA1c was 10.8%. NMR analysis obtained on admission revealed: LDL-P 680nmol/L, Small LDL-P 121nmol/L, Particle size 21.5nm (Pattern A 23.0-20.6), Large HDL-P 0.0 μ mol/L, Large VLDL-P 147.1nmol/L, Apo B 225, Lp(a) < 5. The patient was started on: IV insulin infusion, D5W, 45mg of pioglitazone, 4 grams of omega-3-ethyl esters, 80mg of atorvastatin, 2000mg of nicotinic acid, and 10mg of ezetimibe (the patient was not npo). Two and a half days later, the patient's triglyceride level was 451mg/dl, eight days later and off IV insulin the patient's triglycerides were 138mg/dl with normal serum glucose.

Discussion: This case illustrates that the guidelines provided by the Adult Treatment Panel lack guidance for this degree of triglyceride elevation. In this case first-line monotherapy was not appropriate. Each of the drugs in this regimen played a specific role in decreasing Apo B and VLDL. This case highlights that a multi-drug regimen in a dysmetabolic patient should be standard, include an insulin sensitizing agent and should be initiated immediately upon identification to avoid clinical consequence.

Disclosures: EAC: Clinician, Amylin Pharmaceuticals; Advisory Group Member, Novo Nordisk, Amylin Pharmaceuticals; Speaker Bureau Member, Amylin Pharmaceuticals, Abbott Laboratories, Lilly USA, LLC, Takeda, Novo Nordisk, Merck & Co., Pfizer, Inc., Novartis Pharmaceuticals.

Nothing to Disclose: DPH

P1-458

Madelung's Disease: Report of a Case with Lower Limbs Involvement.

UV Zanardi MD¹, SM Vieira MD/PhD¹, M Nery MD/PhD¹, BL Wajchenberg MD/PhD¹ and ML Correa-Giannella MD/PhD¹.

¹Univ of São Paulo Med Sch São Paulo, Brazil.

Background: Multiple symmetric lipomatosis (MSL), also known as Madelung's disease or Launois-Bensaude syndrome, is a rare disorder characterized by progressive, multiple, symmetric accumulations of nonencapsulated fat masses in the face, neck, trunk, arms and, rarely, in the lower limbs. More than 90% MSL is associated with chronic alcohol abuse in middle-aged men. The etiopathogenesis remains unclear, but an abnormal lipogenesis induced by catecholamines has been observed. Several metabolic and endocrine disturbances are found to be with MSL. Prognosis is dependent on the concomitant presence of a neuropathy with a mortality of 25,8%. Conservative management is based on alcohol abstinence and correction of any associated abnormalities. Surgery is the most effective treatment, although relapses often occur.

Clinical case: A 56-year-old man was referred to the Endocrinology and Metabolism Service at Hospital das Clinicas (University of São Paulo Medical School) reporting the appearance of multiple palpable masses in the neck, trunk, upper and lower extremities over the last 12 years. There was a positive history of moderate alcohol abuse, but the patient denied using medications associated with fat deposition, such as steroidal or antiretroviral agents. Clinical symptoms or signs of somatic or autonomic neuropathy were absent. Physical examination revealed bilateral, symmetric, soft, well-delimited enlargements of the upper arms, shoulders, back, upper and lower trunk, and also upper legs (Fig1). Forearms and distal legs were spared. Laboratorial evaluation showed a slight increase in liver enzymes and hyperuricemia. Other biochemical tests were normal. Therapy with allopurinol was initiated, alcohol abstinence was recommended and the patient was referred to the Plastic Surgery Clinics.



Figure 1. Madelung's Disease: Enlargements of the upper arms, shoulders, back, upper and lower trunk, and also upper legs.

Conclusion: We report a case of Madelung's disease with marked disfigurement and involvement of the lower limbs. Although Madelung's disease usually has no symptoms, sometimes, the progression of adipose tissue growth can cause compression in the aerodigestive tract that needs therapeutic intervention.

Nothing to Disclose: UVZ, SMV, MN, BLW, MLC-G

P1-459

Renal Complications Secondary to Enteric Hyperoxaluria Necessitating Surgical Reversal of Jejunioileal Bypass (JIB) 36 Years Later.

NM Sunnoqrot MD¹, GT Dondlinger MD¹ and SM Pinkstaff MDPhD^{2,3}.

¹Johns Hopkins Univ/Sinai Hosp Internal Med Residency Program Baltimore, MD ; ²Sinai Hosp Baltimore, MD and ³Johns Hopkins Univ Sch of Med Baltimore, MD.

Background: Over 25,000 JIB surgeries were performed in the 1960-70s prior to being banned by the FDA because of severe complications including renal failure. Hyperoxaluria is common after JIB and can result in nephrolithiasis, oxalate nephropathy, and renal failure even after years of medical stability.

Clinical Case: A 60 year-old woman presented in 2001 with a L1 compression fracture, vitamin D deficiency, and a 21 year history of renal stones. In 1973, she underwent a JIB, and in 1980, developed symptomatic nephrolithiasis. She has had more than 100 renal stones, 20 lithotripsies, numerous cystoscopies, and one percutaneous surgery for ureteral obstruction. Creatinine (Cr) levels ranged between 0.9-1.3 mg/dl (0.2-1.3) until 2004 when renal function began to decline with Cr levels varying between 1.4-1.7 mg/dl. In 2008, she suffered acute renal failure due to volume depletion and diarrhea (Cr 2.92 mg/dl) with a GFR of 17.5 ml/min (>60 ml/min). One year later GFR was 32 ml/min. Urinary oxalate excretion was increased at 62 mg/24 hr (4-31). Medical management was advised. Despite care from urologists, endocrinologists, and nephrologists, the progressive course of her renal disease was not recognized to be a complication of her JIB. In 2009, her JIB was reversed with a preoperative GFR of 29 ml/min. Three weeks postoperatively, Cr was 1.34 mg/dl with a GFR of 40 ml/min.

Conclusions: 1. Renal complications due to hyperoxaluria following JIB continue to occur and may progress decades after the initial surgery. 2. Identifying renal complications following JIB allows for timely intervention before renal insufficiency is progressive and irreversible. 3. Although JIB is no longer performed, Roux-en-Y bypass has been reported to cause enteric hyperoxaluria, nephrolithiasis, and renal failure.(1) Any patient who develops renal complications after a malabsorptive surgery should be evaluated and treated for enteric hyperoxaluria. 4. Detailed surgical histories are key in patients with recurrent calcium oxalate stones.

1)Nelson WK,Houghton SG,Milliner DS,et al. Enteric hyperoxaluria,nephrolithiasis,and oxalate nephropathy: potentially serious and unappreciated complications of Roux-en-Y gastric bypass. *Surg Obes Relat Dis* 2005;1:481-485.

Nothing to Disclose: NMS, GTD, SMP

P1-460

Loss of CaMKK2 Enhances Glucose-Mediated Insulin Secretion.

TJ Ribar BA¹, CR Means n/a¹, F Lin BS¹ and AR Means PhD¹.

¹Duke Univ Med Ctr Durham, NC.

The most abundant cell in pancreatic islets is the β -cell, the major function of which is to produce and secrete insulin in response to changes in plasma glucose, although these specialized cells also produce other signaling molecules such as NPY and ghrelin. Control of insulin secretion is sensitive to multiple signaling cascades. One such cascade is dependent upon activation of AMPK, which is also a component of signaling cascades that plays pivotal roles in the hypothalamus to regulate energy homeostasis. We have shown that the AMPK kinase involved in mediating energy balance in the hypothalamus is CaMKK2, which is highly expressed in the NPY/AgRP neurons of the arcuate nucleus. Mice with germ line disruption of the CaMKK2 gene maintain normal circulating insulin levels and remain glucose responsive when fed a high fat diet. We find that in addition to the arcuate nucleus, pancreatic islets express CaMKK2 mRNA and immunocytochemistry revealed that CaMKK2 is predominately localized to β -cells. Thus, we evaluated if CaMKK2 plays a role in regulating β -cell function. In mice fed a normal diet, loss of CaMKK2 neither alters average islet size or basic islet structure relative to WT. We also found that insulin levels in islets from CaMKK2^{-/-} null mice were no different than those in WT islets. However, CaMKK2^{-/-} islets have a marked reduction in NPY mRNA and protein compared to WT which prompted us to evaluate insulin secretion. Whereas static insulin secretion assays performed on isolated islets from adult CaMKK2^{-/-} and WT mice showed no difference in response to basal glucose (2.8 mM), elevated glucose (16.8 mM) or high K⁺ markedly increased insulin secretion from CaMKK2^{-/-} islets relative to WT. In addition, pharmacological inhibition of CaMKK2 in the rat β -cell line INS-1 also resulted in increased insulin secretion in response to high glucose or K⁺. In conclusion, our data reveal that pancreatic β -cells express CaMKK2 and, whereas loss of this enzyme does not change islet structure, it does result in increased glucose-mediated insulin secretion. Thus CaMKK2 may be a viable therapeutic target to combat glucose insensitivity and low insulin secretion that are hallmarks of Type II diabetes.

Nothing to Disclose: TJR, CRM, FL, ARM

P1-461

Localization of the Region of Glucagon-Like Peptide-1 Receptor (GLP1R) and Glucose-Dependent Insulinotropic Polypeptide Receptor (GIPR) Responsible for Ligand Binding.

Mi Jin Moon PhD candidate¹, Jong-Ik Hwang PhD¹ and Jae Young Seong PhD¹.

¹Graduate Sch of Med, Korea Univ Seoul, Korea.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) play important roles in insulin secretion through their secretin-like G-protein coupled receptors. Although GLP-1 and GIP are attractive candidates for the treatment of type 2 diabetes, little is known about molecular interaction with their receptors. In the present study, using chimeric and point-mutated GLP-1/GIP receptors, we attempted to identify domains and/or amino acid residues of rat GLP-1 and human GIP receptors conferring ligand selectivity for GLP-1 and GIP. A chimeric GLP1R having N-terminal domain of GIPR (GLP1R/GIPR_(N)) showed relatively lower affinity than GLP1R to hGLP-1. Moreover, GIPR/GLP1R_(N) lost binding ability to hGIP. This result suggests that the N-terminal domain of GLP-1 and GIP receptors are responsible for ligand binding. hGLP-1 showed lower affinity toward GLP1R/GIPR_(N-TMD2) than GLP1R/GIPR_(N). Replacement of two amino acid residues (Ser¹⁸⁹→Ile and Arg¹⁹⁰→Lys) at the end of transmembrane domain 2 of GLP1R/GIPR_(N-TMD2) rescued the affinity to hGLP. The GIPR/GLP1R_(N-ECL1) chimera exhibited more than 10 times higher affinity to hGLP-1 than GLP1R/GIPR_(N-TMD2). hGLP-1 showed 100 times higher sensitivity to GIPR/GLP1R_(N-ECL2) than GIPR/GLP1R_(N-TMD4). hGIP revealed more than 100 times higher potency toward GLP1R/GIPR_(N-ECL1) than GLP1R/GIPR_(N-TMD2). These results suggest that transmembrane domains 1 and 2 of GLP-1 receptor are responsible for hGLP-1 binding while ECL1 of GIP receptor is probably important for hGIP interaction. This information may provide a clue for development of novel GLP-1 and GIP analogs.

Nothing to Disclose: MJM, J-IH, JYS

P1-462

Hyperinsulinism Linked to a Novel Paternally Inherited Alteration in *KCNJ11*.

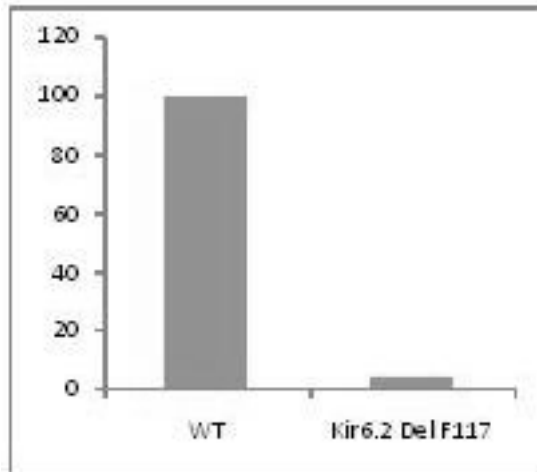
R Dahiya B.Sc (Hons), MBBS¹, S-L Shyng B.S, PhD², I McGown B.Sc³, B Marsh B.Sc⁴, G Leong MBBS, FRACP, PhD¹, M Harris MBBS, FRACP¹, R Lourie B.Sc, PhD³ and A Cotterill MBBS, FRACP¹.

¹Mater Children's Hosp South Brisbane, Australia ; ²Oregon Hlth & Sci Univ Portland, OR ; ³Mater Adults Hosp South Brisbane, Australia and ⁴Inst for Molecular Biosci, Univ of Queensland St Lucia, Australia.

Background Hyperinsulinism (HI) may result from inherited loss-of-function mutations of the ATP-sensitive K⁺ channel (K_{ATP}) an octamer containing the sulfonylurea receptor (SUR1) and a regulated ion pore (Kir 6.2) which are encoded by adjacent genes, *ABCC8* and *KCNJ11*, respectively on chromosome 11p15.1. Inherited defects in the paternal allele of either gene are reported to lead to focal adenomatosis which can be cured by surgical resection. The underlying cause of focal HI and tissue overgrowth is thought to be due to loss of heterozygosity (LOH) at Ch.11p15.

Clinical case A 5 m boy presented with symptoms consistent with HI. Mutation analysis on peripheral blood detected a paternally inherited Phe117del in the *KCNJ11* gene. Nodules were identified by laparoscopic inspection in the tail of the pancreas, resection of which restored blood glucose levels to normal.

Tissue Analysis Histology revealed a 6mm focal area of islet cell adenomatosis in the pancreatic tail with subtle diffuse changes in the surrounding tissue. Increased staining with the tumor suppressor gene, p57^{KIP2}(Ch 11p15.5), was seen in the focal adenoma. Subsequent molecular studies on the affected tissue did not reveal any LOH. Transmission electron microscopy revealed numerous multi-granular bodies, consistent with up-regulated degradation of intracellular insulin content. The organization of the endoplasmic reticulum and Golgi complex appeared similar to normal. Functional studies in COS cells co-transfected with cDNA encoding the mutant Kir6.2 and wild-type *SUR1* showed a complete loss of cell surface channel expression and function.



Chemiluminescence assay showing no surface expression of the mutant K_{ATP} channel

Interestingly, in Western blots, the mutant Kir 6.2 protein was not detected, suggesting premature degradation.

Conclusion The Phe117del in the *KCNJ11* gene is in a highly conserved region has not previously been described in cases of HI gene and is likely to be responsible for this child's hyperinsulinism given the results of the functional studies. The restoration of normal glucose control after resection of the pancreatic tail also confirms that the focal lesion found on histology was responsible for the HI. Loss of heterozygosity however does not appear to explain the focal nature of the HI in this boy. In vitro studies of the mutant channel suggest that the alteration affects folding, and stability of the Kir 6.2 protein, preventing channel formation and activity.

Nothing to Disclose: RD, S-LS, IM, BM, GL, MH, RL, AC

P1-463

Deciphering Protein Codes That Are Essential for Maturation of Pancreatic Islets.

CW Liew PhD¹, JH Lock BA¹, RL Maas MD, PhD² and RN Kulkarni MD, PhD¹.

¹Joslin Diabetes Ctr, Harvard Med Sch Boston, MA and ²Brigham and Women's Hosp, Harvard Med Sch Boston, MA.

A major limitation in the successful treatment of diabetes is to provide patients with a renewable source of insulin that is similar to the endogenous source for maintenance of glucose levels within a physiologic range. Although recent reports have suggested that insulin-producing cells can be derived by a protocol that mimics normal pancreas development or by using small molecule inducers for differentiation of human embryonic stem cells, both approaches work with low efficiency. Indeed, the in vitro differentiated insulin-producing cells are not optimally glucose-responsive – a major limitation for cell-replacement therapy for diabetes. Several observations indicate that immature neonatal mouse islets fail to respond to glucose until after a maturation process, which generally occurs between postnatal (P) days 20 to 28. To gain further insight into the maturation process, mouse islets were isolated from pups at post-natal (P) day 14, P21, P28 or P35 using collagenase digestion. RNA extracted from all samples was amplified by T7 RNA polymerase and hybridized to Illumina Mouse BeadChips-WG6, covering ~48,000 transcripts. Data were normalized with Illumina BeadStudio software and analyzed with MetaCore Pathway analysis software with David 2008 and Gene Ontology (GO) databases. We report significant regulation of genes associated with glucose metabolism, lipid metabolism, insulin biosynthesis, actin cytoskeleton and extra-cellular matrix remodeling. Age-dependent upregulation of key genes including the glucose transporter, Glut2, and proprotein convertases suggest functional maturation of pancreatic islets. Alterations in lipid metabolism in pancreatic islets with age are consistent with the change from a lipid-rich milk diet to chow diet following weaning at P21. We also observe significant changes in genes associated with cytoskeleton, cell-cell adhesion and extra-cellular matrix that are consistent with previous observations that these development and maturational periods are associated with significant morphological change. Taken together, our data suggest that coordinated changes in glucose sensing, insulin processing and cellular remodeling are critical for the functional maturation of pancreatic islets.

Sources of Research Support: In part by NIH RO1 DK 67536 (R.N.K). An NIH training grant (1RL9EB008539-01) (SysCODE) supported C.W.L.

Nothing to Disclose: CWL, JHL, RLM, RNK

P1-464

Loss of Beta Cell-Resident Epidermal Growth Factor Receptor Impairs Glucose-Stimulated Insulin Secretion.

NS Kayton¹, R Aramandla¹, LA Scheving MD¹, WE Russell MD¹ and AC Powers MD¹.

¹Vanderbilt Univ Nashville, TN.

The epidermal growth factor receptor (EGFR) is required for normal exocrine and endocrine pancreas development, as indicated by global EGFR knockout studies. However, the role of EGFR in adult islet physiology and beta cell function is incompletely understood. By immunocytochemistry, we found that EGFR is expressed at higher levels in islets than in surrounding exocrine tissue. RT-PCR shows that EGFR (ErbB1) is the most highly expressed of the four ErbB receptor family members in islets. To investigate the role of EGFR in adult beta cell function, we used the Cre-lox system to inactivate EGFR in beta cells. Mice expressing Cre driven by the *ins2* promoter were bred with EGFR-loxP mice, to generate *ins2Cre+;EGFR^{fl/fl}* (KO) and *ins2Cre-;EGFR^{fl/fl}* (WT). Islet morphology, islet mass, and beta cell mass in adult mice did not differ between the KO and WT animals. As assessed by intraperitoneal glucose tolerance testing (8-10-week-old males and females), glucose tolerance was similar in the KO and WT animals (males: n = 5-6, p=0.1255; females: n = 9, p=0.3860). However, in a perfusion system, islets isolated from 20-week-old female KO mice secreted less insulin than islets from age-matched female WT mice in response to 16.7mM glucose (29.35 ± 7.741 vs. 50.92 ± 4.687 ; n = 5-6, p<0.05). The finding that islets and beta cells develop normally in KO mice suggests either that EGFR-derived signals important for islet differentiation occur prior to inactivation of EGFR by the insulin promoter-driven Cre, or that they can be compensated by other mechanisms. These data also demonstrate that EGFR plays an important role in beta cell function by enabling the beta cell to fully respond to glucose stimulation.

Nothing to Disclose: NSK, RA, LAS, WER, ACP

P1-465

Metabolic Consequences of TCF7L2 Deficiency in Mice.

V Kaur¹, J Wood¹, JL Hall PhD² and MK Thomas MD, PhD^{1,3}.

¹Massachusetts Gen Hosp Boston, MA ; ²Univ of Minnesota Minneapolis, MN and ³Harvard Med Sch Boston, MA.

Polymorphisms within the locus encoding the Wnt signaling pathway member and transcription factor TCF7L2 represent one of the strongest known genetic contributors to risk for the development of type 2 diabetes in humans. The mechanisms by which noncoding *tcf7l2* gene polymorphisms regulate TCF7L2 expression patterns and predispose to the development of diabetes are unknown, but in clinical studies *tcf7l2* risk alleles are associated with impaired insulin production and incretin hormone action. To elucidate mechanisms by which TCF7L2 regulates metabolic and pancreatic islet cell functions, we identified cellular and metabolic phenotypes in mice heterozygous for genetic disruption of the *tcf7l2* gene. *Tcf7l2* heterozygote and littermate wild-type control mice had significant differences in metabolic regulation that varied by age and gender. TCF7L2 deficiency altered insulin production and glucose metabolism, conferring increased metabolic dysfunction with aging. TCF7L2-deficient pancreatic islets acquired progressive changes in proliferative regulatory programs and dysregulation of a network of transcription factors essential for pancreatic beta-cell function. Metabolic phenotypes were indicative of TCF7L2-dependent pancreatic islet cell functions and extrapancreatic regulation of nutrient metabolism. Our studies support a regulatory model in which TCF7L2 governs energy metabolism by coordinating signaling in multiple organ systems, including important regulatory roles in pancreatic islet cell expansion and function. Identification of integrated physiologic functions of TCF7L2 in metabolic regulation will be essential for characterizing molecular mechanisms by which *tcf7l2* gene polymorphisms contribute to the risk of developing diabetes and for designing new therapeutic strategies to address variations in TCF7L2 functions.

Nothing to Disclose: VK, JW, JLH, MKT

P1-466

Primary Murine Islet Cell Tolerance to Elevated Zinc Levels Is Not Diminished by ZnT8 Knockdown.

M El Muayed MD, Dr. med.¹, XM Zhang MD¹, MV Newman¹ and WL Lowe MD¹.

¹Northwestern Univ Feinberg Sch of Med Chicago, IL.

A variation in *slc30A8*, the gene that encodes the zinc transporter ZnT8 has been linked to an increased susceptibility to type 2 diabetes mellitus. ZnT8 is localized on the membrane of secretory vesicles in pancreatic beta cells and is thought to transport zinc (Zn) into these vesicles, thereby facilitating the condensation of insulin. Decreased or absent expression of ZnT8 leads to impaired glucose stimulated insulin secretion in murine islet cells. It can be hypothesized that altered ZnT8-mediated Zn transport into secretory vesicles leads to an accumulation of toxic levels of Zn in the cytoplasm. The goal of this study was to determine whether a decreased ZnT8 expression leads to decreased islet cell viability in the presence normal or elevated ambient Zn concentrations. The studies were performed in murine islets isolated from male C57BL/6 mice. siRNA-mediated knockdown of ZnT8 was used as a model for impaired ZnT8 function. Control cells were transfected with negative control siRNA. Knockdown was verified by western blot analysis of ZnT8 expression. Similar to reports from ZnT8 null mice, glucose stimulated insulin secretion relative to basal insulin secretion was decreased from 2.0 (+/-0.25) to 1.2 (+/-0.21, P<0.05, n=4) without significant changes in total cellular insulin content (17.1 +/-1.5 and 15.3 +/-1.6 5 ng insulin/ μ g protein) after ZnT8 knockdown, thereby validating our model. The effect of a decreased ZnT8 expression on cell viability in the presence of physiologic and supraphysiologic levels of Zn was examined using the Multitox® assay. Zn concentrations of 5, 19, 34, 48, 62, 76, 91 or 105 micromol/l were well tolerated. Significant differences in cell viability between ZnT8 knockdown cells compared to control cells were not observed at any of the concentrations. The cell viability index relative to the control was 1 +/-0 and 0.991 +/-0.014 for the control and ZnT8 knockdown cells respectively at the physiologic Zn concentration of 5 micromol/l. The cell viability index increased to 1.58 +/-0.121 and 1.59 +/-0.2 respectively at the maximum supraphysiologic Zn concentration of 105 micromol/l (n=4). These data suggest that decreased ZnT8 expression does not alter beta cell viability at Zn concentrations up to 105 micromol/l. Thus, the reported increased susceptibility to type 2 diabetes and decreased beta cell function in carriers of certain ZnT8 variants is likely not due to decreased beta cell viability.

Nothing to Disclose: MEM, XMZ, MVN, WLL

P1-467

New Link between the Innate and the Adaptive Immune System in Two Animal Models of Insulin-Dependent Diabetes.

M Ehlers MSc¹, C Papewalis PhD¹, S Schinner MD¹, HS Willenberg AssocProf¹, WA Scherbaum Prof MD¹ and M Schott Prof MD¹.

¹Univ Hosp Duesseldorf Duesseldorf, Germany.

Natural killer (NK) cells belong to the innate immune system and are able to quickly respond to viral infections and tumors. In endocrine autoimmunity the role of NK cells is, however, only ill defined. Recently, a new subset of NK cells with immunosuppressive functions has been identified. These cells express c-kit, they are able to downregulate conventional c-kit negative NK cells in a PD1-ligand dependent manner, and are thought to be responsible for tumor outgrowth.

The aim of our present study was to investigate the impact of this new c-kit positive NK cell subset in autoimmune diabetes. For this purpose, we used two mouse models for insulin-dependent diabetes mellitus (IDDM): 1) the non-obese diabetic (NOD) mouse model, and 2) the low-streptocotozin (STZ) mouse model. In NOD mice, a large increase (6.4 fold) of c-kit positive NK cells was found at 8 weeks of age, a time point of islet cell infiltration. STZ treated mice also showed an increase (12.1 fold) of c-kit positive NK cells in the pancreas at day 8 after STZ administration. *In vitro* analyses revealed that c-kit positive NK cells exhibit a direct lysis activity (> 30%) not only towards conventional NK cells but also towards activated (non-specific) CD8+ T cells and, importantly, towards insulin beta-chain specific CD8+ T cells. In order to investigate the effect of these cells on the development of diabetes *in vivo* we also performed adoptive cell transfer experiments by repetitive intravenous injections of IL-18 generated c-kit positive NK cells (n = 6). In the control group c-kit negative NK cells were administered (n = 6). At 10 weeks of age, all mice (100%) of the c-kit negative group developed diabetes, whereas only 62.5% of the c-kit positive NK cell group had blood glucose levels > 300 mg/dl. Importantly, two mice of the c-kit positive group did not develop diabetes until 20 weeks of age.

To our knowledge, this is the first report describing the role of immunosuppressive NK cells in autoimmunity. Our data provide a direct link between the immunosuppressive function of the innate immune system towards the adaptive immune system in organ-specific autoimmune diseases.

Nothing to Disclose: ME, CP, SS, HSW, WAS, MS

P1-468

Androgen Excess Predisposes to Insulin Deficiency Via AR in β -Cells.

Guadalupe Navarro B.S.¹, Suhuan Liu PhD¹, Karel De Gendt², Guido Verhoeven² and Franck Mauvais-Jarvis MD.PhD¹.

¹Northwestern Univ Chicago, IL and ²Catholic Univ of Leuven Leuven, Belgium.

In women, excess production of the male hormone testosterone is accompanied by insulin resistance and pancreatic β -cell dysfunction. However, limited attention has been devoted to the role of testosterone in β -cell survival and function. We hypothesized that testosterone exerts detrimental effects on β -cell function via the androgen receptor (AR). We observe expression of AR in mouse and human pancreatic β -cells with a predominant extranuclear localization.

In order to address the role of the AR in β -cell in vivo, we generated a β -cell-specific AR knockout mouse (β ARKO^{-/-}) using the Cre-loxP strategy. β ARKO^{-/-} mice are born with the expected mendelian frequency and show no overt abnormality of energy homeostasis. These mice were exposed to excess androgen using the AR selective ligand dihydrotestosterone (DHT) and β -cell stress induced by streptozotocin (STZ), respectively. Following STZ challenge, female control and β ARKO^{-/-} mice were protected from STZ-induced insulin-deficient diabetes and showed minimal alteration in islet mass and pancreas insulin concentrations. Conversely, Control female mice exposed to DHT, became vulnerable to STZ and showed a severe predisposition to insulin-deficient diabetes with dramatic loss of β -cell mass and pancreas insulin concentrations. We observed that the DHT-induced exacerbation of STZ-induced diabetes was significantly reduced in β ARKO^{-/-} mice. Female β ARKO^{-/-} mice exposed to DHT showed a less severe disruption in islet architecture and a more moderate decrease in β -cell mass and pancreas insulin concentration. In conclusion, androgen excess predisposes to β -cell failure at least partially via a direct action on the AR in β -cells.

Sources of Research Support: NIH grants DK074970, HD044405.

Nothing to Disclose: GN, SL, KDG, GV, FM-J

P1-469

Transgenic Mice Expressing an Intestine-Specific Secretory Protein, IBCAP (Formerly CF266), Demonstrates Pancreatic β -Cell Augmenting Activity.

T Yokoo PhD¹, K Watanabe PhD², KT Iida MD, PhD², H Suzuki MD, PhD², H SHIMANO MD, PhD², S Ishikawa MD, PhD¹, M Kawakami MD, PhD¹, N Yamada MD, PhD² and H Toyoshima MD, PhD¹.

¹Saitama Med Ctr, Jichi Med Univ Omiya-ku, Japan and ²Univ of Tsukuba Tsukuba, Japan.

Recent success with GLP-1 analog and DPP IV inhibitory drugs in the clinical application for diabetic patients has highlighted the roles of intestine as a hormone producing organ. Crucial roles of these hormones, including GLP-1, GIP and Ghrelin, in the controls of, energy metabolism and food intake, and their relation with the metabolic syndrome has been brought to worldwide attention. We have identified CF266 as a novel intestine-specific secretory protein using the Oligo-cap Signal Sequence Trap (Oligo-cap SST) strategy developed in our laboratory. We demonstrated that CF266 have insulin secretion promotive effect, and furthermore, adenovirus-mediated expression of CF266 in STZ treated type 1 diabetes model mice was shown to improve the blood glucose control of the animal, and increases the area of pancreatic β -cell in histological analysis. Now we have developed transgenic (Tg) mice expressing CF266 under the control of CAG-promoter, which will lead to the overexpression of the gene. Analysis of the Tg mice have shown marked increase of pancreatic islets, confirming our former findings with the STZ mice treated with CF266 adenovirus. Thus we renamed CF266 as IBCAP; intestine-derived beta-cell augmenting promoter. Further analysis have shown that blood glucose concentration and OGTT test of IBCAP Tg mice are relatively normal compared with their littermate mice. Therefore, IBCAP seems to have promoted the augmentation of islets which are functionally normal. We are now testing whether augmentation of the β -cell is due to an inhibition of apoptosis or due to a stimulation of proliferation. Our findings reveal IBCAP as another potential therapeutic target for diabetes and pancreas β -cell regeneration.

Nothing to Disclose: TY, KW, KTI, HS, HS, SI, MK, NY, HT

P1-470

***ff1b*, the *SF-1* Ortholog, Is Important for Pancreatic Islet Cell Development in Zebrafish.**

JW Powers M.D., HCM¹, JK Mazilu B.E.¹, S Lin Ph.D.¹ and ERB McCabe M.D., Ph.D.¹.

¹Univ of California, Los Angeles Los Angeles, CA.

The adrenal cortex and pancreatic islets have endocrine functions, producing steroid-based hormones and insulin, respectively. Cells of the adrenal cortex originate in the mesoderm while the cells of pancreatic islets originate in the endoderm. The zebrafish is a powerful model for understanding organ development due to its ease of genetic and molecular manipulation, transparent embryos, and large number of progeny for statistically powerful experiments. Like humans, the zebrafish pancreas has both exocrine and endocrine functions; unlike humans, there is only one endocrine islet cell group, instead of multiple islets. We have a transgenic line of zebrafish that has *eGFP* under the *insulin A* promoter, thereby forcing expression of *eGFP* in the pancreatic islet. Using this transgenic line of zebrafish, we have observed that the steroidogenic factor 1 (*SF-1*) ortholog, *ff1b*, which is critical for adrenal cortex development and function in the zebrafish, is also implicated in zebrafish pancreatic islet development. We show that interruption of *ff1b* expression using an *ff1b*-morpholino (*MO*) disrupts development of *eGFP-insulin A* expressing cells. Specifically, *ff1b* morphant embryos show multifocal expression of *eGFP* in contrast to a single site of *eGFP* expression in uninjected embryos. We conclude that *ff1b*-*MO* alters pancreatic islet development in zebrafish, demonstrating the utility of the zebrafish as a model for studying pancreatic development. This work is consistent with previous studies in mouse and human that have suggested *SF-1* participates in the vascular and ductal development of the pancreas, and disruption of *SF-1* function leads to abnormal development of the pancreatic islets due to poor vascularization. JWP and JKM contributed equally to this work.

Nothing to Disclose: JWP, JKM, SL, ERBM

P1-471

Identification and Characterization of Vesicular Nucleotide Transporter (VNUT) in the Pancreatic β Cell.

JC Geisler MS¹, H Chao MD¹ and C Li PhD¹.

¹Univ of Virginia Charlottesville, VA.

ATP plays a critical role in regulating pancreatic β cell function. Intracellularly, ATP regulates insulin secretion by binding and closing ATP sensitive potassium (K_{ATP}) channels. Furthermore, it has been shown that ATP accumulates into insulin secretory granules and is co-secreted upon stimulation of insulin secretion. Consistent with this observation, functional studies show that ATP can serve as an extracellular signal to regulate β cell function via purinergic receptors. Currently the mechanism by which ATP is packaged into insulin secretory vesicles is unknown. However, a vesicular ATP transporter has recently been identified in adrenal chromaffin cells. This protein, designated vesicular nucleotide transporter (VNUT), is a member of the solute carrier family SLC17 and plays a critical role in transporting ATP into secretory vesicles in adrenal chromaffin cells. Thus, it is conceivable that VNUT is expressed in pancreatic β cells to accumulate ATP into insulin secretory granules. To test this hypothesis, we first examined the expression and localization of VNUT in β cells. Using RT-PCR, VNUT mRNA was found to be expressed in mouse pancreas and isolated mouse islets as well as in rodent β cell lines (MIN6, INS-1). To determine VNUT localization inside β cells, a hemagglutinin (HA) tagged VNUT expressing vector was constructed and transfected into MIN6 cells. Immunofluorescence staining showed that the majority of HA-VNUT immunoreactivity (ir) colocalized with insulin, indicating that VNUT is present on insulin secretory vesicles. Furthermore, we found HA-VNUT-ir to be frequently associated with the cell membrane while showing little colocalization with markers for mitochondria and endoplasmic reticulum. To probe the potential function of VNUT in insulin secretion, MIN6 cells were infected with a lentivirus to express VNUT-specific small hairpin RNA (shRNA) to knock down endogenous VNUT expression. The infected cells were then stimulated by various levels of glucose and insulin secretion was measured by ELISA. We found that suppression of VNUT augments glucose-induced insulin secretion. Finally, while we found expression of VNUT mRNA in MIN6 cells to be modulated by glucose, VNUT mRNA expression in the pancreata of *ob/ob* mice was equivalent to that in wild type mice. In summary, our results demonstrate that VNUT is expressed in insulin secretory vesicles of pancreatic β cells and is involved in glucose stimulated insulin secretion.

Nothing to Disclose: JCG, HC, CL

P1-472

Lactogens in the Pathophysiology of Type II Diabetes: Protection Against Glucolipotoxicity (GLT)-Induced β -Cell Death Is Mediated through JAK2/STAT5 Signaling and BclX_L.

Nagesha Guthalu Kondegowda PhD¹, Katoura Williams MS¹, Xiaoying Zhang PhD¹ and Rupangi C Vasavada PhD¹.

¹Univ of Pittsburgh Pittsburgh, PA.

Lactogens acting through the prolactin receptor (PRL-R) are essential for the maintenance of normal β -cell function, proliferation and mass; and also protect β -cells against distinct cell death inducers. The goal of this study was to examine whether lactogens are pertinent in the pathogenesis of type II diabetes, whether they protect β -cells against GLT, and to understand their mechanism of action.

Despite numerous studies, the correlation between plasma PRL and obesity/diabetes in humans and rodents is unclear. We measured plasma PRL levels in leptin-deficient, obese, hyperglycemic C57Bl/6 ob/ob mice at ages 7-11 weeks. PRL levels were significantly lower in ob/ob mice (7.2 ± 0.5 ng/ml) compared to age-matched controls (17.3 ± 2.0 ng/ml). Furthermore, there was a 2-fold increase in PRL-R mRNA in islets from 11-week old ob/ob mice. Whether the changes in PRL/PRL-R levels contribute to the phenotype of these mice is under investigation.

As GLT is a likely contributor to the reduction in β -cell mass in type II diabetes, we assessed the effect of lactogens on GLT-induced β -cell death. Primary β -cells from normal (NL) mice exposed to 25mM glucose/0.5mM palmitate showed a 2.6-fold increase in β -cell death which was completely inhibited in islets of transgenic (TG) mice, expressing mouse placental lactogen-1 (mPL-1) in β -cells. Importantly, in human islets, GLT induced a 3-fold increase in β -cell death which was attenuated to 1.8-fold with human PRL ($p < 0.02$).

To dissect the cellular mechanisms, INS-1 cells were treated with 0.25 and 0.5mM palmitate, resulting in a 15- and 21-fold increase in cell death which was significantly reduced to 10- and 9-fold respectively with PRL. Of the lactogen-activated signaling pathways, our studies with siRNA and dominant-negative mutant show that the JAK2/STAT5 pathway is critical in mediating the pro-survival effect of lactogens. Furthermore, the importance of lactogen-induced expression of the anti-apoptotic molecule, BclX_L, was underscored when BclX_L-siRNA eliminated the protective effect of PRL against GLT-mediated INS-1 cell death. Currently, analysis of gene expression in NL and mPL-1 TG mouse islets \pm GLT, using apoptotic pathway gene arrays shows an increase in expression of distinct anti-apoptotic/anti-inflammatory genes in TG islets under basal and GLT conditions, which is being confirmed.

Taken together these results highlight a potential role of lactogens in β -cell mass expansion in insulin resistance and type II diabetes.

Sources of Research Support: Grants from the National Institutes of Health (DK078060 and DK072264 to RCV; DK055023 and DK07052-32 to AFS) and JDRF Research Awards (1-2008-46 to RCV; 1-2008-39 and 34-2008-630 to AFS). We acknowledge the ICR and JDRF Basic Science Islet Distribution Programs for supplying human islets.

Nothing to Disclose: NGK, KW, XZ, RCV

P1-473

XL903, a Potent, Selective and Orally Bioavailable 11 β -Hydroxysteroid Dehydrogenase Type 1 Inhibitor for the Treatment of Metabolic Disease.

J Dalgard¹, V Bollu¹, B Flatt¹, A Hogue¹, H-J Huang¹, J Li¹, J Li¹, R Mohan¹, I Murray¹, P Willy¹ and KM Ogilvie¹.

¹Exelixis, Inc San Diego, CA.

The enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1) catalyzes the conversion of inactive to active glucocorticoids. Chronically elevated glucocorticoids either in the systemic circulation or produced locally by cells that express 11 β HSD1 results in resistance to the physiological effects of insulin along with obesity, diabetes, and heart disease. Inhibition of 11 β HSD1 activity with small molecules improves metabolic profile in rodents and in early human clinical trials. XL903 is a potent inhibitor of human 11 β HSD1 (in vitro K_i = 7.6 nM) and is 1000 fold selective versus 11 β HSD2. XL903 is also a potent inhibitor of 11 β HSD1 in non-clinical species, including mouse (K_i = 3.2 nM), rat (IC_{50} = 30 nM), cynomolgus monkey (IC_{50} = 59 nM), and dog (IC_{50} = 181 nM), allowing robust characterization of the compound's target-related activities. XL903 has excellent oral bioavailability in non-clinical species and shows high distribution to target tissues such as liver, adipose and muscle. A single oral administration of XL903 at either 1 or 10 mg/kg to C57bl/6 mice inhibited 11 β HSD1 for up to 24 h in both liver and white adipose tissue. The pharmacodynamic effect is enhanced with repeated dosing, yielding nearly 50% inhibition for up to 24 h in both tissues after 4 days of BID dosing at 1 mg/kg. The increase in inhibition did not depend on accumulation of compound in those tissues. In diet-induced obese mice, 28 days of treatment normalized glucose tolerance with maximal effect at a dose of 1 mg/kg BID. Animals dosed once a day at either 1 or 10 mg/kg had a 45% improvement in glucose area under the curve. In summary, XL903 is a potent, selective inhibitor of 11 β HSD1 in multiple species and completely ameliorates glucose intolerance in diet-induced obese mice suggesting that it may be useful in the treatment of human type 2 diabetes and of other pathologies of metabolic syndrome.

Nothing to Disclose: JD, VB, BF, AH, H-JH, JL, JL, RM, IM, PW, KMO

P1-474

Resistance-Only Exercise-Induced Changes in Mitochondrial Function in Older Subjects with Type 2 Diabetes Mellitus.

PR Hungerford MD¹, RL Marcus PhD¹, O Addison DPT¹, A Wende PhD¹ and D McClain MD, PhD¹.

¹Univ of Utah Salt Lake City, UT.

Current practice guidelines recommend resistance exercise (RE) in the management of diabetes. Mitochondrial dysfunction in skeletal muscle has been linked to the development of insulin resistance and type 2 diabetes (T2D) and traditionally, only aerobic exercise has been thought to mitigate mitochondrial dysfunction. Recent evidence suggests that RE also improves mitochondrial function in older adults. We investigated the mitochondrial response to resistance-only exercise training that improved insulin sensitivity in older individuals with T2D. **Methods:** Seven (male=4, female=3) older (mean=68.4±5.9 yrs) individuals with T2D participated in a 16-week RE program, resulting in improved insulin sensitivity (31.6% ± 42.2 max insulin stimulated gluc uptake, p<0.03). Percutaneous biopsies of the vastus lateralis were performed at baseline, 24 hours (post 1), and one week following completion of the exercise program (post 2). Muscle samples were used for measurement of citrate synthase (CS) activity, a marker of aerobic capacity and mitochondrial function in skeletal muscle, by enzymatic assay. MRI scans of the thigh performed at all three time points determined mean muscle cross-sectional area (CSA). Paired t-tests compared CS activity and CSA at all time points. **Results:** CS activity increased between pre and post 1 (p<0.05) and there was no significant difference between pre and post 2 (p=0.30). Thigh muscle CSA increased between pre and post 1 and remained elevated at post 2 (p<0.05). See Table 1.

Table 1

| | Pre ± SEM | Post 1 ± SEM | Post 2 ± SEM |
|------------------------------------|--------------|---------------|----------------|
| citrate synthase (nmol/min/mg mwm) | 80.5 ± 9.6 | 103.6 ± 10.0* | 88.3 ± 8.4 |
| CSA (cm ²) | 108.5 ± 13.3 | 116.8 ± 15.5* | 116.0 ± 14.9** |

*significant difference between pre and post 1, **between pre and post 2, p<0.05

Discussion: Resistance-only exercise increased skeletal muscle CS activity, a marker of aerobic capacity and mitochondrial function, immediately but not 1 week after cessation of training in elders with T2D. Increased CS activity may contribute to improved mitochondrial function in this population. Not traditionally linked to mitochondrial adaptations in skeletal muscle, RE appears to enhance CS activity in older diabetics. The improvement is not solely linked to increased muscle size, as muscle size at 1-week post training remained significantly elevated, while CS activity did not. This suggests a need for frequent RE to maintain improvements in mitochondrial function in elders with diabetes.

Sources of Research Support: Utah BIRCWH Award NIH5K12HD043449-04, University of Utah Center on Aging Pilot Grant and University Research Foundation Seed Grant.

Nothing to Disclose: PRH, RLM, OA, AW, DM

P1-475

Lower Glycemic Load Diet Does Not Improve Insulin Sensitivity.

LL Goree MS, RD, LD¹, WM Granger PhD, RRT¹, P Chandler-Laney PhD¹, K Casazza PhD, RD¹, AC Ellis MPH, RD, LD¹ and BA Gower PhD¹.

¹The Univ of Alabama at Birmingham Birmingham, AL.

Reducing carbohydrate (CHO) content and glycemic load (GL) in one's diet lowers postprandial concentrations of circulating insulin. Whether the reduction in insulin that would occur with sustained consumption of such diets results in an improvement in insulin sensitivity has not been rigorously examined. **OBJECTIVE:** This study was conducted to test the hypothesis that a reduced CHO eucaloric diet (RedCHO) relative to a standard diet (STD) would result in a greater improvement in insulin sensitivity. **METHODS:** Sixty-nine healthy overweight (Body Mass Index = 25.1 - 46.4 kg/m²) men and women were provided with all food for 8 weeks at an energy level calculated to maintain body weight. Participants received either a STD diet (n = 29; 55% calories CHO, 18% calories protein, 27% calories fat) or a RedCHO diet (n = 40; 43% calories CHO, 18% calories protein, 39% calories fat). The RedCHO diet contained foods with a relatively low GL (≤ 45 points/1000 calories). Insulin sensitivity was assessed at baseline and after completion of the 8 week intervention using both an intravenous glucose tolerance test (IVGTT) and a liquid mixed macronutrient meal test (LMMT), administered on separate days. For both tests, mathematical modeling of serum insulin and glucose data was used to generate an insulin sensitivity index. **RESULTS:** Paired t-test indicated no change in insulin sensitivity, as assessed with both IVGTT and LMMT methods, after 8 weeks of consuming either the STD or RedCHO diets. Similarly, ANCOVA indicated no effect of diet on 8 week insulin sensitivity, as assessed with both IVGTT and LMMT methods, after covarying for baseline insulin sensitivity. Results did not differ if gender and ethnicity were included as covariates. Sub-group analysis indicated no interaction with baseline fasting insulin or 30-minute insulin status, as assessed with LMMT method. Insulin sensitivity from the IVGTT was highly correlated with that from the LMMT ($r = 0.64$ at baseline, and $r = 0.74$ at 8 week; $P < 0.0001$ for both). Both insulin sensitivity measures indicated a high degree of repeatability between the baseline and 8 week measures ($r = 0.78$, $P < 0.0001$ for the IVGTT and $r = 0.83$, $P < 0.0001$ for the LMMT). **CONCLUSION:** Using robust methodology, we have shown that 8 weeks of a eucaloric 43% CHO, low GL diet does not improve insulin sensitivity.

Sources of Research Support: Center for Clinical and Translational Science (CCTS) UL1RR025777; P30-DK56336; Diabetes Research and Training Center (DRTC) P60DK079626; NIDDK R01DK67538.

Nothing to Disclose: LLG, WMG, PC-L, KC, ACE, BAG

P1-476

The Effects of Nicotine on Visceral Fat Lipolysis Leading to Hepatic Steatosis and Insulin Resistance.

LA Garcia¹, SA Seasholtz¹, Y Liu M.D. PhD¹, K Lutfy PhD^{1,2}, M Mangubat¹ and TC Friedman¹.

¹Charles Drew Univ of Med and Sci Los Angeles, CA and ²Western Univ of Hlth Scis Pomona, CA.

Nicotine use as well as obesity causes a significant increase in cardiovascular mortality. As smokers however have a lower BMI than non-smokers, it is expected that smoking-induced weight loss should protect against cardiovascular disease, but this is not the case. This contradiction is supported by studies showing smokers are more insulin resistance and have increased rates of diabetes and dyslipidemia. In an effort to understand the mechanisms for the effect of nicotine on insulin resistance and adipose metabolism and localization, 4 groups of adult C57/BL/6 mice were given different treatments of nicotine injections (0.5 mg/kg, 1.5 mg/kg, or an escalating dose designed to avoid tolerance) or vehicle injections, daily, for 8 weeks. In addition to the different nicotine treated groups, the mice were also given either a high fat diet or normal rodent chow to determine the influence or difference diet has in conjunction with nicotine. Our results show a high degree of hepatic steatosis (as measured by oil red staining) in nicotine-treated mice on high fat diet compared to vehicle-treated mice on the same diet. Nicotine led to decreased abdominal body fat (as measured by DXA scan) and lower epididymal fat pad weight compared to the control, especially in the high-fat diet mice despite no significant difference in food intake between the two groups. I proposed that nicotine leads to intra-abdominal fat lipolysis that results in hepatic fat deposition and insulin resistance. Although one would expect that the intra-abdominal fat lipolysis would be beneficial, the hepatic fat deposition, in fact, may be detrimental and may contribute to the high rate of insulin resistance, diabetes and cardiovascular disease seen in smokers.

Nothing to Disclose: LAG, SAS, YL, KL, MM, TCF

P1-477

PPAR δ Activation and Myostatin Inhibition Exert Distinct yet Complimentary Effects on the Metabolic Profile of *ob/ob* Mice.

Barbara L Bernardo¹, Timothy S Wachtmann¹, Patricia G Cosgrove¹, Andrew M Kuhn¹, Alan C Opsahl¹, Kyle M Judkins¹, Thomas B Freeman¹, John R Hadcock¹ and Nathan K LeBrasseur¹.

¹Pfizer Inc Groton, CT.

Pharmacologic and genetic interventions for T2DM have in part aimed to mimic exercise given its robust effects on systemic metabolism. Here we have compared the independent and combined effects of the PPAR δ agonist and putative endurance training mimetic, GW501516, and a myostatin antibody and potential resistance training mimetic, PF-879, on the metabolic profile of the *ob/ob* mouse. GW501516 significantly attenuated body weight and fat mass accumulation, and increased the expression of genes of oxidative metabolism in skeletal muscle and liver. In contrast, PF-879 increased body weight by robustly stimulating muscle growth, and modestly affected the expression of genes involved in insulin signaling and glucose metabolism in skeletal muscle. Despite their differences, both GW501516 and PF-879 significantly improved glucose homeostasis and tolerance in the *ob/ob* mice. GW501516 more effectively improved the HbA1c and lipid profile, and PF-879 uniquely increased energy expenditure, exercise capacity and adiponectin levels. When combined, the independent effects of GW501516 and/or PF-879 on adiposity, HbA1c, triglycerides and exercise capacity were enhanced. In sum, the data confirm that GW501516 improves metabolic dysfunction, and for the first time demonstrate that postnatal inhibition of myostatin not only stimulates muscle growth similar to resistance training, but improves multiple aspects of whole-body metabolism.

Nothing to Disclose: BLB, TSW, PGC, AMK, ACO, KMJ, TBF, JRH, NKL

P1-478

Exostosin 2: A Replicated Quantitative Trait Locus for Insulin Clearance.

MO Goodarzi¹, ND Palmer², D Stefanovski³, KD Taylor¹, MR Jones¹, J Cui¹, X Guo¹, YDI Chen¹, TA Buchanan³, LJ Raffel¹, LE Wagenknecht², SS Rich⁴, AJG Hanley⁵, DW Bowden², RN Bergman³ and JI Rotter¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA ; ²Cedars-Sinai Med Ctr Los Angeles, CA ; ³Wake Forest Univ Sch of Med Winston-Salem, NC ; ⁴Univ of Southern California Keck Sch of Med Los Angeles, CA ; ⁵Univ of Southern California Keck Sch of Med Los Angeles, CA ; ⁶Wake Forest Univ Sch of Med Winston-Salem, NC ; ⁷Univ of Virginia Charlottesville, VA and ⁸Univ of Toronto Toronto, Canada.

While insulin resistance and insulin secretion are well-recognized to be deranged in type 2 diabetes (T2DM), the role of the metabolic clearance rate of insulin (MCRI) is less understood. Most of the susceptibility loci identified for T2DM to date are thought to act by modifying insulin secretion. Whether any of the T2DM loci affect MCRI is currently unknown. In the Insulin Resistance Atherosclerosis Family Study (IRAS-FS), we previously genotyped 17 SNPs from 11 loci that had arisen from the first wave of genome-wide association studies (GWAS) of T2DM and found association of a limited number of loci for measures of insulin secretion [acute insulin response to glucose (AIRg) and disposition index (DI), derived from the frequently-sampled IV glucose tolerance test (FSIGT)] (1). We report here on a subsequent examination of those 17 variants for association with MCRI, derived from the FSIGT by fitting the insulin profile after exogenous injection (at 20 min) to a first-order exponential decay curve (2). Three SNPs in exostosin 2 (*EXT2*) (rs1113132, rs11037909, rs3740878), that were associated with T2DM in a GWAS (3) but not widely replicated, were associated with MCRI in the IRAS-FS Hispanics (1268 subjects) ($P=0.0004$, 0.0004 , 0.0017 , respectively). We then sought to replicate these findings in a second cohort of Hispanics, the Mexican American Hypertension-Insulin Resistance (HTN-IR) cohort (939 subjects), wherein MCRI was quantified from the steady-state insulin level during euglycemic hyperinsulinemic clamps in 739 subjects. In HTN-IR, the 3 *EXT2* SNPs were also associated with MCRI ($P=0.0037$, 0.0068 , 0.0042), with the same direction of effect as in IRAS-FS. In the GWAS (3), the major alleles of these 3 SNPs were the diabetogenic alleles. In our cohorts, the major alleles were associated with increased MCRI. This suggests a mechanism whereby an inherited increase in insulin clearance might predispose to T2DM by reducing endogenous insulin levels, possibly leading to insulin resistance. How *EXT2*, which is mutated in hereditary multiple exostoses and affects cell proliferation and hedgehog signaling, might affect MCRI is unknown. Nevertheless, these exciting results implicate MCRI in the genetic pathogenesis of T2DM, and present a replicated quantitative trait locus for insulin clearance.

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Sources of Research Support: NIH grant R01-DK079888 (to MOG); NIH grant M01-RR000425 (General Clinical Research Center Grant at CSMC); Winnick Clinical Scholars Award (to MOG).

Nothing to Disclose: MOG, NDP, DS, KDT, MRJ, JC, XG, YDIC, TAB, LJR, LEW, SSR, AJGH, DWB, RNB, JIR

P1-479

Stimulation of Insulin Secretion by a Novel Small Molecule Glucagon-Like Peptide-1 Receptor Agonist in Rodent and Human Islets.

KW Sloop¹, FS Willard¹, MB Brenner¹, J Ficorilli¹, K Valasek¹, AD Showalter¹, T Farb¹, JXC Cao¹, MJ Tebbe¹ and MJ Coghlan¹.

¹Lilly Res Labs, Eli Lilly and Co Indianapolis, IN.

Clinical studies show replacement therapy with metabolically stable GLP-1 mimetics improves management of type 2 diabetes mellitus (T2DM), resulting in reduced fasting hyperglycemia, sustained lowering of glycosylated hemoglobin A1c (HbA_{1c}), and often reduced body weight. Unfortunately, GLP-1 analogues are peptides requiring administration by subcutaneous injection. We undertook screening strategies to identify small organic molecules that may be more amenable to developing orally active agents. Compound screening using HEK293 cells stably expressing the human GLP-1 receptor and ex vivo insulin secretion assays using rodent and human pancreatic islets were used to discover a novel class of low molecular weight GLP-1 receptor agonists. These molecules induce GLP-1 receptor-mediated cAMP signaling and stimulate glucose-dependent insulin secretion. In mechanism studies, the compounds activate GLP-1 receptor signaling both alone or in an additive fashion when combined with the endogenous GLP-1 peptide; however, they do not compete with radiolabelled GLP-1 in receptor binding assays. Further, perfusion assays with human islets isolated from a donor with T2DM show near-normalization of insulin secretion upon compound treatment. Molecules possessing these characteristics may offer therapeutic advantage due to their ability to act alone or in combination with GLP-1.

Disclosures: KWS: Employee, Lilly USA, LLC. FSW: Employee, Lilly USA, LLC. MBB: Employee, Pfizer, Inc. JF: Employee, Lilly USA, LLC. KV: Employee, Lilly USA, LLC. TF: Employee, Lilly USA, LLC. JXCC: Employee, Lilly USA, LLC. MJT: Employee, Lilly USA, LLC. MJC: Employee, Lilly USA, LLC.

Nothing to Disclose: ADS

P1-480

Fyn Deficient Mice Are Protected Against High Fat Diet Induced Insulin Resistance and Adipose Tissue Inflammation Despite Low Levels of Regulatory T Cells.

TA Lee MD^{1,2}, H Kwon PhD¹, M Vatish MD, PhD^{1,3}, E Yamada MD, PhD¹, JE Pessin PhD¹ and CC Bastie PhD¹.

¹Albert Einstein Coll of Med Bronx, NY ; ²Children's Hosp at Montefiore, Albert Einstein Coll of Med Bronx, NY and ³Warwick Med Sch, Univ of Warwick Coventry, UK.

Chronic low-grade inflammation is an important factor associated with obesity-related disorders such as insulin resistance and type 2 diabetes. Recent studies suggest that recruitment of CD8⁺T cells followed by macrophage infiltration into the adipose tissue (AT) is responsible for this chronic inflammatory state. Moreover, low levels of Foxp3⁺ CD4⁺ regulatory T cells are associated with local inflammation within AT. Fyn tyrosine kinase is involved in mediating inflammatory responses, in part by altering CD4⁺ T cell cytokine production. In addition, Fyn is involved in insulin signaling as chow diet fed Fyn null mice (FynKO) display decreased adiposity and increased insulin sensitivity. Expression of the anti-inflammatory cytokine IL-10 was dramatically decreased in the AT of FynKO maintained on chow diet compared to the AT of wild type (WT) mice. IL-10 levels also remained lower in the AT of diet-induced obese (DIO) FynKO mice, secondary to a reduction of the Foxp3⁺CD25⁺CD4⁺ regulatory T cell population. In addition, the CD8⁺ T cell fraction was similar in FynKO and WT mice maintained on a high fat diet. However, the glucose clearance was greater and fasting glucose and insulin levels lower in DIO FynKO compared to DIO WT mice. Moreover, whole body glucose uptake was higher in the DIO FynKO during euglycemic-hyperinsulinemic-clamps, confirming that FynKO were more insulin sensitive compared to DIO WT mice. The AT inflammatory (F4/80⁺CD11c⁺) macrophage fraction was reduced and CCL2 and TNF α expression levels were decreased in the DIO FynKO compared to WT mice. Together these findings suggest that FynKO mice do not develop diet-induced insulin resistance despite low levels of Foxp3⁺CD25⁺CD4⁺ regulatory T cells and that AT inflammation is reduced due decreased macrophage activation. These data define a role for Fyn kinase as a regulator of macrophage activation in the context of insulin resistance and suggest that Fyn might be a target to treat the inflammatory component of metabolic disorders.

Nothing to Disclose: TAL, HK, MV, EY, JEP, CCB

P1-481

The Role of Ventromedial Hypothalamic Glucose-Sensing Neurons during Glucoprivation.

E Richardson BSc¹, SS Hussain MA, MB BChir, MRCP¹, JR Counsell BSc¹, E Leavy BSc¹, GA Bewick BSc, PhD¹, SR Bloom MA MD FRCP FMedSci¹ and JV Gardiner BSc, PhD¹.

¹Imperial Coll London, UK.

Hypoglycaemia and hypoglycaemia unawareness severely limit the optimal management of diabetes mellitus and cause recurrent morbidity and even mortality in intensively controlled patients. Altered hypothalamic glucose-sensing has been implicated in the development of defective counter regulatory responses to insulin induced hypoglycaemia and hypoglycaemic unawareness. This change in hypothalamic glucose-sensing has been attributed, at least in part, to an increase in glucokinase activity in the ventromedial hypothalamus (VMH) following insulin induced hypoglycaemia. An increase in VMH glucokinase is also noted in fasting rats. This suggests that VMH glucokinase may have an important role in mediating physiological responses that restore normoglycaemia during glucoprivation.

To test this hypothesis we stereotactically injected recombinant adeno-associated virus (rAAV) encoding glucokinase (n=11) or GFP (n=9, controls) into the VMH of adult male Wistar rats. Glucokinase expression in the VMH was confirmed using *in situ* hybridisation. We tested the role of glucokinase in gradual-onset hypoglycaemia during fasting. Blood glucose was measured, in mmol/l, in the fed state and following a 24hr or 48hr fast. There was no significant difference in blood glucose levels, at baseline (GFP: 5.38 ± 0.38 vs Glucokinase: 5.56 ± 0.48 , $p = 0.39$), 24 hours (GFP: 3.88 ± 0.28 vs Glucokinase 3.92 ± 0.66 , $p = 0.83$) or 48 hours (GFP: 3.87 ± 0.62 vs Glucokinase: 3.74 ± 0.61 , $p = 0.64$). There was no significant difference in body weight (GK $471g \pm 32$ vs GFP: $491g \pm 37$) or weight loss during the prolonged fast ($39.6g \pm 6.2$ vs $39.4g \pm 5.5$).

Altering glucokinase activity in the VMH, using adeno-associated viral gene transfer, offers an attractive and novel method to study the importance of VMH glucose sensing on responses to hypoglycaemia. Our initial results suggest that an increase in VMH glucokinase activity does not affect glucose homeostasis in response to gradual onset hypoglycaemia induced by fasting. Further work is needed to assess the role of VMH glucose sensing in acute glucoprivation, as observed in insulin induced hypoglycaemia.

Sources of Research Support: Integrative Mammalian Biology (IMB) Capacity Building Award and funding from the NIHR Biomedical Research Centre Funding Scheme.

Nothing to Disclose: ER, SSH, JRC, EL, GAB, SRB, JVG

P1-482

Interference with Glucose Metabolism or Inhibition of CtBP Increases Adiposity through a Serotonin-Dependent Mechanism.

EI Manerakis BSc¹, IJ Kurland MD PhD², B Vaitheesvaran PhD², G Palacios PhD² and CV Mobbs PhD¹.

¹Mount Sinai Sch of Med New York, NY and ²Albert Einstein Coll of Med Bronx, NY.

To clarify mechanisms by which glucose signaling in neuroendocrine systems regulate adiposity, we examined the effect of 2-deoxyglucose (2DG), which produces glucopenia and induces pro-obese phenotypes in mammals, on adiposity in *C. elegans*. After 7 days, worms exposed to 2DG exhibited a 2-fold increase in adiposity, as quantified by Nile Red fluorescent dye, accompanied by reduced pharyngeal pump rate, a marker for feeding behavior. Corroborating the Nile Red quantification, 2DG also increased total triacylglyceride, diacylglyceride, and monoacylglyceride by about 2-fold, as assessed by thin layer chromatography. The induction of adiposity by 2DG was completely blocked by inhibition of the transcription factor SREBP, which also regulates adiposity in mammals. Furthermore, the induction of adiposity by 2DG was completely blocked by addition of serotonin in the media, corroborating that the increased adiposity is mediated by neuroendocrine mechanisms. Similarly, RNAi inhibition of C-terminal binding protein (CtBP), which mediates molecular effects of glucose via an NADH-binding domain, also caused increased adiposity reversed by serotonin. These studies corroborate that impairments in glucose sensing can lead to increased adiposity through a neuroendocrine mechanism, and provide a powerful model to examine mechanisms mediating these effects.

Nothing to Disclose: EIM, IJK, BV, GP, CVM

P1-483

Anorexia-Cachexia in Type 2 Diabetic (T2D) Goto-Kakizaki (GK) Rats: Role of Impaired Brainstem Thyrotropin-Releasing Hormone (TRH) Regulation of Vagal Function.

Ai Chen¹, Myungsoo Ko¹, Vay Liang W Go MD¹, Moon K Song PhD², Kaitlyn Liu PhD^{1,2} and Hong Yang MD, PhD^{1,2}.

¹UCLA Los Angeles, CA and ²VA, GLAHS Los Angeles, CA.

Background: TRH-containing terminals innervate dorsal vagal complex (DVC) and sympathetic motor nuclei. Fasting up-regulates brainstem TRH gene expression (1). Intracisternal (ic) injection of TRH analog induces vagal-mediated, ghrelin-dependent food intake (FI) (1). Hyperglycemia inhibits vagal activation by ic TRH analog (2). The GK rat is a non-obese polygenetic T2D model with sympathetic-over-vagal response to ic TRH (3). **Aim:** To study whether abnormal autonomic regulation by brainstem TRH influences FI in GK rats. **Methods and Results:** Glucose (mg%) was 117±4(W) and 257±13(GK) at the beginning of dark phase. The first 2h natural FI (g) was 3.34±0.33 and 0.58±0.24 in male Wistar and GK rats. FI (2h) after overnight fast was 4.49±0.24(W) and 0.79±0.24(GK), which increased glucose from 97±5 to 134±4(W) and 121±7 to 183±9(GK) in 1h. TRH analog ic-induced food intake was reduced by 55% in GK rats. Serum hormones (pg/ml) and brain Fos expression were measured in normally fed, 48h-fasted and fasted+2h refed [FI 4.13(W) and 3.93(GK)] groups. Ghrelin increased from 94±14 to 191±10 in Wistar but not GK rats after fasting while the leptin decrease was less significant in GK rats. Refeeding increased pancreatic polypeptide by 3-fold and gastric inhibitory polypeptide by 6.5-fold (W), both were lower in GK rats [PP1.8-fold, GIP3.3-fold]. Fasting-induced DVC Fos expression was decreased by 66%(NTS, 47±6 vs 138±4), 91%(DMV, 3±1 vs 37±3) and 49%(AP, 18±2 vs 36±2) in GK compared to Wistar rats. Refeeding abolished fasting-induced Fos-expression in the NTS but that in the DVC (24±2) and AP (19±1) kept in Wistar but not GK rats (DVC 0.4±0.3 and AP 4±1). The hypothalamic supraoptic nucleus (SO) and paraventricular nucleus (PVN) receive projections from brainstem TRH-innervated nuclei (4). Fasting-induced Fos in the SO was higher in GK rats (131±15 vs 87±9), which was reduced by refeeding [20±15(W) and 0.7±0.4(GK)]. Fos-induction in the magnocellular division of the PVN was only observed in GK rats (from 48±2 to 127±11) that was reversed by refeeding (44±6). Fasting induced similar brainstem TRH gene expression in both strains. Microinjection of adenovirus-based expression vector for TRH-R1 into the DVC significantly increased body weight (10-30 g) during 1-4 weeks in both Wistar and GK rats compared to control vector-injected rats. **Conclusion:** Reduced DVC neuronal response to TRH is responsible for impaired vagal function during starvation and food digestion in T2D GK rats.

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Sources of Research Support: VA Merit Award.

Nothing to Disclose: AC, MK, VLWG, MKS, KL, HY

P1-484

Prospective Changes in Glucocorticoid Metabolism Predict Alterations in Metabolic Phenotype.

J Gray¹, T McCarthy¹, SV Hughes BSc², BA Hughes BSc², PM Stewart MD² and JW Tomlinson MD PhD².

¹Queen Elizabeth Hosp Birmingham, UK and ²Univ of Birmingham Birmingham, UK.

Glucocorticoid (GC) production rates are elevated in obese, insulin resistant individuals. We and others have demonstrated decreased hepatic cortisol regeneration through reduced 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) activity that converts inactive cortisone to cortisol. In addition, there is enhanced cortisol clearance by A-ring reductases, (notably 5 α -reductase). We have argued that these changes drive the hypothalamo-pituitary-adrenal axis and represent a protective mechanism to decrease tissue specific cortisol exposure to preserve metabolic phenotype. On this background, we established the Birmingham Prospective Obesity, Diabetes and Steroid metabolism cohort (BPODS) to prospectively track changes in GC metabolism with metabolic phenotype. Individuals who are unable to decrease tissue specific cortisol exposure may be at greater metabolic risk.

114 obese patients (75 women, BMI>30kg/m²) were recruited and underwent anthropometric measurements, fasting blood tests, oral glucose tolerance test (OGTT), DXA body composition and 24h urine collections for steroid metabolite analysis (GC/MS). All investigations were repeated 12 months after enrolment.

Over the initial 12-month prospective follow-up period, mean change in weight was +1.1 \pm 0.6kg and BMI

+0.42 \pm 0.22kg/m². 36.7% of patients lost weight and 62.3% gained weight (unchanged in 1 patient). Mean change in glucose tolerance (AUC across OGTT) was -0.17 \pm 0.28mmol/L.min (41.4% deterioration, 58.6% improvement).

Individuals who decreased 11 β -HSD1 activity (urinary GC/MS analysis) lost weight (-2.0 \pm 2.5kg), however, in contrast, those with increased 11 β -HSD1 activity gained weight (+1.7 \pm 0.6kg, p<0.05). Independently, increasing trunk fat mass (DXA) was associated with worsening glucose tolerance (-1.2 \pm 0.5 vs. +0.13 \pm 0.33mmol/L.min, p<0.05) and increasing fasting insulin (-3.3 \pm 2.2 vs. +1.3 \pm 0.9pmol/L, p<0.05). Baseline fasting insulin was unrelated to initial or prospective changes in fat mass. However, those individuals with the highest fasting insulin at baseline, had the lowest incremental increase in fasting insulin at 12 months consistent with already maximal pancreatic beta cell drive (R=0.55, p<0.001). We have demonstrated that 11 β -HSD1 is able to predict individuals with increasing body weight. Furthermore, decreasing 11 β -HSD1 activity tracks with improvements in metabolic phenotype and endorses therapeutic inhibition as a valid treatment target

Sources of Research Support: Medical Research Council and Wellcome Trust.

Nothing to Disclose: JG, TM, SVH, BAH, PMS, JWT

P1-485

Induction of Insulin Resistance by Acute Fatty Acid Elevation Partially Recapitulates Exercise Defects Seen in Diabetes.

IE Schauer MD, PhD¹, LL Herlache MS¹, JG Regensteiner PhD¹ and JEB Reusch MD¹.

¹Univ of Colorado Denver Aurora, CO.

Background/objectives: Physical fitness correlates inversely with CV and all cause mortality. Defects in functional exercise capacity, manifested by decreased peak oxygen consumption (peak VO₂) and slowed VO₂ kinetics, have been demonstrated in individuals with type 2 diabetes (T2D) or insulin resistance (IR), but the mechanism remains unknown. Serum non-esterified fatty acid (NEFA) elevation by lipid/heparin infusion induces IR in healthy adults. We hypothesized that NEFA elevation comparable to that seen in diabetes may recapitulate the exercise defects of T2D. Targeting NEFA levels in diabetes could therefore potentially improve exercise capacity.

Methods: Seventeen lean adults (53% female, age 30±6 years) with normal fasting glucose, HbA1c, and glucose tolerance and no family history of T2D were studied. Insulin sensitivity was measured by hyperinsulinemic euglycemic clamp and exercise capacity by cycle ergometry with a breath-by-breath capable metabolic cart. Subjects were studied after 6 hours of liposyn/heparin infusion vs. saline infusion.

Results: Initial liposyn/heparin infusion resulted in NEFA levels of 2-3mM and the infusion rate was reduced for the remaining subjects resulting in a mean 6-hour NEFA level of 1.08±0.22mM. Insulin sensitivity was impaired by NEFA elevation (glucose infusion rate: 6.57±2.25 vs. 8.50±2.72, p=0.0002). Peak VO₂ was not affected overall or at the lower infusion rate (31.37±5.54 vs. 31.29±5.54, p=0.92), but VO₂ kinetics were significantly slowed (tau₂: 34.7±12 vs. 29.9±9 sec, p=0.023) suggesting a slower physiological adjustment to exercise.

Conclusions: Acute NEFA elevation in non-diabetic individuals induces IR and recapitulates the slowing of VO₂ kinetics seen in T2D. This finding may help explain the exercise abnormalities observed in T2D and suggest targeted treatment options.

Nothing to Disclose: IES, LLH, JGR, JEER

P1-486

Association between Expression of Receptor for Advanced Glycation End Products in Peripheral Blood Monocytes and Circulating Soluble Isoforms of the Receptor in Type 2 Diabetes.

KCB Tan MD¹, HL Tam MPhil¹, MAM Yuen MD¹, SWM Shiu BSC¹ and DJ Betteridge MD².

¹Univ of Hong Kong Hong Kong, Hong Kong and ²Royal Free & Univ Coll London Med Sch London, UK.

Objective: The receptor for advanced glycation end-products (RAGE) plays an important role in the pathogenesis of diabetic complications. The soluble forms of the receptor (sRAGE) lack the transmembrane and cytoplasmic domain of the full-length receptor, and in addition to being a potential biomarker of RAGE-mediated pathology, sRAGE can function as a decoy for RAGE ligands in experimental studies. We have examined whether there is any relationship between membrane bound full-length RAGE expression in peripheral blood monocytes (PBMCs) and circulating levels of sRAGE and esRAGE (a splice variant of sRAGE) in type 2 diabetic patients with poor glycemic control.

Methods: 53 diabetic patients and 52 age-matched healthy controls were recruited. Cell surface bound full-length RAGE expression in PBMCs was measured using flow cytometry. Serum sRAGE and esRAGE was assayed by ELISA.

Results: Mean HbA1c of the diabetic patients was 9.74%. RAGE expression in PBMCs was significantly higher in diabetic patients than controls (3.12 ± 1.02 M.F.I. vs 2.06 ± 0.51 respectively, $p < 0.010$) and there was a correlation between HbA1c and monocyte cell surface full-length RAGE in the diabetic patients ($r = 0.31$, $p = 0.03$). In contrast to the increased level of RAGE expression in PBMCs, serum concentration of sRAGE (573.3 pg/ml [375.7 - 754.3] interquartile range vs 608.1 [405.3 - 940.8], $p < 0.05$) and esRAGE (241.8 pg/ml [154.6 - 356.6] vs 286.5 [202.6 - 390.0], $p < 0.05$) were significantly decreased in diabetic patients. RAGE expression in PBMCs inversely correlated with serum log(sRAGE) ($r = -0.34$, $p = 0.01$) but not with esRAGE.

Conclusions: Poorly controlled type 2 diabetic patients have increased RAGE expression in PBMCs and reduced serum sRAGE and esRAGE levels, thus rendering these patients more susceptible to develop vascular damage from RAGE activation.

Sources of Research Support: Hong Kong Research Grants Council Research Fund (HKU 775708M).

Nothing to Disclose: KCBT, HLT, MAMY, SWMS, DJB

P1-487

Saxagliptin Treatment Reduces sCD40 Levels and Increases Endothelial Nitric Oxide Bioavailability in Obese, Insulin-Resistant Rats.

PR Mason Ph.D.^{1,2}, R Kubant³, AM Jacoby³, RF Jacob Ph.D.², MF Walter⁴, A Bellamine Ph.D.⁵, Y Mizuno^{1,2} and T Malinski³.

¹Harvard Med Sch Boston, MA ; ²Elucida Res LLC Beverly, MA ; ³Ohio Univ Athens, OH ; ⁴Natl Inst of Hlth Bethesda, MD and ⁵Bristol-Myers Squibb Princeton, NJ.

Background: Atherosclerosis is related to inflammation, oxidative stress and endothelial cell (EC) dysfunction. These disease mechanisms are associated with enhanced sCD40 expression and loss of EC nitric oxide (NO) synthase activity. In diabetes, these processes may be reversed with a dipeptidyl peptidase-4 (DPP4) inhibitor through enhanced incretin activity and improved glycemic control. In this study, we tested the effects of the DPP4 inhibitor, saxagliptin, on sCD40 levels and EC function in obese, insulin-resistant Zucker rats.

Methods: Obese, insulin-resistant Zucker rats were maintained on a high-fat diet and treated with 10 mg/kg/day saxagliptin or vehicle for up to 8 weeks. Following treatment, plasma samples were obtained for quantitation of sCD40 levels using ELISA. NO and peroxynitrite (ONOO⁻) release kinetics were directly measured *ex vivo* in aortic and glomerular ECs using amperometric approaches. Changes in inflammatory and EC function were correlated with metabolic parameters.

Results: Fasting glucose levels were significantly elevated in obese animals (136 ± 9 mg/dL) as compared to controls (116 ± 4 mg/dL) following 8 weeks exposure to a high-fat diet. In vehicle treated obese animals, sCD40 levels were 300 ± 206 pg/mL. In obese rats, NO release from aortic and glomerular ECs was reduced by 22% and 31%, respectively. Saxagliptin treatment reduced blood glucose to 115 ± 6 mg/dL while normalizing the response to a glucose challenge. Treatment was also associated with about 10-fold decrease in sCD40 levels (21.6 ± 21.8 pg/mL, *p* < 0.001). Saxagliptin improved aortic and glomerular EC function as evidenced by an 18% and 31% increase in NO release, respectively, while reducing nitrooxidative stress. Saxagliptin also increased the NO/ONOO⁻ ratio, an indicator of NO synthase (eNOS) coupling efficiency, by 40% and 64% in aortic and glomerular ECs, respectively. The EC benefits of DPP4 inhibition were observed before reductions in fasting blood glucose levels.

Conclusion: Obese, insulin-resistant rats had elevated levels of inflammation, oxidative stress and decreased NO bioavailability. Saxagliptin treatment reversed these effects as evidenced by pronounced reductions in sCD40 levels and nitrooxidative stress, concomitant with enhanced NO synthase activity in both aortic and glomerular endothelium. These data indicate that saxagliptin provides direct vascular benefits in advance of normalizing blood glucose levels in obese, insulin-resistant rats.

Disclosures: PRM: Collaborator, Bristol-Myers Squibb. AB: Employee, Bristol-Myers Squibb.

Nothing to Disclose: RK, AMJ, RFJ, MFW, YM, TM

P1-488

Peptide Inhibitors of Albuminuria.

B.K. Singh BS¹, A. Singh PhD² and D. Mascarenhas PhD¹.

¹Mayflower Organization for Res & Education Sunnyvale, CA and ²Emory Univ Atlanta, GA.

BACKGROUND: Signaling events associated with diabetic nephropathy are not well understood. Triangulation of events triggered by unrelated bioactive peptides nephrilin and anephril, both of which inhibit albuminuria in diabetic mice, could reveal a common subset of events associated with albuminuria.

METHODS: db/db mice received 20ug/day anephril or nephrilin for seven weeks. Streptozotocin-treated DBA/2J mice received nephrilin for 26 days. In both studies urine albumin and tissue proteins were measured by ELISA.

RESULTS: Both peptides reduced albuminuria in db/db mice compared to saline-treated animals without affecting plasma glucose or insulin. In kidney tissues the immunoreactivities of IRS2, phospho-PKC and SGK1 were reduced. Nephrilin but not anephril lowered elevated SGK1 in spleens of db/db mice. In streptozotocin-pretreated DBA/2J mice, nephrilin reduced albuminuria and the accumulation of nuclear Rictor, IRS2 and phospho-PKC in kidney. In cultured kidney cells nephrilin disrupted the association of Rictor with IRS proteins and nuclear compartmentalization. Histopathological examination of tissues from mice treated with 20 mg/kg nephrilin daily for 26 days showed no significant pathology compared to saline-treated controls.

CONCLUSIONS: We define a subset of signaling markers most closely associated with albuminuria. mTORC2::IRS complexes may play a role in the nuclear accumulation, and possible downstream transcriptional effects, of phospho-PKC. The activity and apparent safety of nephrilin in rodents suggest a novel intervention strategy for diabetic kidney disease.

Nothing to Disclose: BKS, AS, DM

P1-489

CEACAM1 Repression as an Obligate Step in the Development of Diet-Induced Insulin Resistance.

TA Bowman DPM¹, QY Al-Share PhD¹, AM DeAngelis PhD¹, S Ghosh¹, PR Patel¹ and SM Najjar PhD¹.

¹Univ of Toledo Coll of Med Toledo, OH.

Impaired hepatic insulin clearance causes hyperinsulinemia and secondary insulin resistance. It is unclear whether hyperinsulinemia contributes to worsening insulin resistance by down-regulating insulin receptors and promoting hepatic lipid accumulation. We show that an early event associated with high fat feeding is repression of CEACAM1, leading to impaired insulin clearance prior to the development of a pro-inflammatory state. Transgenic over-expression of CEACAM1 in liver prevents hyperinsulinemia and protects against visceral obesity and the metabolic response to high-fat intake for 3 months, producing reduced serum and hepatic triglyceride, improved insulin clearance, improved glucose tolerance in response to glucose challenge, improved insulin tolerance, and improved glucose uptake in isolated soleus muscle. We propose that hyperinsulinemia resulting from impaired insulin clearance constitutes an early mechanism of diet-induced obesity and insulin resistance, as opposed to a consequence thereof, and that this is mediated by an early and fully preventable reduction in hepatic CEACAM1 level.

Sources of Research Support: NIH grants DK054254 and DK083850 to SMN.

Nothing to Disclose: TAB, QYA-S, AMD, SG, PRP, SMN

P1-490

Free Testosterone (FT) and Sex Hormone Binding Globulin (SHBG) Are Related to Insulin Resistance (IR) and Diabetes (DM) among Men with or at Risk for HIV Infection.

AK Monroe MD, MSPH¹, AS Dobs MD, MHS¹, X Xu MS¹, FJ Palella MD², LA Kingsley DrPH³, WS Post MD, MS¹, MD Witt MD⁴, S Bhasin MD⁵ and TT Brown MD, PhD¹.

¹The Johns Hopkins Univ Sch of Med Baltimore, MD ; ²Feinberg Sch of Med, Northwestern Univ Chicago, IL ; ³Univ of Pittsburgh Graduate Sch of Public Hlth Pittsburgh, PA ; ⁴David Geffen Sch of Med at UCLA Torrance, CA and ⁵Boston Univ Sch of Medicine Boston, MA.

Background

DM is a common co-morbidity in HIV disease. Low testosterone (T) and lower SHBG levels are associated with IR and DM in the general population, but the impact of sex hormones on DM in HIV disease is unknown. We hypothesized that lower T and higher SHBG would be associated with IR and DM in HIV+ men.

Methods

We analyzed data from the Multi-Center AIDS Cohort Study (MACS), which enrolled 6,973 HIV+ and HIV- men who have sex with men starting in 1984. The MACS CV Substudy is a nested cohort of men with no prior CVD (n=947, 856 with hormone data). DM was defined as fasting glucose (FG) ≥ 126 , self-reported DM or use of DM medications; HOMA-IR was calculated from FG and fasting insulin. T and SHBG were measured from archived serum by LC-tandem MS and RIA, respectively. FT was calculated. Multivariable logistic regression, adjusted for HIV status, age, race (black v other), clinic site, and BMI was used to examine the relationship between sex hormones and DM, and adjusted multivariable linear regression was used to examine the relationship between sex hormones and IR.

Results

Compared to the HIV- men (n=318), HIV+ men (n=538) were younger (48.8 v 52.6 yrs, $p < 0.0001$), were more likely to be black (33.5% v 23.9%, $p = 0.003$), and had a lower BMI (25.6 v 26.8 kg/m², $p < 0.001$). Of the HIV+ men, 91.5% were HAART-experienced. HIV+ men had a significantly lower adjusted mean log FT than HIV- men (4.46 v 4.59 ng/dL, $p < 0.01$). Adjusted mean log SHBG was significantly higher in HIV+ men (4.35 v 3.88 mg/dL, $p < 0.01$). DM prevalence was 11.4% in HIV+ men and 8.0% in HIV- men ($p = 0.16$) and mean HOMA-IR was 4.73 in HIV+ men and 3.90 in HIV- men ($p < 0.01$). In adjusted analysis, log T was inversely associated with DM (OR=0.58, 95% CI: 0.34, 0.99); log FT was inversely associated but not significant ($p = 0.37$). Log SHBG was inversely associated with DM (OR 0.50, 95% CI 0.29, 0.88). Log T, log FT and log SHBG were inversely related to IR in adjusted analysis ($p < 0.05$ for all). HIV+ status was significantly associated with DM and IR in all adjusted models. Among HIV+ men, there was no difference in current or nadir CD4 distribution between the DM+/DM- groups and there was no significant correlation between current or nadir CD4 and IR.

Discussion

Despite having higher SHBG concentrations, which confers a protective effect, HIV+ men are more insulin resistant and have a higher prevalence of DM compared to HIV- control men. Lower FT also contributes to increased IR in HIV+ men.

Sources of Research Support: UO1-AI-35042, 5-MO1-RR-00722 (GCRC), M01 RR00425 (GCRC-Harbor-UCLA), UO1-AI-35043, UO1-AI-37984, UO1-AI-35039, UO1-AI-35040, UO1-AI-37613, UO1-AI-35041. NIH (NCAAM) 5K23AT2862(TTB). 2 T32 HP 10025-15-00.

Nothing to Disclose: AKM, ASD, XX, FJP, LAK, WSP, MDW, SB, TTB

P1-491

Elevated Glucagon Levels in Obese Pre-Diabetic Hyperinsulinemic Patients.

Naji Torbay¹ and Rita Nawar².

¹American Univ of Beirut Beirut, Lebanon and ²The Weight Care Clin-Dubai Healthcare City Dubai, United Arab Emirates.

Introduction: Fasting Glucagon (FG) levels are documented to be elevated in Type 2 diabetics. The objective of the present study is to investigate Glucagon levels in the pre-diabetic state.

Methodology: 113 patients coming for weight reduction were randomly selected for determination of Fasting Glucagon. Of these 74 were further selected by inclusion criteria (healthy, on no medications). Patients were divided into 3 groups according to Glucagon levels: Group1 with FG <100pg/ml (n=29), Group2 had FG ≥100 and <200pg/ml (n=31), Group3 with FG ≥200pg/ml (n=14). The following measurements were obtained: weight, age, Insulin, Glucose, total Cholesterol, Triglycerides, SGOT, SGPT, and BMI and HOMA-IR were calculated.

Results: The 3 groups were compared using non-parametric statistical methods due to small sample size. 18.9% of these patients had frank hyperglucagonemia, with FG levels > 200pg/ml. Variables that were significantly different between group 1 and 3 were Fasting Insulin levels (11.70±11.00 vs. 14.55±6.49, p=0.011), and HOMA 2 index (2.78±3.16 vs. 3.28±1.26, p=0.018).

Frequencies & descriptives of Glucagon Group1 & Group3

| Variable | Glucagon <100 (n=29) | Glucagon ≥ 200 | P-value | |
|---------------------------|----------------------|----------------|----------|----|
| Age (yrs) | 40.87±13.08 | 37.36±14.19 | NS | |
| Gender, n (%) | Male | 10 (34.5) | 6 (42.9) | NS |
| | Female | 19 (65.5) | 8 (57.1) | NS |
| Weight (kg) | 85.20±15.61 | 97.86±24.42 | NS | |
| BMI (kg/m ²) | 30.42±4.61 | 32.02±4.40 | NS | |
| Fasting Glucagon (pg/ml) | 66.46±22.73 | 354.36±173.84 | <0.001 | |
| Fasting Insulin (uUI/ml) | 11.70±11.00 | 14.55±6.49 | 0.011 | |
| Fasting Glucose (mg/dl) | 95.52±8.98 | 92.86±9.65 | NS | |
| HOMA-IR | 2.78±3.16 | 3.28±1.26 | 0.018 | |
| Total cholesterol (mg/dl) | 197.07±27.85 | 190.85±41.03 | NS | |
| Triglycerides (mg/dl) | 139.38±95.60 | 138.38±65.60 | NS | |
| SGOT (IU/l) | 23.79±11.53 | 20.18±4.75 | NS | |
| SGPT (IU/l) | 31.76±23.63 | 3.67±20.20 | NS | |

All values are means ± SD unless otherwise indicated. NS: not significant.. P-values between groups by non-parametric tests.

Conclusion: This study demonstrates that Glucagon levels may be elevated even in the pre-diabetic state, and that it is associated to hyperinsulinemia and decreased insulin sensitivity. The clinical significance of these findings is yet to be elucidated. Further studies that measure GLP-1 and DPP-IV in this subcategory of obese non-diabetic hyperinsulinemic subjects need to be performed in order to shed light on the cause of this hyperglucagonemia.

Nothing to Disclose: NT, RN

P1-492

Do Low Vitamin D Levels Increase Risk of Insulin Resistance in Native American Youth?.

JM Erickson RN, CDE¹, JL Beals MD¹, E Ogle¹, PA Nsiah-Kumi MD, MPH¹, M Whiting², F Yu PhD¹, R Broken Leg RN², C Brushbreaker², CD Borgeson BS¹ and JL Larsen MD¹.

¹Univ of Nebraska Med Ctr Omaha, NE ; ²Rosebud Diabetes Prevention Program Mission, SD and ³Univ of Nebraska Med Ctr Omaha, NE.

Background and Objective: Native Americans are among those with the highest incidence of type 2 diabetes among U.S. youth. We have evaluated potential factors contributing to diabetes risk, including the correlation of vitamin D with obesity and insulin resistance in a cohort of Native American youth. **Methods:** We screened Native American youth living in South Dakota (n=167), ages 5-18y, for evidence of insulin resistance, glucose intolerance, and related risks. We measured height and weight, calculated BMI%, and measured fasting glucose, insulin, lipids, and 25-hydroxy-vitamin D level (25VitD) by RIA (Diasorin®). Insulin resistance was assessed by homeostatic model of insulin resistance (HOMA-IR). Most of the screening was done in the summer months. For all values, mean \pm SE are shown. **Results:** Mean age of the cohort (n=167) was 10.7 ± 0.3 years. For 5-12 year olds, mean BMI% was $77.5\% \pm 1.8$ with mean HOMA-IR of 4.3 ± 0.6 (n=96). For 12-18 year olds, mean BMI% was 72.0 ± 3.3 and HOMA-IR was 5.9 ± 0.7 (n=71). Mean 25VitD was 18.3 ± 0.5 ng/ml in 5-11year olds (n=94) vs 16.2 ± 0.7 ng/ml in 12-18 year olds (n=67), with minimum goal 25VitD >30 ng/ml. 25VitD was negatively associated with both BMI% (p=0.008) and HOMA-IR (p<0.0001). Comparing 5-11 and 12-18 year olds, HOMA-IR was higher (p<0.0001) and vitamin D was lower (p=0.01) in the older age group. Females also had a higher HOMA-IR (p=0.00012) and lower 25VitD (p=0.01) compared to males. **Conclusions:** Vitamin D was low overall in Native American youth. As reported in many other populations, 25-hydroxy-vitamin D was negatively correlated with insulin resistance and BMI in Native American youth. While causes of insulin resistance and obesity are complex in all populations, this data suggests that low vitamin D could be a contributing factor to obesity and insulin resistance, and hence with diabetes risk, in Native American youth.

Sources of Research Support: (clinicaltrials.gov NCT00498030; supported by IHS and NIDDK #U26IHS300002).

Nothing to Disclose: JME, JLB, EO, PAN-K, MW, FY, RBL, CB, CDB, JLL

P1-493

Assessment of Vitamin D Status in a Population of Patients with Type-2 Diabetes Mellitus and Its Effect on Glucose Tolerance, Insulin Resistance and Well-Being.

IR Johnson MSc¹ and M Bell MB Bch BAO FRCPI².

¹Natl Univ of Ireland Galway, Ireland and ²Galway Univ Hosp Galway, Ireland.

Vitamin D (VD) is a natural food compound which can also be obtained from skin exposure to direct sunlight. It has been known for almost three decades that VD deficiency can influence pancreatic secretion of insulin.(1). More recently, VD deficiency has been shown to alter insulin synthesis and secretion in humans (2). A meta analysis has demonstrated the association between type-2 diabetes mellitus (T2DM) and VD deficiency (3). In the west of Ireland, deficiency in 25-Hydroxyvitamin D (25-OHD <50nmol/L) has previously been identified in 75.4% of females over 65 years admitted to an acute medical hospital (4). The effectiveness of using parenteral VD for replacement in VD deficiency has also been shown (5). In a cross-section of older adults, VD deficiency was associated with low mood and with impairment in two of four measures of cognitive performance (6).

The objectives of this study were: i) To establish the VD status of patients with T2DM attending an out-patient clinic in the West of Ireland ii) To investigate the effectiveness of VD supplementation in improving glycaemic control and insulin sensitivity in deficient patients iii) To assess the effects of VD status on well-being.

Eligible patients with T2DM not using Insulin or Incretin therapy and who attended the diabetes clinic in Galway University Hospital between June and September 2009 were identified, consented, and had fasting blood and urine samples taken. They were also given a Seasonal Pattern Assessment Questionnaire (SPAQ). Beta cell function (%B), insulin sensitivity (%S) and insulin resistance (IR) were calculated. Patients found to be VD deficient (25-hydroxyVD<50nmol/L) and who had no contraindications to VD supplementation, were offered a single intramuscular injection (cholecalciferol 300,000 IU). A total of 112 patients were recruited with a mean age 64 ± 11 years and 59% were male. 47% of patients were identified as having VD deficiency, of which 94% were given parenteral VD. No significant differences were found between groups for the SPAQ, biochemistry measures or calculated IR and %S. VD deficient patients were found to have greater %B (p=0.007). Conclusion: A large percentage of T2DM patients attending diabetes services at Galway University Hospital were found to be VD deficient. Ongoing follow-up due for completion by April 2010 will determine if providing VD supplementation can improve IR, %S, %B and well-being.

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(2) Gedik A & Akalin S, Diabetologia 1986; 29:142

(3) Pittas AG et al. J Clin Endocrinol Metab 2007; 92:2017

(4) DeLappe E et al., Eur J Clin Nutr 2006; 60:1009

(5) Nugent C et al., Ir J Med Sci 2009; Aug 28

(6) Wilkins CH et al., Am J Geriatr Psychiatry 2006; 14:1032

Sources of Research Support: Health Research Board.

Nothing to Disclose: IRJ, MB

P1-494

Gestational Diabetes Is Linked to Early Postpartum Metabolic Abnormalities: Utility of Assessment of Insulin Sensitivity and Beta-Cell Function by 3 Months after Delivery.

KE Elkind-Hirsch M.Sc,Ph.D.¹, DL Shaler B.S.¹, MS Paterson M.D.¹, BW. Ogden M.D.¹ and BL Schelin M.T.¹.

¹Woman's Hosp Baton Rouge, LA.

Objective: Given the high incidence of type 2 diabetes (DM2) and its comorbidities in women with former gestational diabetes (fGDM), the primary aim of our study was to assess metabolic disorder prevalence in these high-risk women in the early postpartum period.

Design: Ninety women having GDM in their index pregnancies were studied 6-12 weeks after delivery during the interval of April-December 2009. A 75-g oral glucose tolerance test (OGTT) was performed with insulin and glucose measured. Insulin sensitivity and beta-cell secretory capacity derived from fasting values (HOMA-IR) and glucose-stimulated measures (SI_{OGTT} and IGI/HOMA) were calculated. Cholesterol, HDL and LDL cholesterol, triglycerides, liver enzymes, TSH, blood pressure, body mass index (BMI), and body fat distribution (waist-hip ratio) were also assessed. Clinical and obstetric history was compared as well as demographics, variables at GDM diagnosis, metabolic control and results of the pregnancy.

Results: Seventy Caucasian (Cau), 18 African-American (AA) and 2 Hispanic fGDM women were studied. When OGTT glucose results were used, 18 (20%) fGDM women showed some form of impaired glucose tolerance. According to ADA criteria, 71 (80%) fGDM women were normal, 5 (5.55%) had impaired fasting glucose (IFG), 6 (6.7%) had impaired glucose tolerance (IGT), 5 (5.55%) had IFG/IGT, and 2 (2.2%) had DM2 within 3 months of delivery. Using HOMA-IR, 38 (42%) fGDM women had insulin resistance while SI_{OGTT} and IGI/HOMA identified 82 (91%) fGDM women with a postpartum metabolic abnormality. We also observed that 65 (72%) fGDM women had at least one lipid level abnormality. The rates of impaired glucose metabolism after delivery were racially similar but frank DM2 was found only in Cau fGDM women. Abnormal lipid profiles were more prevalent in Cau fGDM women (76%) compared with AA fGDM women (33%). Obesity was highly prevalent with 67 (74%) women having a prepregnancy BMI >25. AA fGDM women were significantly younger, had greater BMI, abdominal adiposity and parity compared to Cau fGDM women but weight gain retained after pregnancy was not racially disparate.

Conclusions: GDM is associated with an increased prevalence of postpartum cardiometabolic dysfunction. Women with fGDM are characterized by having more pronounced insulin resistance and inadequate insulin secretion which persists 3 months after delivery. Additionally, fGDM women have a high incidence of altered lipid profiles in the early postpartum period.

Sources of Research Support: Irene W. and C.B. Pennington Foundation. (ClinicalTrials.gov number, NCT00849849 [ClinicalTrials.gov]).

Nothing to Disclose: KEE-H, DLS, MSP, BWO, BLS

P1-495

There Is No Difference between Sucrose and High Fructose Corn Syrup in Their Propensity To Increase Weight or Induce Insulin Resistance.

J Lowndes MA¹, D Kawiecki MS¹, TJ Angelopoulos PhD, MPH¹, K Melanson PhD² and JM Rippe MD¹.

¹Rippe Lifestyle Inst Celebration, FL and ²Univ of Rhode Island Kingston, RI.

Introduction: It has been suggested that excess consumption of fructose may be a causative factor in the increase in overweight and obesity and the development of associated metabolic complications. As the primary sources of fructose in the American diet, sucrose and high fructose corn syrup (HFCS) have been specifically singled out. Studies on the acute effects of sucrose and HFCS have shown them to be metabolically equivalent by every parameter yet measured in humans, but, to our knowledge, this is the first prospective study to examine the longer term metabolic effects of fructose whether consumed as a component of sucrose or HFCS at normal population consumption levels.

Methods: Sixty-three overweight and obese individuals were placed on a eucaloric (weight stable) ADA exchange diet for 10 weeks (mean age 38.3 ± 11.9 years). Individuals were required to consume either sucrose or high fructose corn syrup sweetened low-fat milk, with the added sugar comprising either 10% or 20% of their daily calories, approximating the 25th and 75th percentile population consumption levels of fructose, respectively. Metabolic rate was estimated using the Harris-Benedict equation, and the caloric target appropriately set to maintain a stable weight. Measurements of body mass, body composition (DEXA) and fasting blood samples were obtained prior to, and at the end of the 10 week intervention. This was a double blind study, so the identity of the sweeteners was hidden from the participants and investigators, with the milk being coded as either group X or group Z. At the time of the current analysis the blind had yet to be broken.

Results: There was no significant increase in weight at either level of either sucrose or HFCS consumption in any group. Similarly, glucose did not significantly increase in any group. There was no change across the cohort in either insulin (6.65 ± 4.50 vs 6.97 ± 5.36 μ U/ml, $p > 0.05$) or insulin resistance as measured by HOMA (1.43 ± 0.96 vs 1.54 ± 1.24 , $p > 0.05$). Significantly, all parameters tested remained in the normal range during both pre and post testing.

Discussion: The absence of any statistically significant differences between the X and Z group and the normal levels of glucose and insulin at both pre and post testing suggest that, when consumed in typical amounts and as part of a weight stable diet, the effects of sucrose (table sugar) and HFCS are equivalent and neither is detrimental to weight or insulin sensitivity.

Sources of Research Support: Corn Refiners Association.

Nothing to Disclose: JL, DK, TJA, KM, JMR

P1-496

Insulin Resistant Subjects Are at Higher Risk of Obstructive Sleep Apnea Than Equally Obese, Insulin Sensitive Subjects.

A Liu MD¹, C Kushida MD, PhD¹ and GM Reaven MD¹.

¹Stanford Univ Med Ctr Stanford, CA.

There is increasing evidence of an existing relationship between obstructive sleep apnea (OSA) and type 2 diabetes, although underlying pathophysiologic mechanisms remain unclear. To evaluate the role of insulin resistance in mediating this association independent of obesity, we assessed the risk of OSA in insulin resistant (IR), as compared with equally obese, insulin sensitive (IS) subjects.

Non-diabetic, overweight/ obese subjects underwent direct quantification of insulin sensitivity by determining steady-state plasma glucose (SSPG) concentrations during the insulin suppression test. Individuals whose SSPG values fell in the top and bottom tertiles of a previously defined reference range were considered to be IR and IS, respectively. The Epworth Sleepiness Scale (ESS) and the STOP and/ or Berlin Questionnaires to evaluate OSA risk were administered to 15 IR and 8 IS subjects.

By definition, mean SSPG values were significantly higher in IR than IS individuals (243 ± 39 vs 87 ± 12 mg/dL, $p < 0.001$). The groups did not differ in BMI (33.5 ± 2.5 vs 31.7 ± 2.9 kg/m², $p = 0.12$). 47% of IR versus 0% IS subjects met criteria for pathologic sleepiness by scoring ≥ 10 points by the ESS ($p = .052$). Mean ESS scores were also greater in IR than IS individuals (8.9 ± 4.4 vs 4.6 ± 2.1 , $p = 0.005$). Finally, 13 of 15 IR subjects were found to be at high risk of OSA by the STOP and/ or Berlin Questionnaires, as compared with 1 IS subject ($p = 0.001$).

In conclusion, apparently healthy, insulin resistant individuals are demonstrated using clinical predictor models to have an increased risk of OSA, independent of obesity.

Nothing to Disclose: AL, CK, GMR

P1-497

The Relationship between Plasma Omentin Levels and Insulin Resistance in Newly Diagnosed Type 2 Diabetic Patients.

NG Kirnap M.D¹, G Gursoy M.D. Assoc.Prof¹, O Esbah M.D¹, Y Acar M.D¹ and B Demirbas M.D.Assoc.Prof.¹.

¹Ministry of Hlth Ankara Education and Res Hosp Ankara, Turkey.

As well as its role in energy storage, adipose tissue produces several hormones and cytokines termed adipokines that have widespread effects on carbohydrate and lipid metabolism. They appear to play important roles in the pathogenesis of insulin resistance, diabetes mellitus and obesity atherosclerosis.

Omentin is a newly identified adipokine that is highly and selectively expressed in visceral adipose tissue relative to subcutaneous adipose tissue. In some recent studies it was shown to be decreased in obesity and in insulin resistant women. In our study, we intended to increase our knowledge about omentin and its relation with type 2 diabetes mellitus, insulin resistance and obesity. We planned to point out the relationship between serum omentin levels and insulin resistance in newly diagnosed type 2 diabetic patients. The study included 80 newly diagnosed type 2 diabetic patients and 40 age matched control subjects. Diabetic group had significantly lower plasma omentin levels than control group ($p < 0,01$). When we grouped our diabetic and control subjects by an indirect measure of insulin resistance such as homeostasis model assessment (HOMA-IR) like $HOMA-IR \geq 2,7$, $HOMA-IR < 2,7$, we found that both diabetic patient and control the groups whose HOMA-IR was $< 2,7$ had significantly lower omentin levels ($p < 0,009$ and $p < 0,05$ respectively). Then we grouped our subjects as $HOMA-IR \geq 2,7$, $HOMA-IR < 2,7$. The diabetic group with $HOMA-IR \geq 2,7$ had significantly lower omentin levels than the control group whose HOMA-IR was $\geq 2,7$ ($p < 0,035$). In the diabetic and the control groups whose HOMA-IR was $< 2,7$ omentin levels were found to be statistically similar.

Nothing to Disclose: NGK, GG, OE, YA, BD

P1-498

Relationship between Serum 25-OH-D Level and Insulin Resistance in Obstructive Sleep Apnea.

N Colak Bozkurt¹, E Cakal¹, E Cakir Ozkaya¹, M Ozbek¹ and T Delibasli¹.

¹Ankara Diskapi Yildirim Beyazit Training and Res Hosp Ankara, Turkey.

Introduction: Obstructive sleep apnea(OSA) and 25-hydroxyvitamin-D3(25-OHD) deficiency are two separate entities both of which inversely associates with prevalence of diabetes and insulin resistance. Vitamin-D deficiency supposed to promote systemic inflammation, impair beta-cell secretory function, alter insulin action(1). Hypoxia induced inflammatory response was imputed to insulin resistance in OSA(2). Vitamin-D deficiency may contribute to insulin resistance in OSA

Methods: Polisomnography, 100g oral glucose tolerance tests were performed in 190 subjects suspicious for OSA. Bioimpedance analyses done to exclude patients with extreme adiposity rates. Patients who had acute infection, cardiovascular disease or on medication had been excluded. Serum 25-OHD and HOMA-IR measured in patients due to apnea-hypopnea indices(AHI) grouped as control(AHI<5, n=47), mild-OSA(5<AHI<15, n=46), moderate-OSA(15<AHI<30, n=47), severe-OSA(AHI>30, n=50)**Results:** 25-OHD levels (11.1-42.9ng/ml) decreased in OSA patients with increasing AHI; 19.9±7.8 (AHI<5) and 16.3±6.9(AHI>30). Diabetes diagnosed in 23 patients with OSA (AHI=43.3) but in 1 control patient. HOMA-IR was 2.14 in controls while 4.29 in severe-OSA's. Vitamin-D level of subjects with HOMA-IR<2.7(n=113, AHI=19.8) was 19.2±6.6 and of HOMA-IR>2.7(n=77, AHI=35.5) was 16.1±7.8 (p<0.01). Mean 25-OH level of 91 nondiabetic subjects' was 19.5±7.4, patients' with impaired glucose tolerance(n=75) was 16.9±6.5, diabetics'(n=24) was 13.8±5.3 (p<0.01). Waist/hip ratio and central fat corrected due to genders were not significantly different between groups while BMI increased significantly parallel to AHI and HOMA-IR with decreasing vitamin-D

Table 1: Patients' characteristics and results

| | Control AHI<5 | Mild OSA 5<AHI<15 | Moderate OSA 15<AHI<30 | Severe OSA AHI>30 | P value |
|--------------------------------|------------------|----------------------|------------------------------|-------------------------|------------|
| Number (n, female/male): | 47 (19/28) | 46 (18/28) | 47 (19/28) | 50 (21/29) | 0.994 |
| Age: | 42.8±9.5 | 47.8±10.3 | 49.8±10.6 | 49.6±10.3 | 0.003 |
| BMI(kg/m ²): | 29.2±4.9 | 29.1±4.1 | 30.7±5 | 33.4±4.6 | 0.001 |
| Waist/hip ratio (cm): | 0.97 | 1.01 | 1 | 0.99 | 0.017 |
| [1]Total fat ratio (%): | 28.5±9.3 | 27.9±7.9 | 29.9±9.1 | 33.1±7.6 | 0.014 |
| [2]Abdominal fat rate (%): | 36.8±9.5 | 37.8±8.5 | 39.2±8.9 | 42.7±7.3 | 0.006 |
| FPG (mg/dl): | 91.8 | 93.9 | 99.8 | 104.1 | 0.001 |
| FPI (μU/ml): | 9.2 | 10.8 | 12.4 | 16.6 | <0.001 |
| HbA1c (%): | 5.52 | 5.52 | 5.74 | 6.01 | <0.001 |
| [3]120 min glucose (mg/dl): | 140±33.2 | 148±35.9 | 148±40.9 | 172±62.9 | 0.003 |
| [4]HOMA-IR: | 2.14±0.7 | 2.53±1.1 | 3.17±2.1 | 4.29±2.8 | <0.001 |
| Non-Diabetics (n, %) | 30 (%32.97) | 24 (%26.37) | 21 (%23.08) | 16 (%17.58) | |
| New Diabetics (n, %) | 1 (%4.17) | 2 (%8.33) | 7 (%29.17) | 14 (%58.33) | 0.001 |
| IFG/IGT (n, %) | 16 (%21.33) | 20 (%26.67) | 19 (%25.33) | 20 (%26.67) | |
| 25-OH-D (ng/ml): | 19.93±7.81 | 18.29±6.48 | 17.55±7.42 | 16.31±6.98 | 0.097 |

[1] Measured by foot-to-foot bio-impedance analysis instrument (Tanita™)

[2] Measured by abdominal bio-impedance analysis instrument (Viscan™)

[3] 100 g glucose tolerance test, [4] HOMA-IR: {[fasting insulin (U/ml)]x[fasting glucose (mmol/l)]}/22.5

FPG (Fasting plasma glucose): 70-110 mg/dl, FPI (Fasting plasma insulin): 2.6-24.9 μU/ml,

IFG, IGT: Impaired fasting glucose (>100 mg/dl)/impaired glucose tolerance (120 min >140 mg/dl)

Conclusion: We suggest that OSA patients have lower vitamin-D level which is related to increased insulin resistance

and prevalence of diabetes correlating with severity of disease. Regarding mechanistic links, vitamin-D insufficiency might play role in insulin resistance and inflammatory response in OSA. Patients primarily with severe OSA and vitamin-D deficiency can be considered for supplementation which might ameliorate beta-cell function and might be of benefit in preventing diabetes

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(2) Esra Tasali et al., Chest 2008;133:496

Nothing to Disclose: NCB, EC, ECO, MO, TD

P1-499

The Effect of Smoking on Insulin Resistance and Serum Resistin Levels.

O Esbah¹, G Gursoy¹, NG Kirnap¹, Y Acar¹ and B Demirbas¹.

¹Ministry of Hlth Ankara Education and Res Hosp Ankara, Turkey.

The relationship between smoking and insulin resistance is controversial. There are conflicting reports as cigarette smoking impairs insulin secretion, augments insulin resistance or it has no effect on glucose metabolism. Resistin is a recently found adipokine, demonstrated to induce insulin resistance in vivo or in vitro. In our study, we intended to examine the relationship of smoking with insulin resistance and resistin. The study included 52 male smokers and 33 age matched non-smoker male control subjects. Smoker group had significantly higher plasma resistin and low density lipoprotein cholesterol levels, body mass index, waist circumference and indirect measure of insulin resistance such as homeostasis model assessment (HOMA-IR) than non-smoker group ($p < 0,05$, $p < 0,01$, $p < 0,05$, $p < 0,02$, $p < 0,01$ respectively). When we grouped smoker and non-smoker groups as HOMA-IR $\geq 2,7$, HOMA-IR $< 2,7$, we found that in the smoker group the men with HOMA-IR $\geq 2,7$ had significantly high resistin levels ($p < 0,05$), but in non-smoker group the HOMA-IR $\geq 2,7$, HOMA-IR $< 2,7$ subjects had similar resistin levels. As we classified smoker and non-smoker groups as their body mass index BMI < 27 , and BMI ≥ 27 , neither smoker, nor non-smoker groups had significantly different resistin levels. In conclusion, we may speculate that smoking seems to be related to resistin levels and this relation may be correlated with insulin resistance.

Nothing to Disclose: OE, GG, NGK, YA, BD

P1-500

Evaluation of Insulin Sensitivity Using Mathematical Models as HOMA- β and HOMA-IR, Compared to Mean Estimated Glucose, HBA1C, Triglycerides in 1101 Non Diabetic Individuals.

LA Naves Dr.¹, MC Reis², LF Abdalla², SSS Costa², LF Castro¹, MP Rodrigues¹ and LA Casulari¹.

¹Univ de Brasilia Brasilia, Brazil and ²Lab Sabin de Analises Clinas Brasilia, Brazil.

Background: The evaluation of insulin sensitivity is an important tool for epidemiological studies and comprehension of Metabolic Syndrome, and Atherosclerosis. The determination of an adequate laboratory method and reference values for each population, is important for precocious intervention. **Objectives:** The aim of this study was to validate and establish the reference values for a 1101 individuals from Brasilia, using the mathematical models HOMA- β and HOMA-IR, using chemiluminescence as a standard method for insulin measurements, and determine the influence of glucose, age, gender, BMI and triglycerides on HOMA-IR and HOMA- β . **Patients and Methods:** We evaluated retrospectively 1101 non diabetic individuals, recruited from the Unit of Endocrinology University of Brasilia and Laboratório Sabin, referred from different physicians to evaluate glucose, insulin, HbA1C, lipidogram from July 2007 to December 2009. The anthropometric data and concomitant treatment were collected at admission. We excluded all individuals taking any hypoglycemic agents or statins. Glucose, HbA1C, lipids, insulin, thyroid function were determined. HOMA-IR, HOMA- β indexes and estimated mean glucose were calculated. Statistical analysis was done by ANOVA. **Results and Discussion:** The gender distribution was 811 women and 290 men, ages 18 to 86 years ($40,59 \pm 14,14$). The patients were categorized by glucose, age and HOMA levels. In normoglycemic group, glucose was $87,33 \pm 6,82$ mg/dl and insulinemia $9,63 \pm 6,62$ μ U/ml, median 8,4 μ U/ml. Mean HOMA-IR was $2,10 \pm 1,52$, median 1,80 (75^o percentil of 2,7), HOMA- β , $152,31 \pm 105,81$, median 126,94. In hyperglycemic group the insulinemia ($14,74 \pm 8,35$ μ U/ml), BMI and HOMA-IR ($4,01 \pm 2,47$) were higher, and HOMA- β ($118,16 \pm 67,52$) was lower than normoglycemic group ($p < 0,05$). The reference values established to individuals with normal BMI, HDL, Cholesterol, Triglycerides and glucose were HOMA-IR of $1,47 \pm 1,44$, median 1,18 (75^o percentil of 1,68 and 90^o percentil of 2,19. Mean levels for HOMA- β were $115,02 \pm 89,11$, median 93,24 (75^o percentil of 155,29 and 90^o percentil of 188,25). **Conclusion:** Age has a strong effect on β cells, with a progressive reduction of HOMA- β according to age groups. The BMI influenced the triglycerides and reduced HDL levels. There were no differences in LDL. Hypertriglyceridemia is associated to insulin resistance and β -cells function.

Sources of Research Support: Nucleo de Apoio a Pesquisa- laboratório Sabin; Universidade de Brasilia; CNPq.

Nothing to Disclose: LAN, MCR, LFA, SSSC, LFC, MPR, LAC

P1-501

Beneficial Effects of Testosterone Added to Diet and Exercise on Glycemic Control and the Metabolic Syndrome Persist 12 Months after Discontinuation of Testosterone.

AE Heufelder MD, PhD¹, MC Bunck MD, PhD², F Saad DVM, PhD^{3,4} and LJG Gooren MD, PhD².

¹Private Practice for Internal Med and Diabetes Munich, Germany ; ²VU Med Ctr Amsterdam, Netherlands ; ³Bayer Schering Pharma Berlin, Germany and ⁴Gulf Med Univ Sch of Med Ajman, United Arab Emirates.

Objective: We previously showed beneficial effects of one-year treatment with transdermal testosterone (50 mg once daily) in addition to diet and exercise (D&E +T) as compared to D&E alone, in hypogonadal men with the metabolic syndrome (MetS) and newly diagnosed type 2 diabetes (T2D) (Heufelder et al., J Androl 2009; 30:726-33). Our current research question was whether the observed beneficial effects on glycemic control and components of the MetS would persist after discontinuation of T treatment.

Research design and methods: Of the original 16 patients receiving D&E+T, 13 discontinued T treatment but continued D&E. Three refused to stop T and were regarded as study drop outs. Twelve months after discontinuation of T (but continuation of D&E), these 13 subjects were compared to the 16 patients who had been treated with D&E alone.

Data patients randomized to D&E+T after 12 months T: (n=13); mean±SE age 57.3±1.4y; BMI 32.1±0.5 kg/m²; waist circumference 107.9±1.3cm; HbA_{1c} 7.5±0.1%; fasting glucose 7.9±0.2 mmol/L; triglycerides 3.2±0.1 mmol/L); data of patients assigned to D&E alone after 12 months: mean±SE age 55.9±1.5y; BMI 32.5±0.6 kg/m²; waist circumference 105.7±1.4cm; HbA_{1c} 7.5±0.1%; fasting glucose 8.3±0.2 mmol/L; triglycerides 3.4±0.3 mmol/L). All patients continued their supervised D&E program.

Results: Following the 12-month off-drug period, the observed beneficial effects of one-year combined D&E+T treatment persisted. This resulted in a statistically significant improvement in HbA_{1c} (between group difference: -0.8±0.1%, p<0.001), FPG (-0.8±0.1 mmol/L, p=0.007), insulin (-21.1±5.7 pmol/L, p=0.001), HDL-C (+0.25±0.04 mmol/L, p<0.001), triglycerides (-0.50±0.11 mmol/L, p<0.001), and waist circumference (-6.5±1.0 cm, p<0.001) as compared to D&E alone for 24-months. Outcome measures in the D&E alone group were also statistically significant improved over baseline values.

Conclusions: Beneficial effects of one-year treatment with T in addition to D&E in hypogonadal men with the MetS and T2D, persisted for at least 12 months after discontinuation of T treatment while patients continued D&E. The outcome measures 24 months after initiation of the study, were better in this group than in the group treated with D&E only.

Disclosures: AEH: Speaker, Bayer Schering Pharma, Berlin, Germany. FS: Employee, Bayer Schering Pharma. LJGG: Speaker, Bayer Schering Pharma.

Nothing to Disclose: MCB

P1-502

FTO rs9930506 Polymorphism Is Associated with Higher Cholesterol and Triglycerides Levels but Not with Non-Alcoholic Fatty Liver Disease in Mexican Subjects.

Dulce Arriola-Lara Master¹, Elva Perez-Luque PhD¹, Juan Manuel Malacara MD and PhD¹, Carlos Radilla-Lopez MD¹ and Amanda Chang-Y-Garcia MD¹.

¹Univ de Guanajuato Campus Leon Leon, Mexico.

Introduction. The increased prevalence of obesity may result from unhealthy diet and physical activity, mainly in genetically susceptible individuals. *FTO* rs90030506 had been associated with BMI, weight and hip circumference. Non-alcoholic fatty liver disease (NAFLD) is a frequent condition in obesity. We studied the association of *FTO* genotypes with obesity, NAFLD, and the metabolic conditions in normal subjects.

Material and Methods. We included 180 subjects in healthy conditions, weight, height, waist and hip circumferences were collected. A fasting blood samples was obtained to measure, lipid profile, insulin, leptin and retinol binding protein 4 (RBP4) levels and for DNA extraction. The *FTO* rs9930506 polymorphisms were determined by PCR and digestion with *Bsa*I enzyme. For analysis we made two groups: wild-type carriers and rsA9930506G or rsG9930506G carriers. Fatty liver was assessed with ultrasonography by a blinded experienced radiologist. It was categorized in three stages (0 to 2).

Results. Volunteers were 40±10.5 years old with and BMI 28.7±6.4. The carriers of rsA9930506G or rsG9930506G genotypes had higher cholesterol (192 mg/dl) and triglycerides level (161mg/dl) than carries of the common rs9930506A allele (p=0.0001; p=0.003 respectively). Normal weight (BMI<25) subjects with genotypes rsA9930506G or rsG9930506G had higher triglycerides (154 mg/dl) and RBP4 levels (35.6 mg/l), (p=0.03; p=0.049 respectively). NAFLD was grade 1 in 16.7% and grade 2 in 2.0% of the subjects. Overweight subjects (BMI≥25 to 30) with genotypes rsA9930506G or rsG9930506G had higher BMI, cholesterol (198 mg/dl) and triglycerides levels (164 mg/dl) than carriers of the common rs9930506A allele (p=0.02; p=0.02 respectively). In this group the NAFLD was: grade 1 in 7.2%, and grade 2 in 11.7%. In obese group (BMI>30) only cholesterol levels were elevated in the carriers of the rsA9930506G or rsG9930506G genotypes (t=-2.87, p=0.005) and NAFLD frequency was: grade 1 in 36.2% and grade 3 in 17.3%. Subjects without NAFLD had lower waist circumference, glucose level, but only grade 2 had lower RBP4 levels than other two grades. The RBP4 levels were associated with triglyceride levels, diastolic pressure and BMI (r=0.29, p=0.003; r=0.22, p=0.02; r=0.19, p=0.04).

Conclusions. Our data show a consistent association of *FTO* rs9930506 gene with cholesterol levels in overweight and obese subjects. Not relationship between *FTO* rs9930506 gene with development with fat liver was found.

Sources of Research Support: Convocatoria Institucional para investigación, Universidad de Guanajuato 2009.

Nothing to Disclose: DA-L, EP-L, JMM, CR-L, AC-Y-G

P1-503

Attenuation of Oxidative Stress and Liver Pathology by a Novel Glutathione Precursor in a Mouse Model of Dietary Steatosis.

I Sinha-Hikim Ph.D.¹, R Shen M.D.¹, H Kim Ph.D.³, AP Sinha Hikim Ph.D.¹, S French M.D.², ND Vaziri M.D.³, A Crum M.D.⁴ and K Norris M.D.¹.

¹Charles Drew Univ, David Geffen Sch of Med, UCLA Los Angeles, CA ; ²LA BioMed, Harbor-UCLA, David Geffen Sch of Med, UCLA Torrance, CA ; ³UC-Irvine Irvine, CA and ⁴Proimmune Rhinebeck, NY.

Background and Objective: Nonalcoholic fatty liver disease (NFLD) is the most common type of liver pathology and is associated with obesity, diabetes and metabolic syndrome. It includes the whole spectrum of fatty liver, including steatosis, steatohepatitis, and eventually cirrhosis. Oxidative stress and the associated hepatocyte apoptosis is believed to play a pivotal role in the pathogenesis of NFLD. We examined the effects of a novel cystine based glutathione precursor fortified with selenium (FT061452 TM or F1) in preventing hepatic steatosis in ApoE^{-/-} mice fed a high-fat diet.

Study Design: Adult (8 weeks old), male ApoE^{-/-} mice were fed a normal diet (ND) or high fat powdered diet (HFD), consisting of 21% fat and 0.21% cholesterol, with or without F1 (0.5g/kg/day) supplementation for 16 weeks.

Results: Compared with ApoE^{-/-} mice consuming ND with or without F1, ApoE^{-/-} mice fed HFD exhibited significant weight gain, hepatomegaly, hypercholesterolemia and hypertriglyceridemia with no change in serum albumin levels. High resolution light microscopy (glutaraldehyde fixed, osmium tetroxide post-fixed, and epoxy embedded) revealed micro-and macro vesicular steatosis in ApoE^{-/-} mice fed the HFD. Image analysis revealed a significant increase in intracellular fat area ($373 \pm 48 \mu\text{m}^2$) compared with ApoE^{-/-} mice fed ND ($143 \pm 20 \mu\text{m}^2$) or ND+F1 ($171 \pm 9 \mu\text{m}^2$). Electron microscopy of hepatocytes revealed a striking increase in lipid-containing vesicles of varying sizes along with a marked decrease in the amount of glycogen and smooth and rough endoplasmic reticulum. Compared with ApoE^{-/-} mice fed the ND with or without F1, liver from ApoE^{-/-} mice fed the HFD also displayed increased 4-HNE levels, hepatocyte apoptosis, and activation of caspases 9 and 3. The adverse effects of HFD on serum triglyceride levels, body and liver weights, and hepatic steatosis were significantly attenuated by F1. Likewise, F1 administration attenuated HFD-induced oxidative stress, apoptosis, and hepatocyte ultra-structural abnormalities.

Conclusions: These results demonstrate that dietary supplementation of F1 ameliorates high fat diet- induced steatosis in ApoE^{-/-} mice and emphasizes the suitability of this model for investigating the mechanisms of diet-induced steatosis.

Sources of Research Support: Award# 1U54RR026138-01, NIH.

Nothing to Disclose: IS-H, RS, HK, APSH, SF, NDV, AC, KN

P1-504

The Major Role of apoB100 in Cycloheximide-Induced Acute Fatty Liver.

M Murakami¹, K Bessho², S Mushiake³, Y Kiyohara¹, H Yamada¹, Y Miyoshi¹ and K Ozono¹.

¹Osaka Univ Graduate Sch of Med Suita, Japan ; ²Cincinnati Children's Hosp Med Ctr Cincinnati, OH and ³Setsuman General Hosp Kadoma, Japan.

Hepatic lipid metabolism is maintained by hepatic lipid uptake, lipogenesis, fatty acid oxidation (FAO) and lipid secretion. Altered balance between these factors is caused by many pathological conditions and leads to fatty liver. Among them, increased hepatic lipid uptake and lipogenesis are the main factors which contribute to nonalcoholic fatty liver disease. On the other hand, impaired FAO is associated with many of the acute fatty liver in human. To establish a mouse model of acute fatty liver in which a novel mechanism is involved, we administered cycloheximide (CHX) to C57/BL6CR mouse. Following 24 hours of intraperitoneal injection of CHX, mean hepatic lipid level was increased by 1.8-fold in CHX-treated mouse compared with controls, which was decreased within 48 hours. Hepatic incorporation rate of radiolabeled Leucine in CHX-treated mice was decreased to 32±13% of controls. Plasma levels of β -hydroxybutyrate (β -OHB) were not decreased, and levels of free fatty acid (FFA) were not increased in CHX-treated mice compared to controls. The expression of acetyl-CoA carboxylase and fatty acid synthase, the major enzymes for hepatic lipogenesis, was not altered throughout the experimental period. These data suggest that impaired FAO, increased hepatic lipid inflow of FFA and increased hepatic lipogenesis are not involved in the pathogenesis of this mouse model. Hepatic triglyceride (TG) secretion rate was significantly decreased to 22.4±6.7% of controls in CHX-treated mice following 8 hours of injection, which gradually returned to the equivalent levels to controls within 48 hours. TG is secreted from the liver in the form of VLDL. Two major proteins, apolipoproteinB100 (apoB100) and microsomal triglyceride transfer protein (MTP) play an essential role for the assembly and secretion of VLDL. Consistent with the result of TG secretion rate, the expression of apoB100 was decreased at 4-16 hours after CHX injection, followed by gradual recovery. On the other hand, MTP was not decreased in CHX-treated mice. Gene expression of apoB in the liver showed no significant difference between controls and CHX-treated mice. Twenty four hours of incubation of HepG2 cells with CHX also led to cellular lipid accumulation along with decreased amount of cellular and secreted apoB100. In conclusion, we demonstrated that decreased hepatic secretion of lipid due to the acute reduction of apoB100 is involved in the pathogenesis of CHX-induced acute fatty liver.

Nothing to Disclose: MM, KB, SM, YK, HY, YM, KO

P1-505

HepG2 Cells as a Model for Hepatocellular Lipid Accumulation - Influence on NAMPT.

A Garten¹, S Petzold MD¹, S Schuster¹, A Korner MD¹, J Kratzsch PhD², R Gebhardt PhD² and W Kiess MD¹.

¹Univ Hosp for Children and Adolescents Leipzig Leipzig, Germany and ²Univ Hosp Leipzig Leipzig, Germany.

Background & Aim: The development of non-alcoholic fatty liver disease (NAFLD) is associated with lipid accumulation in hepatocytes. SIRT1 is a major regulator of hepatocellular lipid metabolism and was found to protect against diet-induced hepatic steatosis in a mouse model. The activity of SIRT1 is regulated by nicotinamide phosphoribosyltransferase (NAMPT). Controversies exist as to whether hepatic NAMPT expression and NAMPT plasma concentrations are decreased or increased in NAFLD. We characterised HepG2 cells as a model for hepatic lipid accumulation after incubation with free fatty acids and questioned whether free fatty acids have an influence on NAMPT expression in and NAMPT release from HepG2 cells.

Methods: NAMPT expression was quantified by western blot and ELISA. The NAMPT Dimer was detected in size-exclusion-chromatography-fractionated cell culture supernatant. NAMPT enzymatic activity was measured using a radioactive filter disc assay. Lipid accumulation in HepG2 cells was induced by incubation with oleate and/or palmitate and fluorimetrically quantified by Nile red staining. Insulin sensitivity of HepG2 cells was determined by detection of phosphorylated insulin receptor and AKT/PKB.

Results: NAMPT is expressed in HepG2 cells. NAMPT protein content in supernatant increases approx. 4fold during 48h. In supernatant the protein occurred primarily at a molecular weight of approx. 110 kDa which corresponds to its dimeric form. The release did not correlate with cell death as evidenced by a cytotoxicity assay. NAMPT enzymatic activity was detected in lysates and supernatants of HepG2 cells. Lipid content in HepG2 cells increased after 24h incubation with oleate (at 0.5 mM approx. 10fold increase) or a mixture of oleate:palmitate 2:1 (at 0.5 mM approx. 7fold increase) compared with control medium. No increase was detectable after incubation with palmitate. NAMPT mRNA expression was not influenced by oleate or palmitate, while NAMPT protein expression was decreased after 24h incubation with 1 mM palmitate, which was probably due to cell death. Insulin sensitivity in HepG2 cells was impaired by incubation with palmitate (0.5 mM), while it was not influenced by oleate.

Conclusion: Hepatocytes may be a significant source of NAMPT in human circulation. HepG2 cells incubated with free fatty acids are a suitable model for the study of hepatocellular lipid accumulation. NAMPT protein expression in HepG2 cells may be impaired by incubation with palmitate.

Nothing to Disclose: AG, SP, SS, AK, JK, RG, WK

P1-506

A Novel Type of Congenital Generalized Lipodystrophy (CGL4) with PTRF Mutations.

S Shastry M.D.¹, MR Delgado M.D.¹, AK Agarwal Ph.D.¹ and A Garg M.D.¹.

¹Univ of Texas Southwestern Med Ctr Dallas, TX.

Context: Congenital generalized lipodystrophy (CGL) is a rare autosomal recessive disorder characterized by near total absence of body fat since birth and predisposition to insulin resistance, diabetes, hypertriglyceridemia and hepatic steatosis. Three CGL loci, *AGPAT2*, *BSC2* and *CAVI*, have been identified previously. Recently, mutations in Polymerase I and transcript release factor (*PTRF*) were reported in six Japanese patients presenting with myopathy and CGL (CGL4).

Objective: To report novel *PTRF* mutations and detailed phenotype of two male and two female patients with CGL4 belonging to two pedigrees of Mexican origin (CGL 7100 and CGL 178).

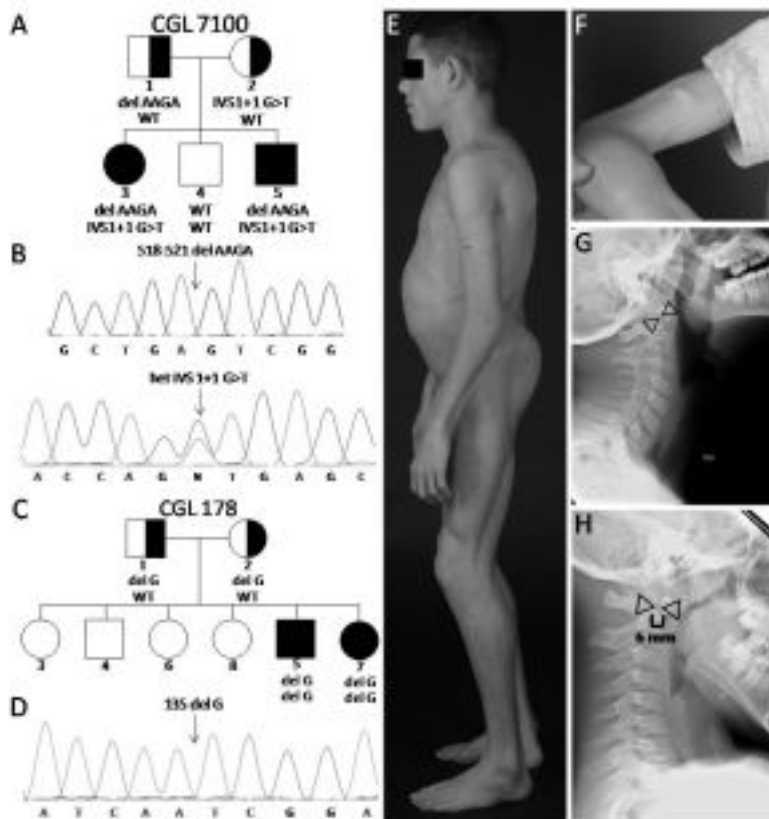
Results: All patients had near total loss of body fat, hypertriglyceridemia and congenital myopathy manifesting as weakness and high serum creatine kinase levels.

Clinical Characteristics of our patients with CGL4

| Characteristic | CGL 7100.3 | CGL 7100.5 | CGL 178.5 | CGL 178.7 |
|--------------------------|------------|------------|-----------|-----------|
| Age (y)/Gender | 14/F | 8/M | 11/M | 1/F |
| Pyloric Stenosis | - | - | + | + |
| Atlantoaxial instability | + | + | + | n/a |
| Acanthosis nigricans | + | + | + | - |
| Hepatomegaly | + | + | + | + |
| Myopathy | + | + | + | + |
| Hypertension | + | - | - | n/a |
| Glucose tolerance | normal | normal | normal | n/a |
| Hypertriglyceridemia | + | + | + | + |
| Echocardiogram | normal | normal | n/a | n/a |

M, male; F, female; +, present; -, absent; n/a, data not available

Three of them had atlantoaxial instability which required surgery in two. Two patients belonging to CGL 178 pedigree required surgery for pyloric stenosis in the first month of life. None of them had any arrhythmias or prolonged QT interval. Three of them had mild acanthosis nigricans but had normal glucose tolerance. All patients had novel null mutations in *PTRF* gene.



A. CGL 7100 pedigree. B. Chromatograms showing two heterozygous *PTRF* mutations in CGL 7100. C. CGL 178 pedigree. D. Chromatogram showing homozygous *PTRF* mutation in CGL 178. E. Lateral view of CGL 178.5 showing generalized lipodystrophy and protuberant abdomen. F. Percussion myoedema. G. and H. Lateral radiographs during extension and flexion of cervical spine showing atlantoaxial instability. Abbreviations: WT, wild-type allele

Conclusions: Mutations in *PTRF* result in a novel phenotype that includes generalized lipodystrophy, myopathy, atlantoaxial dislocation, pyloric stenosis and arrhythmias. How mutations in *PTRF*, which plays an essential role in formation of caveolae, cause variable phenotypes, remains to be elucidated.

Nothing to Disclose: SS, MRD, AKA, AG

P1-507

A Novel Syndrome with Mandibular Hypoplasia, Deafness, Progeroid Features and Generalized Lipodystrophy.

S Shastry MD¹, V Simha MD¹, K Godbole MD², P Sbraccia MD³, S Melancon MD⁴, CS Yajnik MD⁵, G Novelli MD³ and A Garg MD¹.

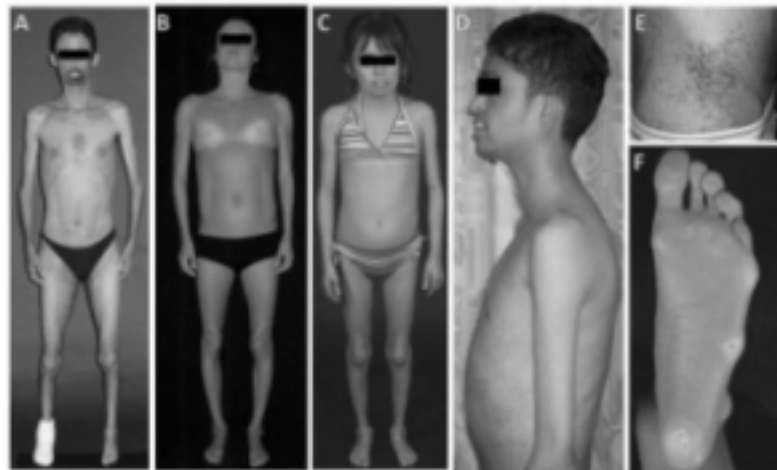
¹UT Southwestern Med Ctr Dallas, TX ; ²Deenanath Mangeshkar Hosp Pune, India ; ³Tor Vergata Univ Rome, Italy ; ⁴McGill Univ Hlth Ctr Montreal, Canada and ⁵King Edward Memorial Hosp Pune, India.

Context: Mandibuloacral dysplasia (MAD) is an autosomal recessive progeroid disorder due to mutations in lamin A/C (*LMNA*) or zinc metalloproteinase (*ZMPSTE24*) genes. It is associated with Type A (partial) or B (generalized) lipodystrophy.

Objective: To report a novel syndrome with some overlapping features with MAD.

Results: We report six patients with mandibular hypoplasia, deafness, progeroid features and generalized lipodystrophy (MDP). These patients have similar features to MAD patients such as hypoplastic mandible, beaked nose, stiff joints and sclerodermatous skin.

Figure 1



Patients with MDP. A, anterior view of a 36-yr-old man showing generalized lipodystrophy, reduced muscle mass and prominent maxillary incisors. B, anterior view of a 39-yr-old Italian female showing generalized lipodystrophy and lack of breast development. C, anterior view of a 10-yr-old French Canadian girl with bilateral coxa-waiga and lack of lipodystrophy. D, profile view of a 25-yr-old Indian man showing generalized lipodystrophy, muscle wasting and prominent maxillary incisors. E, Telangiectasias on the abdomen. F, loss of fat from the sole and multiple calluses of the patient shown in panel A.

However, they did not have any mutations in the *LMNA* or *ZMPSTE24* and showed distinct characteristics such as sensorineural hearing loss, absence of clavicular hypoplasia and acro-osteolysis. All males with MDP were hypogonadal.

Clinical Features of MDP patients compared with MAD due to *LMNA* or *ZMPSTE24* mutations

| Clinical Features | MAD LMNA | MAD ZMPSTE24 | MDP |
|-----------------------|-------------|--------------|---------------------|
| N | 28 | 6 | 6 |
| M/F | 12/16 | 2/4 | 4/2 |
| Mandibular hypoplasia | 28 (100%) | 6 (100%) | 6 (100%) |
| Dental overcrowding | 21/25 (84%) | 3/3 (100%) | 6 (100%) |
| Bird-like facies | 25/26 (96%) | 4/4 (100%) | 6 (100%) |
| Sparse hair | 2/3 (67%) | 3/3 (100%) | 0 (0%) |
| Sclerodermatous skin | 5/5 (100%) | 2/2 (100%) | 6 (100%) |
| Atrophic skin | 9/10 (90%) | 4/4 (100%) | 6 (100%) |
| Lipodystrophy | partial | partial | partial/generalized |
| Clavicular hypoplasia | 25 (89%) | 4/4 (100%) | 0 (0%) |
| Acro-osteolysis | 26/27 (96%) | 5/5 (100%) | 0 (0%) |
| Contractures | 25 (89%) | 5/5 (100%) | 6 (100%) |
| Deafness | 0 (0%) | 0 (0%) | 6 (100%) |
| Male Hypogonadism | 1/2 (50%) | 0 (0%) | 4/4 (100%) |
| Growth Retardation | 20/24 (83%) | 0 (0%) | 6 (100%) |

Skinfold thickness, DEXA, and MRI evaluation of body fat showed a generalized lipodystrophy in most patients.

Conclusions: MDP patients have a few overlapping but some distinct clinical features as compared to MAD suggesting that it is a novel syndrome. The genetic basis of MDP remains to be elucidated.

Nothing to Disclose: SS, VS, KG, PS, SM, CSY, GN, AG

P1-508

Drosophila as a Model Organism for Congenital Lipodystrophy.

RA Stewart MD¹, S Pulipparacharuvil PhD¹, V Rai MS¹ and K Bharucha MD, PhD¹.

¹Univ of Texas Southwestern Med Sch Dallas, TX.

Berardinelli-Seip congenital lipodystrophy is a rare metabolic disease that results in near complete absence of adipose tissue at birth. It is an autosomal recessive disorder caused by mutation of the *BSCL2* gene, and also results in metabolic derangements that include severe insulin resistance, diabetes, dyslipidemia, hepatic steatosis, and hypertension. *BSCL2* encodes the protein seipin, and the function of the seipin protein has yet to be fully understood; thus, the pathophysiology of seipin deficiency has not been completely elucidated. Seipin is widely conserved across species, allowing the use of *Drosophila melanogaster* as a model organism for elucidating the molecular mechanisms involved in this most severe form of congenital lipodystrophy. *Drosophila* has emerged as a powerful model organism to study metabolic pathways, and many aspects of lipid homeostasis are conserved between humans and *Drosophila*. In addition, the *Drosophila* fat body serves many of the same functions as mammalian adipose tissue, and is the major repository of energy storage in the form of triglycerides. We have initiated both *in vivo* and *in vitro* studies of seipin function. Our *in vivo* results show that *Drosophila* seipin (dseipin) is required to maintain proper lipid droplet distribution and morphology in the larval fat body. Cell-based studies confirm that dseipin is an ER-associated protein, paralleling the cellular distribution of its mammalian homologs. Furthermore, our findings suggest an intimate association of dseipin with lipid droplets in *Drosophila* S2 cells. Taken together, our results suggest that dseipin may play a critical role in lipid droplet function in *Drosophila*, and may provide new insights into the pathogenesis of complete congenital lipodystrophy.

Nothing to Disclose: RAS, SP, VR, KB

P1-509

Maternal Protein Restriction (MPR) Leads to Augmented Cholesterol Due to Chromatin Silencing of the Hepatic *Cholesterol 7 α Hydroxylase (CYP7A1)* Promoter during Early Development of the Rat Offspring.

G Sohi B.Sc. Hons^{1,2,3}, K Marchand M.Sc.³, A Revesz B.Sc. Hons^{1,2,3}, E Arany Ph.D³ and D. B. Hardy Ph.D^{1,2,3}.

¹The Univ of Western Ontario London, Canada ; ²The Children's Hlth Res Inst London, Canada and ³The Lawson Hlth Res Inst London, Canada.

High circulating cholesterol remains one of the main risk factors associated with adult cardiovascular disease (CVD) (1). The major site for the regulation of cholesterol homeostasis occurs in the liver, primarily through the conversion of cholesterol to bile acids via the enzyme, cholesterol 7 α hydroxylase (*CYP7A1*) (2). Emerging evidence also suggests that the risk of developing CVD is inversely related to birth weight, independent of genetics, lifestyle, and diet (3). In rodent models, maternal protein restriction (MPR) results in low birth weight and symptoms of CVD in adulthood (4,5). Therefore, we **hypothesized** that MPR derived offspring are at a higher risk of developing hypercholesterolemia in adult life due to developmentally impaired *CYP7A1* expression. Pregnant Wistar rats were either fed a control 20% or a low 8% protein diet throughout pregnancy and weaning, and subsets of rats were sacrificed at embryonic day 19 (e19), postnatal day 21 (preweaning, d21) and day 130 (postweaning, d130). Blood was extracted for cholesterol analysis and fetal and postnatal livers were excised, weighed, and flash frozen for the determination of *CYP7A1* expression. In addition, chromatin immunoprecipitation (ChIP) was performed to investigate whether the *in vivo* binding of RNA Polymerase II and posttranslational histone modifications were altered at the promoter of *CYP7A1*. MPR during pregnancy and lactation led to low birth weight offspring with decreased liver to body weight ratios at e19 and d21. Moreover, circulating cholesterol levels increased in both sexes at d21 and exclusively in the male offspring at d130. This coincided with corresponding decreases in the expression of *CYP7A1*. ChIP revealed that this decrease was accompanied by diminished acetylation and enhanced methylation of Histone H3 (K9,14), markers of chromatin silencing, at the promoter region of *CYP7A1* in the male offspring at both d21 and d130. This was concomitant with a decrease in the recruitment of RNA Polymerase II at both ages. The decrease in *CYP7A1* expression may also be due to a decrease in hepatic JMJD2B mRNA, a Histone H3 (K9) demethylase. Collectively, these findings suggest that increased cholesterol in the MPR offspring is due in part to permanent chromatin silencing at the promoter of *CYP7A1* during early development. Future work will examine the expression and binding of histone H3 (K9) methyltransferases/demethylases to the promoter of *CYP7A1* during normal and abnormal development.

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Sources of Research Support: CIHR/Sick Kids Foundation New Investigator Award; NSERC, UWO Obstetrics and Gynaecology Graduate Scholarship.

Nothing to Disclose: GS, KM, AR, EA, DBH

P1-510

Mouse Models of Human Cholesterol Metabolism for Pre-Clinical Studies of Lipid Homeostasis.

MD Hayward PhD¹, W Campbell BA¹, S Karagrigoriou BA¹, D Chen BA¹, C Chu BA¹, A Wozniczka BA¹, M Macbride PhD², S Petralia PhD², DS Grass PhD¹ and O Buiakova M.D, Ph.D.¹.

¹Taconic Cranbury (Xenogen Biosciences Corp) Cranbury, NJ and ²Taconic Hudson, NY.

Rodent cholesterol metabolism differs from human cholesterol metabolism, at least in part, due to rodents' deficiency in cholesterol ester transfer protein (CETP). Because of this difference, rodent HDL-C levels are normally much higher relative to LDL-C levels, which contrasts with what is observed in humans. The CETP enzyme is responsible for the transfer of cholesteryl esters between HDL-C and LDL-C particles. In order to develop a mouse model that more closely resembles human cholesterol metabolism, a transgenic mouse model was created that expressed the CETP enzyme under the control of the ApoA1 promoter (1) and this transgenic line has been commercially available through Taconic (Taconic model #1003). Recently, these mice have been backcrossed to congenicity on the B6N background (Taconic models #3715). Male and female CETP transgenic mice were compared on both the mixed background and congenic B6N backgrounds with respect to serum total cholesterol, triglycerides, and cholesterol particle distribution. The amount of LDL-C was similar in the male transgenic mice on both backgrounds. In addition, the WT B6N mice had much lower LDL levels than in the WT mixed background. This resulted in a much larger relative increase in LDL-C in the transgenic mice on the B6N background, compared to their respective WT, than the transgenic mice on the mixed background. Further, these mice were fed a diet containing 1% niacin (vitamin B₃) and the same measurements were repeated, specifically measuring the HDL-elevating effects of niacin.

To further alter murine cholesterol metabolism so that it more closely resembles that observed in humans, this line of transgenic mice was crossed onto a transgenic line that expresses ApoB, the protein component of LDL, thus maximizing the amount of circulating LDL-C in the resulting mice. These double transgenic mice, which have been available commercially for several years (Taconic model #1007), are on a mixed background of C57BL/6N and SJL. Recently, these mice have been backcrossed to congenicity on the C57BL/6N background (Taconic model #3716). Male transgenic mice congenic to the B6 background and containing the single CETP transgene, ApoB transgene, or both transgenes were profiled using a battery of tests which examine lipid metabolism and glucose homeostasis on the atherogenic Western diet or on a regular diet. Data from this phenotypic characterization will be presented.

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Disclosures: MDH: Employee, Taconic Inc. WC: Employee, Taconic Inc. SK: Employee, Taconic Inc. DC: Employee, Taconic Inc. CC: Employee, Taconic Inc. AW: Employee, Taconic Inc. MM: Employee, Taconic Inc. SP: Employee, Taconic Inc. DSG: Employee, Taconic Inc. OB: Employee, Taconic Inc.

P1-511

Inhibition of Endoplasmic Reticulum Stress in Hepatocytes by High-Density Lipoprotein (HDL).

Rosalyn Alcalde M.D.¹, Emad Naem M.D.¹, Senan Sultan M.D.¹, Abdul-Razzak Alamir M.D.¹, Michael J. Haas Ph.D.¹ and Arshag D. Mooradian M.D.¹.

¹Univ of Florida - Jacksonville Jacksonville, FL.

Endoplasmic reticulum (ER) stress and the inability to efficiently process and secrete secretory proteins have been implicated in promoting dyslipidemia and diabetic β -cell dysfunction. Since the liver contributes substantially to the regulation of plasma protein levels and to lipid and glucose metabolism, ER stress may have a role in promoting liver dysfunction. Treatment of HepG2 hepatocytes with tunicamycin and thapsigargin induced ER stress in a dose-dependent manner, as measured by ER stress responsive alkaline phosphatase (ES-TRAP) using the plasmid pSEAP2.Control, which expresses a secreted form of placental alkaline phosphatase (SAP). Likewise, tunicamycin and thapsigargin also induced a dose-dependent decrease in albumin secretion and subsequent intracellular albumin accumulation, which correlated with the changes in SAP activity. While high-density lipoprotein (HDL) has been shown to induce NO synthesis and inhibit oxidative stress in a receptor-dependent manner, it is not clear if HDL modulates ER stress. To determine if HDL inhibits ER stress, HepG2 cells were treated with 0.1 μ M tunicamycin, 50- or 500- μ g/ml HDL (purified from human serum), 27 mM dimethylsulfoxide (DMSO), or 1 mM 4-phenylbutyrate (both inhibitors of ER stress.) Similar to DMSO and 4-phenylbutyrate, HDL inhibited tunicamycin-induced ER stress in a dose-dependent manner. Similar results were obtained with thapsigargin suggesting that the response to HDL is not unique to tunicamycin. These results suggest that HDL inhibits ER stress in hepatocytes and by extension, may be of benefit to other cell types that are sensitive to ER stress, such as coronary artery endothelial cells and pancreatic β -cells.

Nothing to Disclose: RA, EN, SS, A-RA, MJH, ADM

P1-512

Comparison of the Effects of Metabolic Syndrome Risk Factors on the Changes of TG and Cholesterol Content in VLDL and LDL Subfractions after 10 Weeks of Fructose Consumption in Older Overweight/Obese Adults.

RI Menorca¹, KL Stanhope¹, SC Griffen¹, K Nakajima², NL Keim³, EJ Schaefer², AA Bremer¹, JM Schwarz⁴ and PJ Havel¹.

¹Univ of California, Davis Davis, CA ; ²Tufts Univ Sch of Med Boston, MA ; ³United States Dept of Agriculture, Western Human Nutrition Res Ctr Davis, CA and ⁴Univ of California, San Francisco San Francisco, CA.

We have reported that 10 weeks of fructose consumption at 25% of energy requirements increases postprandial plasma TG and fasting apolipoprotein B, LDL-C, and small dense LDL-C (sdLDL-C) concentrations in overweight/obese adults (43-70 years), while 10 weeks of glucose consumption does not. We also reported that the increase of sdLDL-C was more than two times larger in subjects with 3 metabolic syndrome risk factors (MSRF) than in subjects with 0-2 MSRF. To determine the risk factor most associated with the development of fructose-induced dyslipidemia, we investigated the effects of fasting TG and HDL concentrations and weight circumference on the changes of the TG and cholesterol content in VLDL and LDL subfractions in subjects consuming fructose. Fasting and postprandial (5 h post-dinner) plasma collected during baseline and 10 wk intervention was analyzed by HPLC for cholesterol and TG content in 5 VLDL and 6 LDL subfractions separated by particle size. In separate ANOVAs, subjects were divided based on normal and high waist circumference (W_B), normal and high fasting TG concentrations (TG_B), or low and normal fasting HDL concentrations (HDL_B) at baseline based on the NCEP ATP III Guidelines. Subjects with low HDL_B had greater increases of postprandial TG content in large VLDL particles and lower increases in small VLDL particles compared with subjects with normal HDL_B ($P = 0.0009$, $HDL_B \times size$). Subjects with high TG_B exhibited greater increases of postprandial TG content in all VLDL subfractions than subjects with normal TG_B , especially in large VLDL particles ($P = 0.005$, $TG_B \times size$). Postprandial TG content was less increased in large LDL particles and more increased in small LDL particles in subjects with high compared with normal TG_B ($P = .0066$, effect of $TG_B \times size$), whereas there were no differences between subjects when grouped by HDL_B or W_B . The changes of cholesterol content in the fasting LDL subfractions were also affected by TG_B , but not by W_B and HDL_B . Fasting LDL cholesterol content was more increased in all LDL subfractions in subjects with high TG_B than subjects with normal TG_B ($P = .019$, $TG \times size$), with the differences between the 2 groups being significant in the smallest LDL particles ($P < 0.05$). Thus, fasting TG concentrations >150 mg/dl appear to be more closely associated with the processes that lead to increased levels of sdLDL-C than other MSRF in overweight/obese subjects after 10 weeks of fructose consumption.

Nothing to Disclose: RIM, KLS, SCG, KN, NLK, EJS, AAB, JMS, PJH

P1-513

Effect of RANKL Inhibition by Denosumab on Serum Cholesterol in Postmenopausal Women with Osteoporosis.

MA Bolognese¹, N Daizadeh², A Wang², C Haller², A Daniels², S Siddhanti² and L Grazette².

¹Bethesda Hlth Res Ctr Bethesda, MD and ²Amgen Inc Thousand Oaks, CA.

ApoE knockout mice lacking osteoprotegerin (OPG), a RANKL decoy receptor, develop advanced vascular plaque progression and calcification in addition to osteopenia. In epidemiological studies, alterations of the OPG/RANKL/RANK system have been inconsistently associated with altered lipid profiles and atherosclerotic calcification. Denosumab (DMAb), a fully human mAb against RANKL, reduced fracture risk in the phase 3 FREEDOM study of postmenopausal women with osteoporosis (Cummings et al, NEJM 2009;361:756). In that study, more DMAB than placebo (Pbo) subjects reported hypercholesterolemia (7.2% vs 6.1%) as an adverse event (AE), although this was without laboratory confirmation. The incidences of all, serious, and positively-adjudicated cardiovascular AEs were balanced between the groups. To further explore the effect of RANKL inhibition by DMAB on cholesterol levels, posthoc analyses were conducted on stored serum samples from 2 studies.

In the phase 3 FREEDOM study, postmenopausal women with osteoporosis were randomized to Pbo or DMAB 60 mg Q6M for 36 mos. In the phase 2 study, postmenopausal women with low BMD were randomized to Pbo, DMAB 60 mg Q6M, or alendronate 70 mg QW for 12 mos. Total cholesterol was measured in phase 3, non-fasting, stored samples from 450 randomly selected subjects (224 Pbo, 226 DMAB) and total cholesterol, HDL, and LDL were measured in phase 2, fasting, stored samples from 157 subjects (79 Pbo, 78 DMAB) at baseline, 6 mos, and 12 mos. Cholesterol values at baseline, 6 mos, and 12 mos, and changes in cholesterol values from baseline at 6 and 12 mos were not statistically different between the Pbo and DMAB groups in either study (Table). Subgroup analyses of the phase 3 data focusing on subjects with prior history of hypercholesterolemia or prior use of cholesterol-lowering drugs showed similar results.

Data from these posthoc analyses demonstrate that RANKL inhibition by DMAB is not associated with increases in serum total or LDL cholesterol.

| | | | Baseline | 6 months | 12 months | Change at 6 months | Change at 12 months |
|---------------|---------|---------|----------------|----------------|----------------|--------------------|---------------------|
| Total (mg/dL) | Phase 3 | Placebo | 234 (198, 261) | 226 (197, 259) | 230 (199, 251) | -6 (-24, 9) | -5 (-23, 13) |
| | | DMAB | 236 (209, 263) | 228 (202, 258) | 233 (205, 261) | -6 (-21, 11) | -3 (-21, 15) |
| | Phase 2 | Placebo | 237 (202, 262) | 230 (204, 256) | 240 (202, 257) | -1 (-18, 12) | 2 (-14, 14) |
| | | DMAB | 226 (212, 249) | 229 (207, 252) | 223 (205, 243) | -1 (-13, 18) | -3 (-17, 12) |
| HDL (mg/dL) | Phase 2 | Placebo | 60 (52, 71) | 60 (50, 72) | 60 (50, 75) | -1 (-4, 3) | -1 (-4, 4) |
| | | DMAB | 62 (53, 74) | 61 (50, 71) | 63 (50, 72) | -1 (-6, 3) | -1 (-6, 3) |
| LDL (mg/dL) | Phase 2 | Placebo | 145 (123, 176) | 139 (115, 166) | 148 (113, 162) | -6 (-20, 10) | -5 (-18, 9) |
| | | DMAB | 143 (118, 166) | 139 (119, 157) | 138 (124, 153) | -5 (-17, 12) | -5 (-16, 11) |

Values are medians (Q1, Q3)

Sources of Research Support: Amgen Inc.

Disclosures: MAB: Consultant, Lilly USA, LLC, Amgen; Speaker, Lilly USA, LLC, Roche Pharmaceuticals, Novartis Pharmaceuticals, Amgen. ND: Employee, Amgen. AW: Employee, Amgen. CH: Employee, Amgen. AD: Employee, Amgen. SS: Employee, Amgen. LG: Employee, Amgen.

P1-514

Effect of L-Carnitine (LC) on Postprandial Lipid and Insulin Levels in HIV-Positive Patients on Highly Active Antiretroviral Therapy (HAART).

AS Semrad DO¹, S Devaraj PhD¹, TJ Semrad MD¹, E Anuurad MD, PhD¹, DM Asmuth MD¹ and L Berglund MD¹.

¹Univ of California, Davis Sacramento, CA.

Background: HIV patients on HAART have increased risk of coronary artery disease (CAD). Triglycerides (TGs) rise in the proinflammatory postprandial state and recent evidence associates hypertriglyceridemia with the development of CAD. LC facilitates the transfer of long-chain acyl groups into the mitochondria and reduces fasting TGs. Since mitochondrial metabolism is altered in HIV patients, we hypothesized that LC would improve postprandial lipid responses in HIV(+) patients on HAART.

Methods: We conducted a double-blind randomized placebo controlled study in HIV(+) patients undergoing stable protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitor (NNRTI)-based HAART for at least 6 months. After a 2-week low fat dietary run in period, patients were randomized to 3 grams of LC or placebo once daily for 4 weeks while continuing the diet. After 4 weeks of treatment, subjects returned for a 10-hour metabolic study evaluating the response to a fatty shake. Blood samples were taken at 0 (pre-shake), 3, 5, 7 & 10 hours to measure insulin, glucose, TG, nonesterified fatty acids (NEFA), remnant like particles (RLP)-C and total cholesterol (TC) levels.

Results: Sixteen subjects were enrolled and 13 completed the study (7 placebo, 6 LC). At enrollment, the two groups were well-matched for height, fasting glucose and lipid levels, but BMI and weight were higher in the placebo group ($p < 0.05$). For all subjects at time 0, HDL correlated with NEFA ($r = 0.59$) and negatively correlated with RLP-C ($r = -0.67$), and insulin ($r = -0.66$, all $p < 0.05$). The two groups had similar postprandial levels of TG, RLP-C and TC. However, the total AUC for NEFA was significantly lower ($p = 0.04$) in the LC group compared to the placebo group. Furthermore, the total ($p = 0.01$) and incremental ($p = 0.07$) area under the curves (AUC) for insulin were lower in the LC group as was insulin resistance after treatment, measured by HOMA-IR (2.37 vs. 0.95, $p = 0.05$).

Conclusion: Postprandial insulin and NEFA levels are lower in HAART-treated HIV(+) patients taking LC compared to placebo. However, there is no difference in postprandial TG or RLP-C levels. These results suggest that LC may lower insulin resistance in HIV(+) patients on HAART.

Sources of Research Support: California Research Center for the Biology of HIV in Minorities; Grant Number UL1 RR024146 from the National Center for Research Resources.

Nothing to Disclose: ASS, SD, TJS, EA, DMA, LB

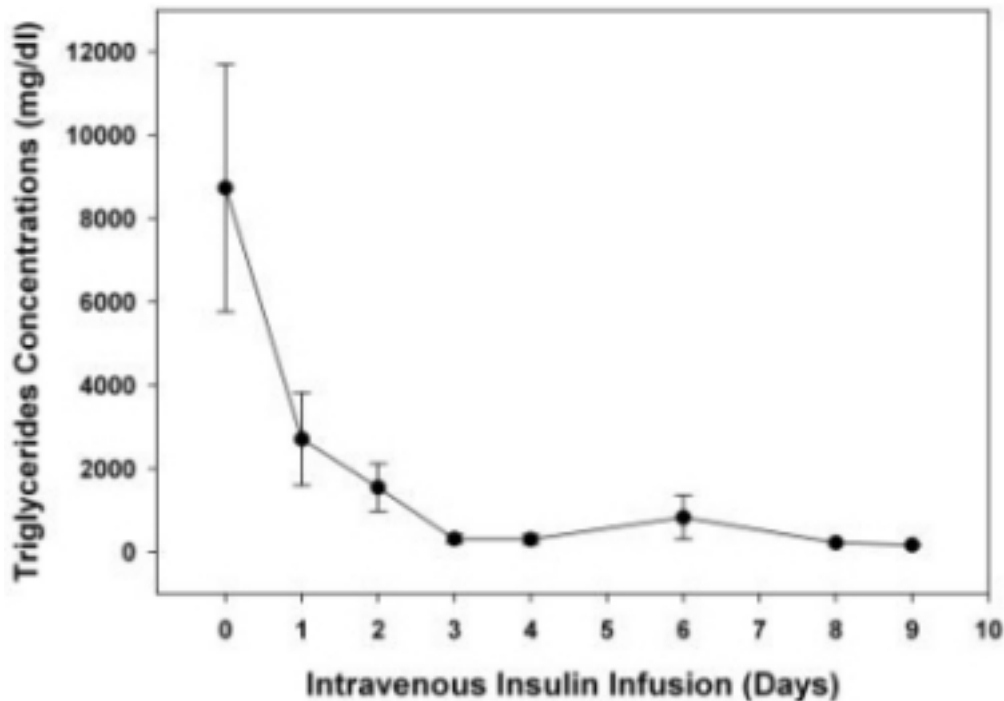
P1-515

Treatment of Severe Hypertriglyceridemia with an Intravenous Insulin Infusion.

Toufic Abdo¹, Mehul Vora¹, Deepti Rawal¹, Sandeep Dhindsa¹, Ajay Chaudhuri¹ and Paresch Dandona¹.

¹SUNY at Buffalo Buffalo, NY.

Severe hypertriglyceridemia is associated with considerable morbidity including acute pancreatitis, sepsis and respiratory failure. Oral triglyceride lowering drugs, subcutaneous insulin, plasma exchange and apheresis have variously been used. In view of the ability of insulin to activate lipoprotein lipase and to increase the clearance of triglyceride, we used the infusion of insulin intravenously in a series of 7 patients with severe hypertriglyceridemia (median: 9,500; 777- 17, 250 mg/dl). Six of these patients had diabetes mellitus while 4 had acute pancreatitis. One patient had a pancreatic pseudocyst. One of these patients developed renal failure and needed hemodialysis while another developed acute respiratory failure. Insulin was infused intravenously at a rate of 2U/hr in most patients with glucose to maintain normoglycemia. Triglyceride concentrations fell by >50% within 24 hours, reverted to less than 1000mg/dl within 4 days in most of the patients and diminished to <10% of the baseline by 8 days. All patients recovered from their pancreatitis and other co-morbid conditions and were discharged from the hospital. Long term treatment was continued with oral triglyceride lowering agents and with basal insulin as appropriate. We conclude that intravenously infused insulin is a rapidly and consistently effective triglyceride lowering drug, is simple to administer. The known potent anti-inflammatory action of insulin may be an additional benefit. Intravenous insulin may be ideal way to treat severe hypertriglyceridemia.



Disclosures: AC: Speaker, Lilly USA, LLC, Merck & Co., Novo Nordisk, Sanofi-Aventis; Research Funding, Sanofi-Aventis. PD: Speaker, Sanofi-Aventis; Research Funding, Sanofi-Aventis, GlaxoSmithKline, Amylin Pharmaceuticals; Speaker, Lilly USA, LLC, GlaxoSmithKline, Amylin Pharmaceuticals.

Nothing to Disclose: TA, MV, DR, SD

P1-516

Beyond LDL: Residual Cardiovascular Risk at a Diabetes Clinic Serving a Low Income Hispanic Population.

EO Beale MD¹, P Situ NP², T Luna-Lollie RN CDE² and W-A Lee DO¹.

¹Univ of Southern California Los Angeles, CA and ²Edward R Roybal Comprehensive Hlth Ctr Los Angeles, CA.

Background: In patients with longstanding DM there is little evidence that tight glycemic control improves macrovascular outcomes, the major cause of mortality. By contrast it is now recognized that there is residual CVS risk from non-LDL lipids. Optimizing levels of LDL, HDL and TGs can be expected to significantly improve outcomes in populations with a high prevalence of longstanding DM, such as low-income Hispanics.

Aim: To ascertain the effects of current management on LDL, HDL and TGs and the drugs used in management, at a protocol-driven, nurse-supervised diabetes clinic serving a predominantly uninsured Hispanic population in East Los Angeles with longstanding DM.

Method: Ttest and chi-squared analysis of prospectively collected data from patients attending the clinic in 2009 who had more than 1 visit to the clinic. Data includes patients who were discharged for achieving clinic A1c goal (<8%), non-adherence, lost to follow-up and other reasons.

Results: 446 patients, 44.4% male, 51.7(9.6)years, duration of diabetes 10.7(7.7)years, adm. BMI 31(4), adm. A1c 10.2(2.1) discharge A1c 8.2(1.8), p<0.001. Ave stay in clinic 6.5(3.9) months. See tables. LDL was above goal on adm. and fell into the goal range with significant increase in statin alone and in combination with ezetimibe. HDL remained below goal in both genders, but improved significantly in females. TGs fell significantly with no change in fibrate use, likely due to improved glycemic control.

Comparison of Admission and Discharge Lipids (n=399)

| | LDL in | LDL out | HDL (m:f) | HDL out | TGs in | TGs out |
|-------------------|--------|---------|-----------------|------------------|--------|---------|
| Mean | 107.0 | 95.1 | 43.6(41.4:45.6) | 44.6(41.2:47.2) | 209 | 174 |
| SD | 39.6 | 37.4 | 11.2(11.6:11.6) | 12.8(12.07:13.4) | 202 | 163 |
| p-value in vs out | | <0.001 | | 0.05(0.2:0.006) | | <0.001 |

in=on admission out=on discharge

Use of Lipid Lowering Agents on Admission to and Discharge from Clinic

| | Statin in | Statin out | Statin-Ezetimibe Combo in | Statin-Ezetimibe Combo out | Fibrate in | Fibrate out |
|------------------------|-----------|------------|---------------------------|----------------------------|------------|-------------|
| % of Patients on Agent | 53.0 | 74.9 | 3.6 | 7.3 | 6 | 6 |
| p-value in vs, out | | <0.001 | | 0.02 | | 0.3 |

in=on admission out=on discharge

Discussion: In a low-income Hispanic population at high risk for macrovascular disease attending a protocol-driven clinic, the ave LDL is at goal on discharge. TGs and HDL improve but remain slightly above goal. Attention should be given to improving these factors with lifestyle strategies and medication, particularly fibrate and niacin.

Nothing to Disclose: EOB, PS, TL-L, W-AL

P1-517

Gene Polymorphisms of ENPP1 and MTHFR as Risk Factors to Cardiovascular Disease in Turner Syndrome Patients.

KC Oliveira MSc¹, B Bianco PhD¹, AD Guedes MD PhD¹, BB Galera PhD², MF Galera MD PhD², ITN Verreschi MD PhD¹ and MVN Lipay PhD¹.

¹Fed Univ of Sao Paulo Sao Paulo, Brazil and ²Cuiabá Univ Cuiabá, Brazil.

Epidemiological studies had revealed a decrease on live expectance of until 13 years of Turner syndrome (TS) patients related to normal women. The main death cause is cardiovascular diseases (CVD), which are a common, multifactorial disorder likely to be influenced by multiple genes of modest effect. Risk factors to CVD, as systemic arterial hypertension and glicidic metabolism abnormalities have a higher frequency on TS carriers. The A121C variant of the ectonucleotide pyrophosphatase/ phosphodiesterase 1 (*ENPP1*) gene is associated with obesity, insulin resistance, type 2 diabetes and cardiovascular morbidity in various ethnic populations.

Abnormal folate/homocysteine metabolism due to polymorphisms in genes involved in this pathway, including the two most commonly occurring variants, C677T and A1298C, in the *MTHFR* gene, has been implicated as an etiologic factor in CVD. In a previous study, our group had observed that the *MTHFR* gene polymorphism C1298C, that reduces the enzyme efficiency, was more frequent in TS patients, suggesting its involvement in chromosomal imbalances (Oliveira et al., Arq Bras Endocrinol Metabol. 2009; 53:115). Considering the association between these gene polymorphisms with CVD, therefore, we hypothesized that they might also be associated with these conditions on TS patients.

A hundred and twenty-five TS patients and 294 unrelated healthy controls, with no familiar history of CVD were evaluated for *ENPP1* A121C gene polymorphism and also for C677T and A1298C, in the *MTHFR* gene. Genotypes were assessed by PCR-RFLP and qPCR.

The frequencies of AA, AC and CC genotypes of the *ENPP1* polymorphism in TS patients were 43%, 47% and 10% ($p=0.5655$), and 44%, 43% and 13% in control group. Regarding the *MTHFR* C677T polymorphism, the frequencies of genotypes CC, CT and TT in TS patients were 50%, 44% and 8% ($p=0.0088$) and 56%, 30% and 14% in control group. Considering the *MTHFR* A1298C, the frequencies of genotypes AA, AC and CC in TS patients were 44%, 47% and 18% ($p<.0001$) and 65%, 30% and 5% in control group. No significant associations were observed for the *ENPP1* polymorphisms on TS patients which could reflect a specific risk of occurrence. However, both the *MTHFR* gene polymorphism at position 1298 and 677, was more frequent in the group of TS patients. The findings of the present study support the hypothesis that the polymorphisms in *MTHFR* gene are associated with the genetic susceptibility for CVD in patients with TS.

Oliveira et al., Arq Bras Endocrinol Metabol. 2009; 53:115.

Sources of Research Support: FAPESP (Grants No. 2008/03597-0 and 2009/05250-0).

Nothing to Disclose: KCO, BB, ADG, BBG, MFG, ITNV, MVNL

P1-518

Growth Hormone Treatment but No Hormonal Replacement Therapy Influences Favorably Lipid Profile in Women with Turner Syndrome.

Tomasz J. Irzyniec MD, PhD^{1,2} and Wacław Jez MD, PhD³.

¹Silesian Univ Sch of Med Katowice, Poland ; ²Hosp of the Ministry of Interior Affairs Katowice, Poland and ³The Specialized Hosp No 2 Bytom, Poland.

Introduction. Turner syndrome (TS) is one of the forms of gonadal malfunction. Women with TS show a higher risk of developing cardiovascular diseases (CD), including arteriosclerosis that occur due to also carbohydrate and lipid metabolism disorders. The aim of a hormone therapy is not only treatment of short stature (growth hormone-GH) and sex hormone deficit compensation, but also counteraction of the deficit effects, including the reduction of the risk of CD. *Aim.* This study aimed the assessment of the lipid and carbohydrate metabolisms in TS women in context of the hormone replacement therapy (HRT) as well as the use of the GH in their childhood.

Materials and methods: One hundred and sixty four TS-women (age 25±8y) were investigated in the years 1995-2004. TS was diagnosed and confirmed by karyotyping. Karyotype 45,X was presented by 88 of them (54%). The information about the application of HRT or previous use of GH was collected during the interviews. In all patients gonadotropins (FSH and LH) and 17 beta-estradiol (E2) levels were measured. Also the total cholesterol (TC), high and low density lipoprotein (HDL and LDL), and triglycerides (TG) as well as glucose in blood serum were assessed.

Results: Only 95 patients (58%) out of the total of 164 declared they currently received the HRT. Although in these women observed significantly lower levels of gonadotropins (FSH 32,1±22,1 vs. 44,2±23,3 IU/l p<0.001 and LH 20,8±17,5 vs. 26,6±18,1 IU/l p=0,04) as well as higher levels of E2 (136±148 vs. 89,9±100 pmol/l p<0.03) compared to TS-women who did not receive HRT, no differences in the lipid parameters were found. TS women who received the HRT only showed slightly no statistically significant lower glucose level (5±0,7 vs. 5,2±1 mmol/l p=0.08). In the group of patients with TS who were treated with GH in childhood (33 women-21,5±5 y), there were statistically significant more favourable results regarding the lipid metabolism parameters compared to the patients (113 persons-23,3±5 y) and no GH therapy (TC 4,5±0,8 vs. 5,0±1,1mmol/l p=0,02, HDL 1,4±0,4 vs. 1,5±0,5 mmol/l - ns, LDL 2,9±0,7 vs. 3,3±0,9 mmol/l - p=0,03 and TG 0,8±0,3 vs. 1,1±0,6 g/l - p=0,012, respectively).

Conclusions: 1. The hormone replacement therapy in women with TS does not significantly affect the lipid metabolism parameters. 2. The use of the growth hormone in childhood of women with TS favorably influences their lipid profile.

Nothing to Disclose: TJI, WJ

P1-519

Effect of Testosterone on Inflammatory Markers in Development of Early Atherogenesis in the Testicular Feminised Mouse (Tfm) Model.

DM Kelly BSc¹, DJ Sellers BSc, PhD¹, MN Woodroffe BSc, PhD¹, TH Jones BSc, MBChB, MD, FRCP² and KS Channer BSc, MBChB, MD, FRCP³.

¹Sheffield Hallam Univ Sheffield, UK ; ²Barnsley Hosp NHS Foundation Trust Barnsley, UK and ³Sheffield Teaching Hosps NHS Foundation Trust Sheffield, UK.

Objectives Atherosclerosis is a chronic inflammatory disease characterised by leukocyte infiltration and adhesion, and cytokine production surrounding a lipid core. Men with cardiovascular disease have a high prevalence of low testosterone which is associated with a greater progression of atherosclerosis over time. Testosterone replacement therapy (TRT) can improve several cardiovascular factors including insulin resistance and visceral obesity. This study investigates whether the known atheroprotective effect of testosterone in the testicular feminised (Tfm) mouse model¹ is associated with an effect on inflammatory factors.

Methods Tfm express a non-functional androgen receptor (AR) and low levels of circulating testosterone. Tfm mice were fed a high cholesterol diet (42% butterfat, 1.25% cholesterol and 0.5% cholate) ad libitum for 28 weeks and received either physiological testosterone replacement (intramuscular mixed testosterone esters, Sustanon 100®, 25mg/kg) or placebo (saline) and were compared to wild type littermate controls. Aortic root serial sections were analysed by oil red O staining and percentage lipid deposition calculated. Evidence of inflammatory cells was investigated by immunohistochemistry. Blood was analysed for testosterone, 17 β -estradiol, lipids and cytokines (TNF α , IL-6, IL1 β , IL-10 and MCP-1). Investigators were blinded to treatment group throughout sample analysis. (ANOVA).

Results TRT significantly reduced lipid deposition in Tfm mice receiving a high cholesterol diet compared to placebo-treated controls (2.15 \pm 0.17% versus 4.70 \pm 1.04%, respectively; $P=0.05$). No significant differences were detected in any serum cytokine measured. Immunocytochemistry detected monocyte infiltration locally adjacent to lipid streaks.

Discussion The protective effect of testosterone on fatty streak formation in Tfm mice may possibly act through the modulation of lipid metabolism but may also mediate the local inflammatory response. An effect of TRT on circulating cytokines may not have been observed as lipid streaks were localised to the aortic root and insufficient to produce a systemic inflammatory response.

1 Nettleship JE et al., Circulation 2007;116:2427.

Sources of Research Support: Biomedical Research Centre, Sheffield Hallam University.

Nothing to Disclose: DMK, DJS, MNW, THJ, KSC

P1-520

Characterization of Postprandial Lipemia among Filipino Coronary Artery and Cerebrovascular Disease Patients with Normal Fasting Lipid Levels on Treatment.

AM Galia MD¹, BC Chua MD¹, MB Platon RND¹, KI Llave RND¹, MB Zacarias MD¹ and LB Mercado-Asis MD PhD¹.

¹Univ of Santo Tomas Hosp Manila, Philippines.

Background: Postprandial hyperlipemia has been observed in healthy individuals.⁽¹⁾⁽²⁾ Among patients with major cardiovascular or cerebrovascular events (CAD/CVD) maintained on statins, postprandial peaking may still be noted even with normal fasting lipid levels.

Objective: To characterize postprandial lipemia among CAD/CVD individuals with normal fasting lipid levels maintained on statins.

Methodology: Six individuals (age $63 \pm 1.5y$; 3 males) who had documented myocardial infarction or cerebral infarct in not less than six months and maintained on statin (atorvastatin 20-80mg/d, simvastatin 20-40mg/d x 6months or more) and with normal fasting lipid levels based on NCEP-ATP III guidelines were studied. They received a standard set of meals consisting of 35% fat divided into three meals and two snacks. Fasting, preprandial, and 4-hr postprandial lipid determinations were done. No postprandial peaking was noted in the total cholesterol, LDL, and HDL. In both triglyceride (TG) and VLDL levels, 5 significant peaks were noted, with 4 peaks above target levels. Fasting TG (fTG) vs 4th, 6th, 8th, 10th, and 12th hours were as follows: 115.5 vs 193.7, 192.5, 162.7, 143.7, and 146.0 mg/dL ($p < 0.05$). Fasting VLDL (fVLDL) vs 4th, 6th, 8th, 10th, and 12th hours were as follows: 23.1 vs 38.7, 38.5, 32.5, 28.7, and 29.2 mg/dL ($p < 0.05$). A sustained elevation of TG and VLDL levels from baseline was noted for ten hours.

Conclusion: Significant postprandial peaking of TG and VLDL sustained for ten hours was observed in individuals with CAD/CVD maintained on statins.

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(2) Gabriel F., Abejuela Z., Mercado-Asis L, Zacarias M, Sumpio J, Llave KI, Platon B. Orlistat and Postprandial Lipemia. 2008 (submitted for publication, International Journal of Endocrinology and Metabolism)

Nothing to Disclose: AMG, BCC, MBP, KIL, MBZ, LBM-A

P1-521

Metabolic Syndrome in Patients with Acute Ischemic Stroke.

C Matos MD¹, F Mota MD¹, ML Pereira MD¹, JR Fontes MD¹ and A Frias MD¹.

¹Hosp Braga Braga, Portugal.

INTRODUCTION: The combination of metabolic changes in an individual, known as Metabolic Syndrome (MS) is associated with a high cardiovascular risk, with most studies reporting an increased risk of coronary heart disease.

OBJECTIVES: To assess the prevalence of MS in patients with acute ischemic stroke.

METHODS: Prospective study of patients consecutively admitted to the Stroke Unit during a one year period. The anthropometric data and lipid profile were collected in the first 24 hours after admission, to avoid the consequences of prolonged rest and nutritional changes. In patients without known diabetes mellitus, a 75 g oral glucose tolerance test was carried out after the 4th day of hospitalization, to allow clinical stabilization. For the definition of MS, the IDF (2005) criteria of MS for the European population were used and compared with the results obtained using the recommended criteria for the US population.

RESULTS: Seventy eight patients were studied, 41% female and 59% male. Age was 62.10 ± 13.82 years. The overall prevalence of MS was 46% (35% in men and 63% in women). 94% (30/32) of women had high waist circumference, compared with 65% of men (30/46). In addition to waist circumference, the more prevalent criteria for MS were: hypertension, decreased HDL cholesterol, diabetes mellitus or elevated fasting glucose and hypertriglyceridemia, respectively. As expected, the prevalence of MS was lower, using the US criteria for waist circumference.

DISCUSSION: This group of patients illustrates the high prevalence of MS in patients with acute ischemic stroke. The higher prevalence in women than in men (63% vs 35%) may be explained by the increased frequency of high waist circumference in women, according to the IDF criteria for sex.

Nothing to Disclose: CM, FM, MLP, JRF, AF

P1-522

Triglyceride: HDL-Cholesterol Ratio as a Surrogate Marker for Insulin Resistance and Inflammation in Pediatric Obesity.

O Kawadry D.O.¹, J Kichler PhD¹, R Hoffmann PhD¹ and R Alemzadeh M.D.¹.

¹Children's Hosp of Wisconsin Wauwatosa, WI.

Background/Aims: Cardiovascular risk factors tend to precede the development of atherosclerosis in obese children and adults. The triglyceride (TG):HDL-cholesterol (TG:HDL-C) ratio has been identified as a marker to predict the risk of coronary artery disease, but its relationship with indices of insulin resistance remains inconclusive. To date, the relationship between the TG:HDL-C ratio and markers of insulin resistance and inflammation while controlling for potential confounding factors such as body adiposity, gender, and ethnicity has not been studied.

Materials and Methods: Fasting serum glucose, insulin, lipids, homeostatic model assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), highly sensitive c-reactive protein (hs-CRP), and hemoglobin A_{1c} (HbA_{1c}) were evaluated in 163 subjects (F/M), aged 13.1 ± 2.8 years [66 Caucasian (C), 50 Hispanic (H), and 47 African American (AA)] [body mass index (BMI) 36.8 ± 7.9]. Body composition was determined by bioelectrical impedance.

Results: There were no differences in BMI, fat mass (FM), serum glucose, insulin, QUICKI, HOMA-IR, lipid profile, and HbA_{1c} among the three ethnic groups. Subjects with HOMA-IR ≥ 4.0 had higher BMI (p < 0.001), FM (p < 0.0001), TG:HDL-C (p < 0.02), and hs-CRP (p < 0.02) than those with HOMA-IR < 4.0. However, subjects with TG:HDL-C ≥ 3.0 did not display a higher degree of BMI, insulin, adiposity, HbA_{1c}, HOMA-IR, and QUICKI values than those with TG:HDL-C < 3.0. Nevertheless, the TG:HDL-C ratio correlated with HOMA-IR (r = 0.20, p < 0.01), QUICKI (r = -0.20; p < 0.01), and hs-CRP (r = 0.46, p < 0.0001) when controlling for FM, gender and ethnicity.

Conclusions: The TG:HDL-C ratio is correlated with markers of insulin resistance and inflammation even after controlling for potential confounding factors such as adiposity, gender, and ethnicity. Therefore, the TG:HDL-C ratio can be utilized not only as an independent marker of insulin resistance, but also as a reliable tool for identification of atherosclerosis risk in obese children and adolescents.

Nothing to Disclose: OK, JK, RH, RA

P1-523

Carotid Intima to Medial Thickness (IMT) Measurements in Normal, Healthy Children Using Radio Frequency (RF) Technique.

SG Badrieh MD¹, A Vijayasarithi MS³, CS Stump MD, PhD¹ and SJ Goldberg MD¹.

¹The Univ of Arizona Tucson, AZ.

Objective: Common Carotid artery Intimal-Media Thickness (CCA-IMT) is the distance between the carotid intima and muscular wall and detects thickened intima and/or lipid deposition in that space. CCA-IMT is considered a surrogate for coronary artery atherosclerosis (3). CCA-IMT is currently measured by a laborious point to point method or an ultrasound enveloping B mode technique. Recently, a radio frequency (RF)-based technique has emerged. RF analysis appears to improve precision, is more time efficient and user independent for deriving IMT (1). RF analyzes unprocessed raw RF signals with fewer assumptions (2). Our goal was to establish the first RF derived normal CCA-IMT values in healthy, normal weight children.

Methods: We studied healthy volunteers, ages 5-20 years. We specified that subjects should be of normal weight, but planned to accept all responders. Parents or the subject (if >18 years) consented to an IRB approved protocol, and provided family and diet history. All subjects had a bilateral RF CCA-IMT measurement of the middle half of the CCA performed by a single, experienced ultrasonographer. Mean value for the CCA-IMT was determined automatically from the RF signal imposed on the B-mode image.

RESULTS: Our method allowed enrollment of 84 consecutive volunteers. BMI in 76 subjects was <25. 8/84 had a BMI in (25-28) range. Mean age=12.2; SD=4.2 years. Mean BMI was 19.2; SD=4.1. Male/female distribution was 64.2%/35.8%. Mean IMT for the population was 0.424; SD=0.04mm. No significant differences were detected when right and left CCA-IMT were compared (0.419; SD=0.04 and 0.428; SD=0.04; p=0.22). Male vs female had a mean of 0.426; SD=0.04mm and 0.419; SD=0.04mm; p=0.44. CCA-IMT for age more or less than 12 years showed respective means of 0.429; SD=0.037mm vs 0.419; SD=0.04mm; p=0.24. No significant difference in CCA-IMT was found for those with or without family history for coronary disease and for those who frequented fast food restaurants as opposed to those who did not. Our RF determined mean values were identical to those determined by prior methods (4-7).

Conclusion: 1) CCA-IMT values for the RF technique were comparable to values obtained by investigators using B mode techniques. 2) Mean CCA-IMT did not progress between 5 and 20 years in normal weight subjects. 3) No significant gender differences were found. 4) Our results can serve as a normal reference for comparing RF CCA-IMT data in patients of similar age groups.

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Sources of Research Support: The University of Arizona research foundation; Partial support from Southern Arizona Veterans Affairs Health Care System.

Nothing to Disclose: SGB, AV, CSS, SJG

P1-524

***In Vitro* and *In Vivo* Anticancer Effects of Metformin and the AMP-Activated Protein Kinase (AMPK) Activator 5-Aminoimidazole-4-Carboxamide-1- β -D-Ribofuranoside (AICAR): Enhanced Activity in the Presence of the Local Microenvironment, Activated Akt/mTOR Pathway and Exposure to Glycolysis Inhibitors.**

Melissa Ooi MD¹, Douglas W. McMillin PhD¹, Vassiliki Kotoula MD, PhD², Joseph Negri¹, Jake Delmore¹, Constantine S. Mitsiades MD, PhD¹, Kenneth C. Anderson MD¹ and Nicholas Mitsiades MD, PhD¹.

¹Dana-Farber Cancer Inst, Harvard Med Sch Boston, MA and ²Sch of Med, Aristotle Univ of Thessaloniki Thessaloniki, Greece.

Context: Obesity and insulin resistance are associated with increased risk for several malignancies, possibly due to hyperinsulinemia and increased circulating free IGF-I levels that can have growth-promoting and anti-apoptotic effects on cancer cells. Epidemiologic studies have suggested that type 2 diabetic patients treated with metformin have lower cancer incidence and lower cancer-related mortality than diabetics who receive sulfonylurea monotherapy or insulin. Even more intriguing is the recent finding that metformin can have direct anticancer activity *in vitro* against prostate and breast carcinoma cell lines.

Objective/Methods: We investigated the direct effects of metformin and AICAR on a panel of 17 hematopoietic (multiple myeloma) and 9 solid tumor (thyroid, prostate, breast carcinoma) cell lines *in vitro* using MTT, flow cytometry, annexin V-PI, immunoblotting, as well as compartment-specific bioluminescence assays in the absence or presence of bone marrow stromal cells or differentiated osteoclasts. The *in vivo* anticancer activity of AICAR was evaluated in a xenograft model in NOD.CB17 SCID mice.

Results: Metformin and AICAR exerted on all cell lines tested *in vitro* a dose-dependent growth-suppressive effect, that was preserved or even enhanced in the presence of stromal cells or osteoclasts. Mitochondrial membrane depolarization and apoptosis (evidenced by Annexin V staining and cleavage of caspases and PARP) were induced by AICAR, but not metformin. AICAR-induced apoptosis was attenuated by overexpression of Bcl2 and enhanced by activation of Akt. Treatment with metformin or AICAR increased phospho-AMPK and decreased phospho-p70S6K, phospho-S6 ribosomal protein and cell cycle protein (Cyclin D1, Cdk4 and Cdc2) levels. AICAR treatment significantly decreased tumor burden and prolonged survival in our xenograft model. Two glycolysis inhibitors, 3-bromopyruvate and 2-deoxyglucose, exhibited synergistic cytotoxicity and apoptosis induction in combination with AICAR or metformin.

Conclusions: We demonstrate a direct, growth-suppressive effect of metformin and AICAR in various malignancies, involving activation of AMPK and inhibition of p70S6K, that can be potentiated by interactions with the local microenvironment, the Akt pathway and glycolysis inhibitors. These data support the clinical evaluation of metformin and other AMPK activators as anticancer agents, especially for malignancies with an active Akt/mTOR pathway.

Nothing to Disclose: MO, DWM, VK, JN, JD, CSM, KCA, NM

P1-525

Vitamin D Metabolism Genes CYP27A1 and CYP24 Are Present in Thyroid Carcinoma Cell Lines.

SE Wakeley MD¹, RG Bennett PhD^{1,2}, FG Hamel PhD^{1,2} and WS Goldner MD¹.

¹Univ of Nebraska Med Ctr Omaha, NE and ²Veterans Administration Med Ctr Omaha, NE.

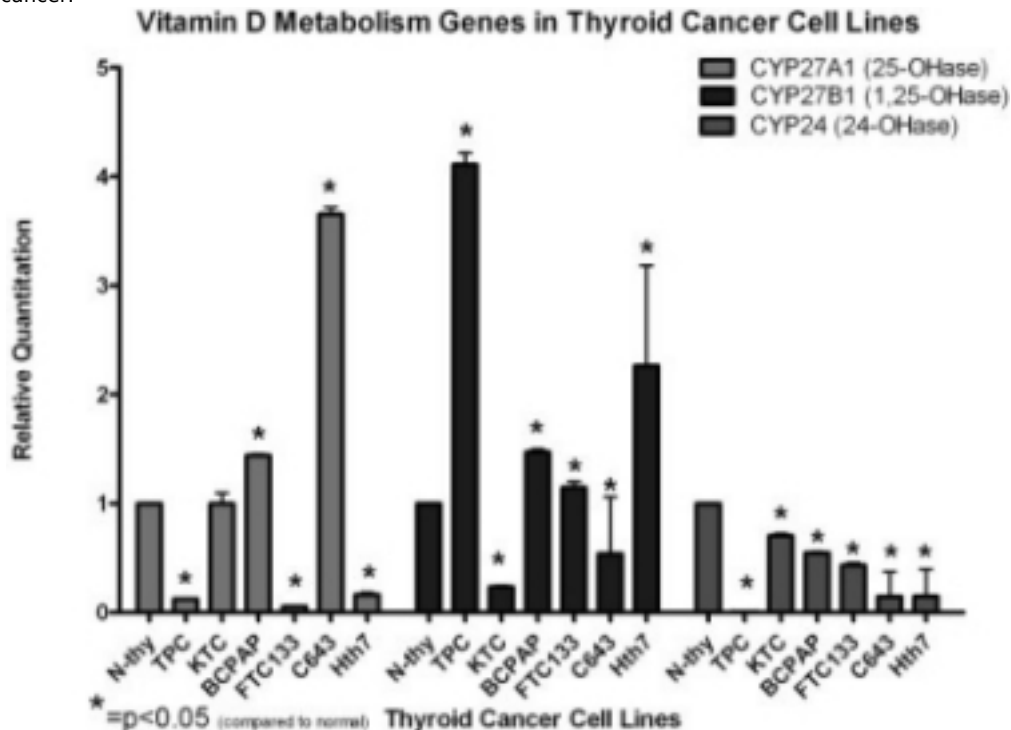
Vitamin D deficiency has been implicated in the pathogenesis of many cancers but not definitively linked to thyroid cancer (1). However, treatment with active Vitamin D (1,25(OH)₂D) has been reported to reduce the aggressiveness of thyroid cancer in animal models (2). Vitamin D metabolism involves many enzymatic steps. D₂ and D₃ are converted to 25(OH)D, then to 1,25(OH)₂D and broken down to 1,24,25(OH)₂D. This metabolism is regulated by the genes CYP27A1, CYP27B1, and CYP24 respectively (3). Vitamin D receptors and CYP27B1 expression are present in thyroid cells (4,5). To our knowledge, there is no data on the presence or absence of CYP27A1 and CYP24 in thyroid cells. Methods: At

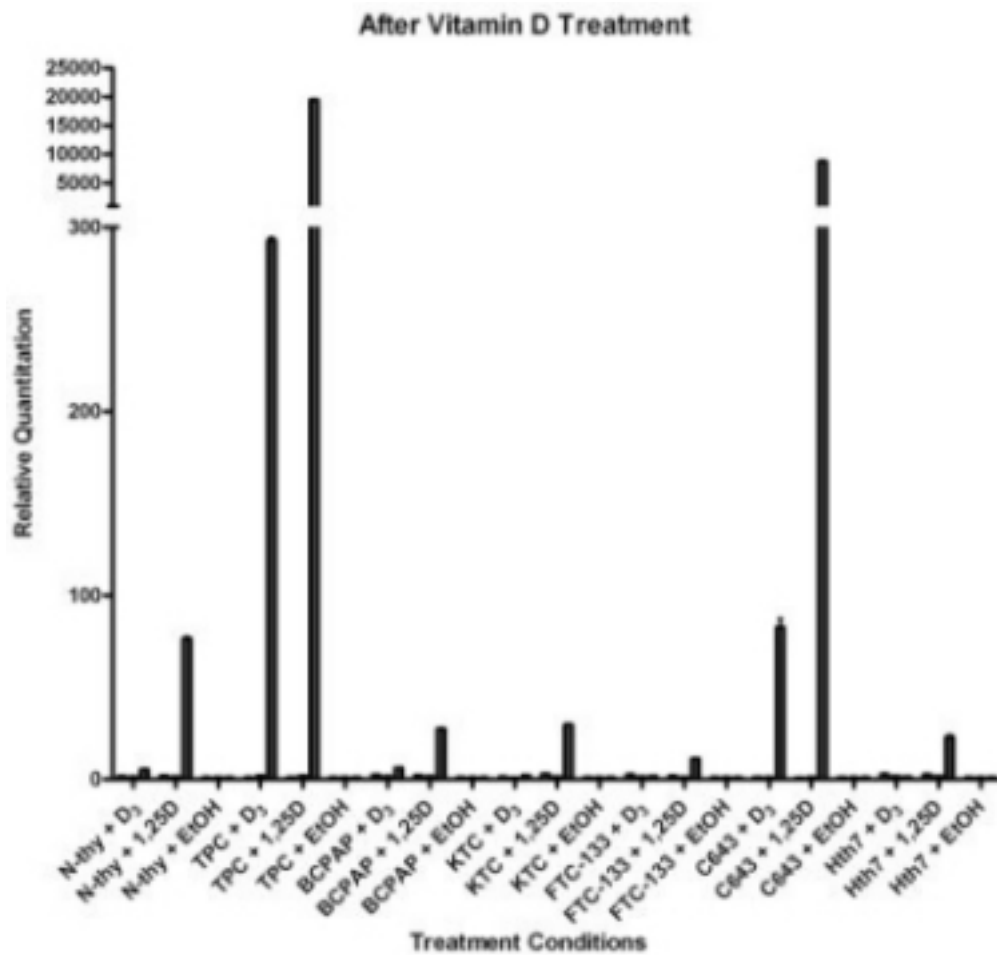
baseline and after treatment with vitamin D₃ (10⁻⁵ M) and 1,25(OH)₂D (10⁻⁷ M), we evaluated gene expression of CYP27A1, CYP27B1, and CYP24 using real time RT-PCR, in 6 thyroid cancer cell lines and 1 normal thyroid cell line.

Thyroid Cancer Cell Lines

| Thyroid Cancer Type | Cell Line | Mutation |
|---------------------|-------------|-------------|
| Papillary | TPC-1 | RET/PTC1 |
| Papillary | BCPAP | BRAF(V600E) |
| Papillary | KTC-1 | BRAF(V600E) |
| Follicular | FTC-133 | - |
| Anaplastic | C643 | HRAS(G13R) |
| Anaplastic | Hth7 | BRAF(WT) |
| Normal | Nthy-ori3-1 | - |

Results: CYP27A1, CYP27B1 and CYP24 were present in all cell lines, yet level of gene expression varied amongst different thyroid carcinoma cell lines compared to normal thyroid cells. Vitamin D treated cancer cells showed variable expression of both CYP27A1 and CYP27B1 and marked differences in CYP24. Conclusion: We report the novel finding that CYP27A1 and CYP24 are present in thyroid cancer cells and expression varies depending on the histologic type and molecular mutation of the cell line. Further study is needed to understand the role of vitamin D metabolism in thyroid cancer.





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Nothing to Disclose: SEW, RGB, FGH, WSG

P1-526

CXCL11 Chemokine Is Up-Regulated, by Cytokines, in Papillary Thyroid Cancer, and Modulated by PPARgamma Agonists.

P Fallahi MD¹, SM Ferrari¹, A Corrado¹, I Ruffilli¹, M Minuto MD¹, P Miccoli Prof¹ and A Antonelli MD¹.

¹Univ of Pisa - Sch of Med Pisa, Italy.

Context. CXCL11 is one of three chemokines known to bind the receptor CXCR3, the two others being CXCL9 and CXCL10. CXCL11 differs from the other CXCR3 ligands in both the strength and the particularities of its receptor interactions: it has a higher affinity, is a stronger agonist, and behaves differently. In papillary thyroid carcinomas (PTC), oncogenes activate a transcriptional program in papillary thyroid cancer cells (PTCs) that includes upregulation of the CXCL10 chemokine, which in turn stimulates proliferation and invasion. PPARgamma activators thiazolidinediones (TZDs), modulate CXCL10 secretion, and inhibited PTC growth. However, until now, no study evaluated CXCL11 in PTC.

Objective and Methods. The effects of IFNgamma and TNFalpha stimulation on CXCL11 secretion in PTCs and normal thyroid cells (TFC) were tested. Furthermore, the effect of PPARgamma activation by thiazolidinediones (TZDs), rosiglitazone and pioglitazone, on CXCL11 secretion and proliferation in these cell types was studied.

Results. In primary cultures of TFC and PTCs, CXCL11 production was absent under basal conditions; a similar dose-dependent secretion of CXCL11 was induced by IFNgamma in both cell types. TNFalpha alone, induced a slight but significant CXCL11 secretion only in PTCs. The stimulation with IFNgamma or TNFalpha+IFNgamma induced a synergistic CXCL11 release in TFC; a significantly higher (2-3 times higher) CXCL11 secretion was induced in PTCs. Treatment of TFC with the TZDs, dose-dependently suppressed IFNgamma+TNFalpha-induced CXCL11 release, while stimulated CXCL11 secretion in PTCs. A significant antiproliferative effect by TZDs was observed only in PTCs.

Conclusions. IFNgamma and TNFalpha induce a huge release of CXCL11 by PTCs, suggesting that oncogenes activate a transcriptional program in PTCs that includes upregulation of the CXCL11 chemokine, which in turn may stimulate proliferation and invasion. A discrepancy between the stimulatory effect of TZDs on CXCL11 secretion and the inhibitory role on PTCs proliferation is shown.

Nothing to Disclose: PF, SMF, AC, IR, MM, PM, AA

P1-527

The t-Boc Derivative of 7-(O)-Carboxymethyl Daidzein Is Cytotoxic to the Human Follicular Thyroid Carcinoma Cell Line WRO and a Negative Growth Modulator in Non-Malignant Human Goiter Cells *In Vitro*: Potential Role of Reactive Oxygen Species (ROS).

Meital Grafi-Cohen MSc¹, Orli Sharon BSc¹, Zaki Kraiem PhD², Fortune Kohen PhD³, Naftali Stern MD¹ and Dalia Somjen PhD¹.

¹Tel-Aviv Sourasky Med Ctr and the Sackler Fac of Med Tel-Aviv, Israel ; ²Technion, Haifa, India and ³The Weizmann Inst Rehovot, Israel.

The incidence of thyroid cancer is up to three fold higher in women than in men, suggesting that estrogenic effects may be involved in the pathogenesis of this malignancy. We previously reported that a novel phytoestrogen derivative generated in our laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein [cDtbc], possesses potent anti-cancer effects in several human cancer cell types expressing preferentially mRNA for estrogen receptor (ER) β relative to ER α . These earlier studies indicated that cDtbc exerted cytotoxic effects in thyroid cancer by the induction of apoptosis and not through cell necrosis. In the present study, we compared the effect of cDtbc in the follicular thyroid carcinoma cell line WRO and cultured primary goiter cells originally harvested from 3 different patients. Both WRO and primary thyroid cells expressed ER α and ER β with only slightly higher abundance of ER β over ER α . In both WRO and goiter cells, DNA synthesis and cytotoxic to thyroid cancer cells and, to a lesser extent, to non-malignant goitrous cells, acting in part via induction of ROS formation. Since WRO cells are clearly estrogen sensitive, thus resembling the estrogen-sensitivity of other thyroid carcinoma cell lines reported by us earlier (NPA, MRO and ARO), this property can be utilized to design highly effective estrogen-related, anti-thyroid cancer drugs. Further, the present study in goiter cells opens creatine kinase (CK: a marker of genomic response to estrogen agonists) increased in response to E₂, the ER α agonist PPT and the ER β agonist DPN. Very significantly, cDtbc abolished these effects completely in WRO but only partially in non-malignant goiter cells. Acting in the absence of E₂, cDtbc alone was cytotoxic to WRO, as determined by DNA synthesis indices, the XTT assay and direct microscopic visualization, and much less so to human non-malignant goiter cells. A functionally critical effect of cDtbc was its ability to markedly increase reactive oxygen species (ROS) formation, which was particularly prominent in WRO, but was also detectable in goiter cells. The NADPH-oxidase inhibitor DPI not only abolished ROS formation but also partially inhibited the cytotoxic effects of cDtbc on WRO. Hence, cDtbc possesses some antiestrogenic properties in thyroid cancer cells and is involved in pathways through which the growth of non-malignant goiters can possibly be modulated.

Nothing to Disclose: MG-C, OS, ZK, FK, NS, DS

P1-528

Treatment of Dkk-1 Inhibits Thyroid Cancer Cell Growth by Regulating Wnt/ β -Catenin Signaling.

SH Kwak MD¹, SW Cho MD, PhD¹, YJ Park MD, PhD¹, SH Kim¹, HY Ahn MD¹, MJ Kim MD¹, SH Ahn MD, PhD¹, SY Kim MD, PhD¹ and BY Cho MD, PhD¹.

¹Seoul Natl Univ Coll of Med Seoul, Korea.

Aberrant activations of Wnt/ β -catenin pathway is one of the common pathogenesis of various human cancers. Dickkopf (Dkk)-1 is a secretory protein which inhibits Wnt/ β -catenin signaling by binding to Wnt co-receptor, LRP5/6. In this study, we investigated the effect of Dkk-1 in papillary thyroid cancer in vitro. We demonstrated that human Wnt3 gene was up-regulated in 6 papillary thyroid cancer tissue samples and protein expression levels of total β -catenin was also increased in these samples. Treatment of Dkk-1 at 20 to 100nM strongly inhibited cell growth in papillary thyroid cell line in dose-dependent manners and also increased protein expression levels of cleaved caspase-3. Low expression levels of total β -catenin and phospho β -catenin protein were observed in Dkk-1 treated cell lysate. BHP10-3 cell lines underwent parallel experiments, and also showed the inhibitory effect on cell growth but not significant. Retrovirally mediated expression of constitutively active β -catenin restored this inhibitory effect of Dkk-1 to thyroid cancer cell line. Collectively, our results indicate that Dkk-1 inhibited thyroid cancer cell growth by altered Wnt/ β -catenin pathway in vitro.

Nothing to Disclose: SHK, SWC, YJP, SHK, HYA, MJK, SHA, SYK, BYC

P1-529

NF- κ B-Dependent Growth in an Orthotopic Mouse Model of Advanced Thyroid Cancer Suggests a Role for Interleukin-8 and Angiogenesis.

KT Bauerle BS¹, G Deep PhD¹, RE Schweppe PhD¹, R Agarwal PhD¹ and BR Haugen MD¹.

¹Univ of Colorado Denver - Anschutz Med Campus Aurora, CO.

Approximately 1,700 people die each year in the U.S. from advanced thyroid cancer. Recent understanding of molecular signaling has uncovered potential therapeutic targets for these patients. Our laboratory has focused on the role of NF- κ B signaling in thyroid cancer cell growth, invasion, and angiogenesis. Three anaplastic cancer cell lines (8505c, SW1736, C643) and two papillary cancer cell lines (BCPAP, TPC1) were used to establish tumors in an orthotopic nude mouse model with injection of cancer cells directly into the thyroid. Tumor formation was observed only with the BCPAP and 8505c cell lines. To investigate the role of NF- κ B in tumor progression, we inhibited NF- κ B via overexpression of a dominant-negative I κ B α (mlkB), and *in vivo* tumor growth of both cell lines was significantly inhibited by mlkB (Final Tumor Volume = [BCPAP vector (276.8 mm³) vs mlkB (75.4 mm³); [8505c vector (172.7 mm³) vs mlkB (62.8 mm³)]. Blockade of NF- κ B signaling resulted in 8505c cell growth arrest *in vitro* through S-phase arrest, but had no effect on the BCPAP cells. We therefore investigated another mechanism by which NF- κ B inhibition blocks BCPAP tumor growth *in vivo*. Using an *in vitro* model of angiogenesis, capillary tube formation by endothelial cells (HUVECs) was inhibited when exposed to conditioned media from mlkB-expressing cells compared to control. Dot-blot analysis of secreted proteins (Angiogenesis Antibody Array, R&D Systems) from conditioned media of cells demonstrated that IL-8 secretion was blocked by mlkB. We then measured secreted levels of IL-8 and transcript levels in all five cell lines. IL-8 mRNA and protein levels were significantly reduced in BCPAP, SW1736, TPC1 and C643, but not 8505C cells. Interestingly, secreted IL-8 levels were ten-fold higher in the tumorigenic BCPAP and 8505C cell lines, compared with the non-tumorigenic cell lines. In summary, the inhibition of *in vivo* BCPAP tumor growth by NF- κ B signaling blockade in our orthotopic model is likely due to decreased tumor angiogenesis and is associated with decreased IL-8 levels. Furthermore, high local IL-8 levels may be important for *in vivo* tumor growth. Taken together, these data suggest that, while NF- κ B signaling appears to inhibit thyroid cancer growth through different mechanisms, its regulation of IL-8 expression may ultimately lead to tumor angiogenesis and growth, making it an attractive therapeutic target in the treatment of advanced thyroid cancer.

Nothing to Disclose: KTB, GD, RES, RA, BRH

P1-530

A New Mouse Transgenic Model To Monitor Adult Thyroid Stem Cells.

D DelPrete PhD¹, H Ye PhD¹, G Barila BSc¹, L Joannas BSc¹ and DL Altschuler PhD¹.

¹Univ of Pittsburgh Pittsburgh, PA.

Stem cells have the ability to self-renew and give rise to different cell types. Recently, adult thyroid stem cells have been identified in human goiter and normal mouse thyroids; however, their role in thyroid homeostasis and/or thyroid tumor formation is for the moment unknown, awaiting the development of appropriate lineage tracing tools.

We have recently developed a new reporter mouse line for lineage tracing studies; LoxP/FRT sites positioned in opposite orientation, surround a cassette encoding a GFP unit followed by dsRed in an antisense orientation. The reporter strain ubiquitously expresses GFP under the CMV promoter, giving green fluorescence in almost every cell type examined. CRE/Flp recombinase is able to "flip" a piece of DNA when LoxP/FRT sites are positioned in opposite orientation. Therefore, when the reporter strain is crossed with a tissue-specific inducible form of CRE/Flp, dsRed is appropriately positioned for expression, and a switch from green to red fluorescence occurs, both transiently (inducible) and in a cell type-specific manner.

Thyroids analysis from this line revealed that although follicular cells (PAX8+, Tg+) were GFP+, a small cell population was GFP-. Interestingly, these GFP- cells scored positive for SCA1, CD133 and CD34, and negative for thyroid markers PAX8 and Tg.

Thyroid single cell suspensions form spheres when plated in non-adherent and serum-free conditions (i.e. thyrospheres). Sorted GFP- cells were however unable to form spheres unless mixed with GFP+ cells. Conditioned medium from GFP+ cells rescues the spheroid-forming activity of GFP- sorted cells, indicating an active communication between the stem cell and differentiated thyroid compartments.

All thyrospheres were SCA1+; however, while the central cell in spheres was always GFP- (GFP- Sca1+ CD34+ Tg-), all peripheral cells were GFP+ (GFP+ SCA1+ CD34- Tg+), consistent with a central renewable stem cell compartment maintaining a peripheral differentiation program.

Thyrospheres can differentiate in vitro to follicular cells (TSHR+ NIS+ TPO+) and can form functional follicles when grown under collagen-embedded conditions. Their ability to reconstitute a functional T4-producing unit is currently examined using thyroidectomized syngeneic mice. Moreover, the participation of thyroid Sca1+ cells in thyroid homeostasis and tumorigenesis is currently under study utilizing our reporter line in combination with Sca-CRE-ER and Tg-Flp-ER mice.

Sources of Research Support: NIH DK063069 to DLA.

Nothing to Disclose: DD, HY, GB, LJ, DLA

P1-531

Translocator Protein (TSPO) in Human Thyroid Tumors: Correlation with Thyroid Cancer Phenotype, Akt Activation and Resistance to Apoptosis.

J Klubo-Gwiedzinska MD, PhD¹, K Jensen MD², MJ Hardwick PhD¹, KD Burman MD¹, L Wartofsky MD¹, D Van Nostrand MD¹ and VV Vasko MD, PhD².

¹Washington Hosp Ctr Washington, DC ; ²Uniformed Services Univ of the Hlth Scis Bethesda, MD ; ³Washington Hosp Ctr Washington, DC and ⁴Washington Hosp Ctr Washington, DC.

Introduction

The translocator protein (TSPO) is a peripheral benzodiazepine receptor that regulates mitochondrial cholesterol transfer, mitochondrial membrane potential and apoptosis. TSPO over-expression has been implicated in the progression of epithelial tumors including breast and prostate cancers. TSPO ligands are being investigated in clinical trials for use in positron emission tomography (PET) imaging.

Material and Methods

We characterized TSPO expression and Akt activation in 120 human thyroid tumors including 25 follicular adenomas (FA), 15 follicular cancers (FCs), 70 papillary cancers (PCs), 10 medullary cancers (MCs), 26 lymph node metastases (LNM) and 2 distant metastases (DM) by immunohistochemistry. Using rtPCR, Western blot and immunostaining we assessed TSPO expression in FC, PC and MC-derived cell lines and in cells transfected with AKT constructs. In an H₂O₂-inducible oxidative stress model, changes in mitochondrial membrane potential were evaluated with fluorogenic cation (JC-1). Cell proliferation and apoptosis assays were performed after cell treatment with TSPO ligands (PK11195 and Ro5-4864) and PI3K/Akt inhibitor (LY-294002).

Results

TSPO expression was detected in 10 of 56 normal thyroid tissue samples. Compared to normal thyroid, TSPO expression was increased in 6/25 FAs, 3/15 FCs, 40/70 PCs and in 9/10 MCs. The intensity of TSPO immunostaining was higher in PCs (p= 0.003) and MCs (p=0.0001) compared to follicular tumors. High level of TSPO expression was found in 21/26 LNM and in 2/2 DM and was similar to corresponding primary tumors.

TSPO expression correlated with Akt activation in thyroid cancer cell lines. Forced over-expression of Akt was associated with increased TSPO expression. Oxidative stress was associated with Akt inhibition and depolarization of mitochondrial membranes. MC-derived cells were more resistant to H₂O₂ compared to FC or PC-derived cells. Pharmacological inhibition of PI3K/Akt signaling was associated with increased sensitivity to oxidative stress in all thyroid cancer cells. TSPO-ligands prevented H₂O₂ inducible mitochondrial membrane depolarization.

Conclusion

TSPO expression is increased in PCs and MCs and correlates with Akt activation in thyroid cancer cells. High level of TSPO in metastatic samples suggests the possible application of TSPO ligands for PET imaging of metastases, especially in patients with medullary cancer.

Nothing to Disclose: JK-G, KJ, MJH, KDB, LW, DVN, VVV

P1-532

CYP24A1 Haplotypes Are Associated with Differentiated Thyroid Carcinoma.

M Penna-Martinez PhD¹, E Ramos-Lopez MD¹, J Stern¹, H Kahles MD¹, N Hinsch MD¹, M-L Hansmann MDPHD¹, I Selkinski MD¹, F Gruenwald MDPHD¹, C Vorlaender MD², WO Bechstein MDPHD¹, S Zeuzem MDPHD¹, K Holzer MDPHD¹ and K Badenhoop MDPHD¹.

¹Univ Hosp Frankfurt am Main, Germany and ²Buergerhospital Frankfurt am Main, Germany.

Background: Previously we reported an association of vitamin D receptor (VDR) polymorphisms and differentiated thyroid cancer (DTC) risk. The aim of the present study was to investigate other vitamin D pathway genes such as CYP27B1, CYP2R1 and CYP24A1 which code for enzymes that, respectively, synthesize and degrade 1,25(OH)₂D₃ in patients with DTC and healthy controls (HC). Also its influence on 25(OH)D₃ and 1,25(OH)₂D₃ plasma levels in DTC was evaluated. **Patients and methods:** Two hundred fifty five patients with DTC [papillary (PTC): n = 205; follicular (FTC): n = 50] and HC (n = 269) German origin, were genotyped for CYP2R1, (rs10741657), CYP27B1 (rs10877012) and CYP24A1 (rs2248137, rs2296241) using real time PCR. Furthermore, the 25(OH)D₃ and 1,25(OH)₂D₃ plasma levels in patients were measured by radioimmunoassay. **Results:** No difference was observed between DTC patients and HC in the genotype frequencies of the named polymorphisms. However the haplotype rs2248137C/ rs2296241A for CY24A1 (13.9 % vs. 20.4%; p corrected (pc) = 0.01) was less frequent in the PTC whereas the haplotype rs2248137C/rs2296241G (57.8 % vs. 41.4%; pc = 0.01) was more frequent in the FTC compared to HC. In addition there was no correlation between 25(OH)D₃ or 1,25(OH)₂D₃ levels and the rs10741657, rs10877012, rs2248137 and rs2296241 polymorphisms in DTC patients. **Conclusion:** Haplotypes within of the CYP24A1 gene appear to be associated with differentiated thyroid cancer in Germans. While the haplotype "CA" for CYP24A1 polymorphisms confers protection from PTC, the haplotype "CG" is associated with an increased FTC risk. These findings suggest further alterations in the vitamin D system that possibly influence the pathogenesis of DTC. Such an alteration of vitamin D pathways may affect the thyroid tissue and not be apparent in the circulation. Since this is the first report associating CYP24A1 polymorphisms with thyroid carcinoma, these findings need to be confirmed in studies with larger numbers of patients and in other populations.

Nothing to Disclose: MP-M, ER-L, JS, HK, NH, M-LH, IS, FG, CV, WOB, SZ, KH, KB

P1-533

Forkhead Box A2 Is Down-Regulated in a Microarray Analysis of Korean Papillary Thyroid Cancers.

Eui Dal Jung MD¹, Ho Sang Shon MD, PhD¹, Ji Hyun Lee MD, PhD¹ and Chang Ho Cho MD, PhD¹.

¹Sch of Med, Catholic Univ of Daegu Daegu, Korea.

Background: Papillary thyroid cancer (PTC) is the most common malignancy in the thyroid. Several molecular mechanisms are involved in the development and progression of PTC. BRAF V600E point mutation was identified as the most common genetic abnormality in PTC. BRAF mutation was known to be more prevalent in Korean PTC patients than in patients from other countries. We investigated distinct genetic profiles in Korean PTC using cDNA microarray analysis.

Methods: Transcriptional profiles of 5 PTCs and corresponding normal tissues were generated using cDNA microarrays. The tumors were genotyped for BRAF V600E point mutation. The results of cDNA microarray gene expression were confirmed by real-time polymerase chain reaction (PCR) and immunohistochemistry in 35 PTC patients. **Results:** Four of 5 patients had BRAF V600E point mutation. The cDNA microarrays of 5 PTCs showed that 96 gene expressions were increased and 16 gene expressions were decreased in cDNA microarrays of 5 PTCs. Real-time RT-PCR showed increased expressions of *SLC34A2*, *TM7SF4*, *COMP*, *KLK7*, and *KCNJ2* genes and decreased expressions of *FOXA2*, *SLC4A4*, *LYVE-1*, and *TFCP2L1* genes in 35 PTCs compared with paired normal tissue. Especially, *FOXA2* is down-regulated by showing a cytoplasmic accumulation in the immunohistochemistry of 35 PTCs. **Conclusion:** These findings demonstrate both similarities and some differences between our results and previous result. In Korean PTC, *FOXA2* is down-regulated by cytoplasmic expression. Further study is needed to confirm the relationship between *FOXA2* and *BRAF* mutations in Korean PTC. These findings may provide insight into the molecular pathways involved in Korean PTC.

Nothing to Disclose: EDJ, HSS, JHL, CHC

P1-534

X-Linked Inhibitor of Apoptosis (XIAP) in Papillary Thyroid Carcinoma.

JH Yim M.D.¹, WG Kim M.D.¹, G Gong M.D., Ph.D.¹, EY Kim M.D.¹, TY Kim M.D., Ph.D.¹, JH Joon M.D.¹, SJ Hong M.D., Ph.D.¹, WB Kim M.D., Ph.D.¹ and YK Shong M.D., Ph.D.¹.

¹Asan Med Ctr, Univ of Ulsan Coll of Med Seoul, Korea.

Backgrounds: The X-linked inhibitor of apoptosis (XIAP) is one of potent inhibitors of apoptosis and known to be associated with aggressiveness of cancer. In this study, we evaluated the expression of XIAP and the clinical impacts of XIAP in papillary thyroid carcinoma (PTC).

Patients and Methods: XIAP expression was examined by immunohistochemical staining in 162 specimens from conventional PTC and 82 specimens from other malignant or benign thyroid tumors. We evaluated the association of XIAP with clinicopathologic factors of conventional PTC. V600E mutation status in BRAF gene was examined by direct sequencing from amplified genomic DNA samples.

Results: XIAP was positive in 128 of 162 conventional PTC (79%) and 3 of 18 follicular variant of PTC (17%). The positive rates of XIAP in follicular, medullary, poorly differentiated and anaplastic thyroid carcinoma were 7%, 30%, 57%, and 30%, respectively. XIAP was negative in follicular adenoma and nodular hyperplasia. The expression of XIAP was associated with cervical lymph node metastasis ($p=0.004$) and BRAF V600E mutation ($p=0.025$) in conventional PTC. However, there was no difference in disease free survival according to expression of XIAP.

Conclusion: The expression of XIAP was more prominent in conventional PTC and more aggressive thyroid malignancy. Although XIAP was not independent prognostic factor, XIAP expression was commonly associated with BRAF V600E mutation and cervical lymph node metastasis.

Nothing to Disclose: JHY, WGK, GG, EYK, TYK, JHJ, SJH, WBK, YKS

P1-535

A Web-Based Thyroid Tumor Database Coupled with a Biospecimen Bank Is a Unique Resource Available for Epidemiological and Translational Studies.

KJ Treude BS¹, S Sherman PhD¹, O Shats MS¹, JM Erickson RN, MS¹, AS Kueh MS¹, WM Lydiatt MD¹, RB Smith MD¹ and WS Goldner MD¹.

¹Univ of Nebraska Med Ctr Omaha, NE.

The steady increase in thyroid cancer incidence rates in the U.S. requires novel studies of risk factors causing this disease. However, existing cancer registries often lack detailed demographic, lifestyle, and dietary data, as well as data on medical and family histories. To overcome this deficiency, we developed a web-based, secure database linked with a biospecimen bank, the Thyroid Tumor and Cancer Collaborative Registry (TCCR, <http://tccr.unmc.edu>, clinicaltrials.gov # NCT00693368) that is aimed at collecting demographic, lifestyle, dietary and clinical information, as well as biospecimen obtained from patients diagnosed with thyroid cancer and nodules. **Methods:** Adults with a history of thyroid carcinoma or ultrasound confirmed thyroid nodules are eligible to participate in the TCCR-based studies. Once informed consent is obtained, a subject is given a choice to fill out a secure online questionnaire or its paper equivalent. The questionnaire consists of the following sections: demographics; occupational, environmental, and lifestyle exposure history; medical, gynecological, medication, and family history; dietary habits; and a quality of life survey. Medical personnel enter data on diagnostic procedures, clinical presentation, pathology, and treatment. Subjects are asked to provide a blood sample (plasma, serum, and DNA) and a urine sample. TCCR, linked with the caBIG biobank software caTissue, allows cross-referencing with biologic specimens. **Results:** The registry commenced with IRB approval in March, 2008. Since its inception we have approached 728 eligible patients, and 84.8% (86%F/14%M) have consented to take part in the registry. 98.2% of consented patients also consented to genetic studies. 92.6% are Caucasian, 4.2% African American, 1.9% Hispanic, 1.0% Asian or Pacific Islander, 0.3% American Indian. 41.7% of the subjects in the registry have thyroid cancer. 54.5% of the thyroid cancer and 44.2% of the nodule patients have completed their questionnaires. **Conclusion:** Recruitment for the TCCR has been highly successful and the majority of subjects have consented to genetic research. The integration of a web-based database with a biobank provides a unique resource for translational research. The modern technical approaches, standardized questionnaires, and built-in multi-center functionality create great potential for collaboration with outside investigators from multiple institutions.

Nothing to Disclose: KJT, SS, OS, JME, ASK, WML, RBS, WSG

P1-536

The Role of the Grb14 Adaptor Protein in RET Signaling in Thyroid Cancer.

Katalin Balogh¹, Sylvia Asa¹ and Shereen Ezzat¹.

¹Ontario Cancer Inst Toronto, Canada.

We have previously shown that members of the fibroblast growth factor receptor (FGFR) family play opposing functions on the immediate substrate FRS2 α . Gene expression profiling has identified the Grb14 adaptor protein as a target of the FGFR2-IIIb isoform. This adapter binds avidly to several tyrosine kinases, protecting their kinase loops from dephosphorylation. In particular, Grb14 inhibits insulin receptor (IR) and insulin-like growth factor receptor (IGF-IR) catalytic activities and impedes their peptide substrates binding. Grb 7 and 10 have already been shown to interact directly with the RET kinase through their SH2 domain in a phosphorylation-dependent manner. To determine the functional role of Grb14 in RET signaling, we used three thyroid cancer cell lines (TT, TPC-1 and WRO) where specific Grb14 expression was identified at the mRNA and protein levels. Co-immunoprecipitation confirmed physical interaction between RET and Grb 14 in the presence of serum stimulation. Stable knockdown of Grb14 resulted in the anticipated protection of the insulin receptor 1158/1162/1163 sites from de-phosphorylation. In contrast, RET/PTC phosphorylation was diminished without impact on Erk1/2 phosphorylation, consistent with a more selective role for Grb14 in interrupting signals. Moreover, loss of Grb14 resulted in diminished cell proliferation and invasion with re-expression of the TTF-1 differentiation marker. These findings uncover a new role for the most restricted member of the Grb7/10/14 family in modulating survival signals in thyroid cancer.

Sources of Research Support: Canadian Institutes of Health Research.

Nothing to Disclose: KB, SA, SE

P1-537

MicroRNA Expression Profiling of Medullary Thyroid Carcinomas.

A Boichard MS¹, JM Bidart PharmD PhD¹, A Al Ghuzlan MD¹, B Caillou MD¹, G Meurice¹, C Orear¹, M Talbot¹, S Broutin PharmD¹, C Dupuy PhD¹, M Schlumberger MD PhD¹ and L Lacroix PharmD PhD¹.

¹Gustave Roussy Inst Villejuif, France.

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor derived from C-cells. Activating mutation of RET oncogene are found in most hereditary cases and in half of sporadic cases. Other molecular mechanisms independent of RET remain still unclear. Small, endogenous, non-coding microRNAs (miRNAs) are potent regulators of many biological processes. Deregulations of miRNAs have been observed in many human cancers including epithelial thyroid carcinomas (1) and specific miRNA profiles have been described for invasive tumors (2). The aim of the current study is to explore miRNA expression in MTC.

MiRNA expression profile was analyzed in 53 frozen samples, including sporadic (n=39) and familial (n=14) MTC, using Agilent® miRNA chips. Clinical records indicated that 16 patients had no initial invasive features (TxN0M0) and 37 had lymph node or distant metastases. When present, RET mutations are located at codon 918 (n=13), 634 (n=12) or at other codons (n=6). Differences in miRNA expression between these groups of tumors were analyzed with Significance Analysis of Microarray (SAM) algorithm.

A signature of twenty three miRNAs allowed to distinguish non invasive from invasive tumors. A significantly higher expression (with a fold-change > 2 and q-values < 0.05) was observed in invasive tumors for 18 miRNAs including hsa-miR-21, hsa-miR-214, hsa-miR-155, and hsa-miR-199. On the other hand, 5 miRNAs, including hsa-miR-137, hsa-miR-224 and hsa-miR-129, had a significantly higher expression in non invasive tumors. There was no relationship in miRNA expression and RET status or familial history.

We conclude that miRNA expression profile in MTC is related to tumor aggressiveness. Among these miRNAs, some have already been involved in cell growth, proliferation and invasiveness of malignant cells (i.e. hsa-miR-21 (3)). Overall, our study suggests that miRNA profile could be used for prognosis assessment in MTC. Investigations are being carried out on functions of miRNA in MTC oncogenesis.

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Nothing to Disclose: AB, JMB, AAG, BC, GM, CO, MT, SB, CD, MS, LL

P1-538

Successful Localization of Medullary Thyroid Carcinoma and Its Metastases Using CCK-2 Receptor Scintigraphy with ^{99m}Tc-Demogastrin 2.

A.C. Froberg MD¹, W.W. de Herder MD PhD¹, B.A. Nock PhD², T Maina PhD², M de Jong Prof¹, C.H. van Eijck Prof¹, M Smits MD¹, M Verrijsseldonck¹, W.A. Breeman PhD¹ and E.P. Krenning Prof¹.

¹Erasmus MC Rotterdam, Netherlands and ²I/R-RP, NCSR "Demokritos" Athens, Greece.

Objectives

In medullary thyroid carcinoma (MTC) serum calcitonin is a very sensitive and specific tumor marker. However, with the present available diagnostic imaging modalities, localization of recurrent tumor or metastases often is unsuccessful. MTC show over-expression of cholecystokinin-2 receptors (CCK2R) in >90%; these might be viable targets for radionuclide scintigraphy. In animal studies promising results were obtained with ^{99m}Tc-N₄-Gly-(D)Glu-(Glu)₅

-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH₂ (^{99m}Tc-Demogastrin 2) and studies in patients (pts) were started.

Patients and Methods

17 out of 32 scanned pts with MTC had no known tumor localization before scintigraphy. Results of these pts (calcitonin levels 1.2-143 (median 10) times the upper reference value) are reported. Scintigraphy was performed at 3-4 and/or 8 and/or 24 h post injection of ± 800 MBq ^{99m}Tc-Demogastrin 2. Positive and equivocal findings were correlated with CT, MRI and/or ultrasound and if feasible fine needle aspiration cytology of neck lesions was done.

Results

Of 9 out of 17 pts scans were judged positive. In 7 of them, lesions not only in the neck but also in mediastinum/lung-hilum, bone, lung and/or liver were confirmed (at least 1 lesion in each pt). The smallest proven MTC metastasis (neck) was 2,9 mm. In 2 pts neck lesions have not yet been confirmed (in 1 no clear retention of radioactivity over time). Three scans showed equivocal lesions, in these pts correlating imaging did not show evidence of tumor. Five scans were judged negative. In 2 pts non-visualized neck lesions were subsequently discovered by other means and resected. In these metastases in-vitro autoradiography showed lack of clear CCK2R over-expression.

Conclusions

Total body CCK2R scintigraphy with ^{99m}Tc-Demogastrin 2 is a new and very promising diagnostic tool to localize MTC and its metastases in pts with elevated calcitonin levels as even very small metastases (< 5 mm) and lesions at unexpected locations could be visualized. Further studies are needed to further define its value in patient management.

Nothing to Disclose: ACF, WWdH, BAN, TM, MdJ, CHvE, MS, MV, WAB, EPK

P1-539

Analysis of the p27Kip1 Gene in a RET-Negative Family with Medullary Thyroid Carcinoma.

VC Longuini PhD¹, RA Toledo PhD¹, DM Lourenco, Jr MD¹ and SP Toledo MD¹.

¹Univ of Sao Paulo Sao Paulo, Brazil.

Context: The vast majority of cases with familial medullary thyroid carcinoma (FMTC) occurs in the context of multiple endocrine type 2 (MEN2) and is associated with activating mutations of the RET protooncogene. However, rare RET-negative families with FMTC and MEN2 have been reported. Recently, it has been reported that mutations in the p27Kip1 gene is responsible for MENX syndrome in rats, which includes MEN1- and MEN2-related tumors, including MTC.

Objective: To verify the germline status of p27Kip1 in human FMTC.

Patients: Three siblings with MTC carrying no mutations in the RET.

Methods: PCR amplification and automatic sequencing were performed.

Results: Three siblings with FMTC were referred to our service to perform RET mutation analysis. No mutation was found in the hot-spot exons of RET, so the analysis was extended to the remaining exons. The entire RET protooncogene (21 exons) was analyzed and no pathological changes were observed. Therefore we sequenced the 2 exons of the candidate gene p27Kip1. The common polymorphism V109G but no pathological change was identified in patients DNA samples.

Conclusions: So far, the role of p27Kip1 in RET-negative patients with FMTC remained unknown. Our data suggest that a gene other than RET and p27Kip1 would be implicated in the etiology of this MTC family.

Nothing to Disclose: VCL, RAT, DML, SPT

P1-540

Detection of Circulating Tumor Cells (CTCs) in Patients with Thyroid Cancer.

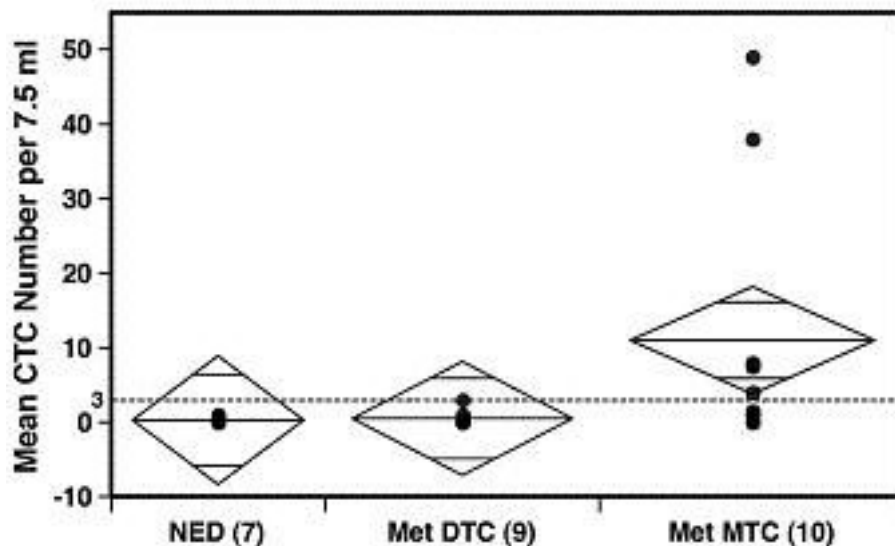
CL Michaelis MD^{1,2}, GJ Cote PhD¹, L Santarpia MD, PhD¹, MJ Klein RN¹, TG Gornet¹, HA Fritsche PhD¹, NL Busaidy MD¹, SG Waguespack MD¹, ME Cabanillas MD¹, MI Hu MD¹, C Jimenez MD¹ and SI Sherman MD¹.

¹MD Anderson Cancer Ctr Houston, TX and ²Baylor Coll of Med Houston, TX.

The presence of CTCs is associated with a poor prognosis in breast, prostate and colon cancer, because they may be the primary mediator for development of hematogenous metastases. This study's aim was to determine if the Veridex CellSearch System can detect CTCs in whole blood from thyroid cancer patients. The CellSearch System identifies CTCs by immunomagnetic cell selection using EpCAM as a capture antigen, with immunohistochemical characterization to detect epithelial-derived cells that stain for cytokeratin and DAPI and not for the lymphocyte marker CD45.



Captured cells are visually confirmed and counted with a microscope. An in vitro study was done with 4 thyroid cancer cell lines: TPC-1, TT, MZ-CRC-1, and BHT-101. Cultured cells (10) were microscopically drawn into a micropipet and added to 7.5 mL tubes of blood from a healthy male. Quantitative recovery was found for the cell lines derived from papillary and medullary (MTC) cancers, but not anaplastic (ATC) or unspiked blood. The patient study group included 10 subjects with metastatic MTC, 9 with metastatic differentiated thyroid cancer (DTC), and 7 with no evidence of disease for ≥ 5 years (NED). Data duplicates were available for 21/26 patients. Elevated CTCs (≥ 3) were found in 6 patients with metastatic MTC, while only one patient with metastatic DTC and none in the NED group had ≥ 3 detectable CTCs.



The sensitivity and specificity for detecting CTCs in subjects with MTC or DTC were 37% and 100%, using a cut-off of 3 total cells; ROC analysis suggested that other cutoffs were less optimal. This study is the first to report detection of CTCs in thyroid cancer patients using the CellSearch system. Although EpCAM has been reported expressed in both DTC and MTC (but not ATC), our results suggest that EpCAM-based detection methods may only identify CTCs in metastatic MTC.

Disclosures: MEC: Researcher, Johnson & Johnson, Procter & Gamble, Eisai. SIS: Researcher, Genzyme Corporation, AstraZeneca, Amgen, Eisai, V Foundation for Cancer Research, National Cancer Institute; Consultant, Bayer, Inc., Genzyme Corporation, Lilly USA, LLC, Oxigene, Celgene, Exelixis, Plexxikon, Semafore, Veracyte; Speaker, Genzyme Corporation, Exelixis.

Nothing to Disclose: CLM, GJC, LS, MJK, TGG, HAF, NLB, SGW, MIH, CJ

P1-541

Economic Impact of a Novel Molecular Diagnostic Test in Evaluation of Thyroid Nodules with Indeterminate FNA Cytology Results.

Richard B. Lanman MD¹, Giulia C. Kennedy PhD¹, Natalia Olchanski MS², Charles Mathews MPP², C. Charles Wang MD¹ and Paul W. Ladenson MD³.

¹Veracyte, Inc South San Francisco, CA ; ²Boston Healthcare Boston, MA and ³Johns Hopkins Univ Sch of Med Baltimore, MD.

Background: Of patients evaluated for thyroid nodules with fine needle aspirate cytology (FNA), 22% are classified as indeterminate and most undergo thyroid surgery, even though 70% are ultimately found to be benign on surgical pathology.(1-8) A novel, highly specific molecular test has been developed using microarray analysis of RNA expression and a predictive algorithm to better identify which cytologically indeterminate nodules are benign.

Methods: A health economic model was constructed to compare current management of cytologically indeterminate thyroid nodules with incorporation of a novel molecular test. These pathways and their outcomes were based on guidelines (ATA, NCCN), and epidemiological, claims, and clinical research data. Direct medical costs were estimated over a two-year time frame, including additional preoperative testing, surgery, and postsurgical follow-up and complications. Cost estimates were based on analysis of 2007 Medicare claims, adjusted to 2010 values, the Medicare fee schedule, published literature, and procedure rate trends from 2006-2008 claims. A range of price points for the novel test were evaluated, targeting net cost savings of 25%.

Results: Among 216 million insured U. S. adults, an estimated 432,000 patients would be evaluated annually for thyroid nodules, 95,040 of whom would prove to have a cytologically indeterminate nodule. Following the current clinical pathway, 79% of indeterminate nodules currently have thyroid surgery, 75,082 patients would be operated upon with 22,525 nodules identified postoperatively as malignant (30%) and 52,557 as benign (70%). Assuming preoperative use in cytologically indeterminate nodules of a novel molecular test with 75% specificity and 95% negative predictive value for malignancy(9), only 37,468 patients would undergo surgery (-50%), among whom 22,832 would prove to have malignancy (+1%) and only 14,636 to have benign nodules (-72%). Total direct medical costs over 2 years with the current clinical pathway would be \$1.026 billion, compared with \$787.5 million (-23%) with use of the novel test for nodules with indeterminate cytology.

Conclusions: Incorporating a novel molecular diagnostic test with 75% specificity and 95% negative predictive value for malignancy into current clinical management of cytologically indeterminate thyroid nodules would reduce the number of surgeries on patients with benign thyroid nodules by 72% and reduce healthcare costs by 23%.

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Sources of Research Support: Veracyte, Inc.

Disclosures: RBL: Employee, Veracyte, Inc. GCK: Employee, Veracyte, Inc. NO: Consultant, Veracyte, Inc. CM: Consultant, Veracyte, Inc. CCW: Employee, Veracyte, Inc. PWL: Medical Advisory Board Member, Veracyte, Inc.

P1-542

A Multi-Gene Test for Accurate Classification of Thyroid Nodules.

Jl Wilde Ph.D.¹, N Rabbee Ph.D.¹, D Chudova Ph.D.¹, H Wang Ph.D.¹, C Friedlander Ph.D.¹, E Wang Ph.D.¹, M Pagan Ph.D.¹, E Tom¹, J Reynolds¹, CT Rigl Ph.D.¹, CC Wang M.D.¹, L Friedman R.N.¹, RB Lanman M.D.¹, M Zeiger M.D.², E Kebebew M.D.³, J Rosai M.D.⁴, VA LiVolsi M.D.⁵ and GC Kennedy Ph.D.¹.

¹Veracyte, Inc South San Francisco, CA ; ²Veracyte, Inc South San Francisco, CA ; ³Johns Hopkins Sch of Med Baltimore, MD ; ⁴Natl Cancer Inst Bethesda, MD ; ⁵Ctr Consulenze Anatomica Patologica Oncologica Milan, Italy and ⁶Univ of Pennsylvania Sch of Med Philadelphia, PA.

Fine-needle aspiration (FNA) of thyroid nodules allows definitive cytopathology diagnoses in 70-80% of cases; however, the remaining 20-30% often lead to ambiguous results. Since a more definitive diagnosis on FNAs would allow better management of patients with atypical or suspicious thyroid nodules, we set out to develop a molecular test on thyroid FNAs that provides accurate diagnostic information on nodules with indeterminate cytopathologic features. Many studies have used molecular analysis to try to determine which indeterminate cytology samples are malignant. We used a different approach; we tried to identify those indeterminate nodules which are benign. We used genome-wide mRNA expression analysis to measure >247,186 transcripts, including alternatively-spliced genes, in 849 thyroid nodules comprising subtypes which result in indeterminate cytopathology. Thyroid nodules were diagnosed by expert surgical pathology (i.e., gold standard). With this training set, machine-learning algorithms were used to develop multi-gene molecular classifiers that accurately distinguish benign from malignant thyroid lesions.

The algorithm utilizes 100-200 gene transcripts and multi-dimensional analytical approaches to achieve an overall 10-fold cross-validated accuracy of >95% on prospectively collected thyroid FNAs. Preliminary performance characteristics of this test show ROC curve AUC values of 0.94, indicating reasonable sensitivity as a function of specificity. Furthermore, the false negative rate we observe with our molecular classifier is no greater than that of FNAs diagnosed as benign by cytopathology^{1,2}. Using several different classifiers we have identified a subset of samples whose surgical pathology diagnoses are highly inconsistent with their molecular profiles. These discordant calls are counted as classifier errors, but in fact may be due to 1) inadequate sampling of the thyroid nodule during the FNA process and/or 2) ambiguous surgical pathology diagnoses. A multi-center clinical trial is ongoing which will allow us to validate these results on an independent test set.

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Disclosures: JIW: Employee, Veracyte, Inc. NR: Employee, Veracyte, Inc. DC: Consultant, Veracyte, Inc. HW: Employee, Veracyte, Inc. CF: Employee, Veracyte, Inc. EW: Employee, Veracyte, Inc. MP: Consultant, Veracyte, Inc. ET: Employee, Veracyte, Inc. JR: Employee, Veracyte, Inc. CTR: Employee, Veracyte, Inc. CCW: Employee, Veracyte, Inc. LF: Employee, Veracyte, Inc. RBL: Employee, Veracyte, Inc. MZ: Study Investigator, Veracyte, Inc. EK: Study Investigator, Veracyte, Inc. JR: Study Investigator, Veracyte, Inc. VAL: Study Investigator, Veracyte, Inc. GCK: Chief Scientific Officer, Veracyte, Inc.

P1-543

BRAFV600E Analysis on Fine Needle Aspiration Biopsy Increases Diagnostic Accuracy for Papillary Thyroid Cancer in Clinically Unsuspected Nodules.

M Rossi MD¹, G Trasforini MD¹, S Leoni MD¹, F Tagliati BS¹, R Rossi MD¹, E Roti MD², EC Degli Uberti MD¹ and MC Zatelli MD¹.

¹Section of Endocrinology, Univ of Ferrara Ferrara, Italy and ²Ospedale di Suzzara SpA Suzzara, Italy.

Papillary thyroid cancer (PTC) represents the majority of differentiated thyroid cancers, presenting in 29-83% of cases the activating BRAF V600E mutation. The aim of our study was to analyze the influence of BRAF V600E mutation analysis on diagnostic accuracy for PTC of fine needle aspiration biopsies (FNAB) in clinically unsuspected thyroid nodules. We therefore enrolled 497 patients (388 females, 109 males; mean age 50.5 years), for a total of 672 samples. FNAB were evaluated by the cytologist and the syringe wash out was employed for BRAF mutation analysis, performed after DNA isolation by direct sequencing and allelic discrimination. Among the 672 samples, BRAF V600E mutation was found in 35 samples, including 25 diagnosed as PTC also cytology-wise (confirmed by histology). Total thyroidectomy was also performed in 10 patients with BRAF V600E mutation but negative cytology, all confirmed as PTC at histology. Among the 637 samples with negative BRAF V600E mutation, 629 had benign cytology, while 8 were consistent with PTC then confirmed by histology. Statistical analysis showed an incidence of 74% for the BRAF V600E mutation in PTC cases; sensitivity was 72.9% and specificity was 100%. Positive and negative predictive values were 100% and 98.1%, respectively. Diagnostic accuracy of cytology was 97.8%, and increased to 99.3% when combined with BRAF mutation analysis. These data indicate that BRAF V600E mutation analysis can significantly improve diagnostic accuracy in clinically unsuspected cases. On the other hand biomolecular analysis is complementary to cytology, since it is not sufficient to diagnose every malignant case.

Sources of Research Support: Progetto Università - Regione della Regione Emilia-Romagna.

Nothing to Disclose: MR, GT, SL, FT, RR, ER, ECDU, MCZ

P1-544

BRAF^{V600E} Mutation Analysis in FNAC Specimens for Evaluation of Thyroid Nodule: A Large Series in a BRAF^{V600E}-Prevalent Area.

Jl Lee MD¹, SW Kim MD,PhD¹, HJ Kim MD¹, HK Kim MD¹, HY Jang MD¹ and JH Chung MD, PhD¹.

¹Samsung Med Ctr, Sungkyunkwan Univ Sch of Med Seoul, Republic of Korea.

Background: The BRAF^{V600E} mutation is highly specific to papillary thyroid carcinoma (PTC). A test for this mutation may increase the diagnostic accuracy of fine needle aspiration cytology (FNAC), especially in a BRAF^{V600E} mutation-prevalent area.

Methods: This prospective study enrolled 1074 patients with thyroid nodules who underwent both FNAC and BRAF^{V600E} mutation analysis by DPO-based multiplex PCR in FNA specimens.

Results: The ancillary test for BRAF^{V600E} significantly improved the sensitivity of FNA procedure, from 67.5% with FNAC alone to 89.6 % with FNAC and the DPO-based multiplex PCR analysis combined. Diagnostic accuracy increased from 90.9% to 96.6%. Nine cases of PTC were detected only by BRAF^{V600E} mutation analysis. Unexpectedly, the preoperative DPO-based multiplex PCR produced five false positive results, which surgery showed to represent benign nodules.

Conclusions: Molecular testing for the BRAF^{V600E} mutation in FNA thyroid nodule specimens increases diagnostic value when applied in a BRAF^{V600E} mutation-prevalent area. However, when using this potentially powerful technique, we must consider both its strengths and its weaknesses.

Nothing to Disclose: JIL, SWK, HJK, HKK, HYJ, JHC

P1-545

BRAF^{V600E} Mutation Is Highly Associated with Malignant Ultrasonographic Features in Korean Patients with Papillary Thyroid Cancer.

EJ Lee MD¹, DL Kim MD, PhD¹, SK Kim MD, PhD¹ and TS Whang MD, PhD¹.

¹Konkuk Univ Sch of Med Seoul, Korea.

Background

Ultrasound (US) – guided fine needle aspiration biopsy (FNAB) is the most important tools in evaluating thyroid nodules (1). BRAF^{V600E} mutation is a useful diagnostic marker for differentiating malignant thyroid nodules from benign of thyroid nodules in BRAF^{V600E} prevalent area (2).

Objective :

The aim of this study was to evaluate the association of BRAF^{V600E} mutation with US features in thyroid nodules in Korean patients.

Methods

From July 2007 to December 2009, we retrospectively analyzed the US features of 993 thyroid nodules in 822 patients who had been examined with US-guided FNAB and BRAF^{V600E} analysis. Cytological diagnoses of the US-guided FNAB samples were made according to the American Thyroid Association guidelines, 2009. The US features that suggested malignancy included microcalcification, irregular margin, marked hypoechogenicity, and a shape that was taller than wide. The presence of the BRAF^{V600E} mutation was investigated by pyrosequencing.

Results

On the basis of cytological analysis, the 993 thyroid nodules were classified into benign (n=598, 60.2 %), malignant (n=171, 17.2 %), suspicious malignant (n= 53, 5.3%), indeterminate (n= 165, 16.6 %), and nondiagnostic (n=6, 0.6 %) categories. BRAF^{V600E} mutation was positive in 252 cases (25.4 %) of 993 thyroid nodules : 158 malignant, 42 suspicious, 51 indeterminate, and 1 benign cytology. BRAF^{V600E} mutation was associated with following US features : taller than wide shape [odds ratio (OR) 1.901, 95% CI : 1.233-2.931, *P* = 0.004], irregular margin [OR 4.542, 95% CI : 3.013-6.847, *P* < 0.001], markedly hypoechogenicity [OR 28.435, 95% CI : 13.273-60.917, *P* < 0.001], and presence of microcalcification [OR 3.330, 95% CI : 2.068-5.361, *P* < 0.001], but not associated with gender, nodular size, composition and macrocalcification.

Conclusion

BRAF^{V600E} mutation was associated with malignant US features, especially taller than wide shape, margin irregularity, markedly hypoechogenicity and microcalcification.

(1) Ravetto C et al.,Cancer 90:357

(2) Chung KW et al.,Clin Endocrinol (Oxf) 65:660

Nothing to Disclose: EJL, DLK, SKK, TSW

P1-546

Preoperative Ultrasonographic Tumor Characteristics as a Predictive Factor of Tumor Stage in Papillary Thyroid Cancer.

MR Kim MD¹, JE Huh MD¹, JY Mok MD¹, YK Jeon MD¹, SS Kim MD¹, BH Kim MD¹, YK Kim MD² and IJ Kim MD¹.

¹Pusan Natl Univ Hosp Busan, Korea and ²Kim Yong Ki Internal Med Clin Busan, Korea.

Background: It is important to predict the status of extrathyroid extension and lymph node metastasis preoperatively for determining of the scope and extent of initial surgery. The aim of this study was to evaluate the usefulness of tumor characteristics on ultrasonography (US) for predicting the pathologic tumor stage of papillary thyroid cancer.

Methods: This study was performed at Pusan National University Hospital from January 2007 to December 2008. 178 patients who underwent surgery for papillary thyroid carcinoma less than 2 cm in size were evaluated. We analyzed the preoperative US findings such as tumor size, shape, margin, echogenicity, multifocality, calcification, vascularity, and the status of contact to the thyroid capsule.

Results: The tumor size, shape, margin, echogenicity, and contact status with capsule were significantly associated with pathologic extrathyroid extension. And the tumor size, echogenicity, and contact status were significant parameters for lymph node metastasis. Using multivariate logistic regression, the tumor size (>1 cm), markedly hypoechoic echogenicity, and the contact with protrusion were predictive for the presence of extrathyroid extension and lymph node metastasis, respectively. When a tumor do not contact with capsule on US, the cut-off value for the shortest diameter to capsule associated with extrathyroid extension was 1.05 mm (sensitivity 71.4%, specificity 65.2%).

Conclusion: Preoperative tumor characteristics on US correlated with several prognostic factors for papillary thyroid cancer and may serve as preoperative supplementary markers for determining the optimal extent of papillary thyroid cancer surgery.

Nothing to Disclose: MRK, JEH, JYM, YKJ, SSK, BHK, YKK, IJK

P1-547

Surgical Result According to Management Guideline Based on BRAF^{V600E} Mutation Status of Thyroid Nodules.

SK Kim MD,PhD.¹, DL Kim MD,PhD.¹, KH Song MD,PhD.¹, EJ Lee MD.¹ and TS Hwang MD,PhD.¹.

¹Konkuk Univ Sch of Med Seoul, Republic of Korea.

Background : BRAF^{V600E} mutation analysis has been proposed as a valuable adjunctive tool for refining the cytological diagnosis of thyroid nodule and exclusively associated with papillary thyroid carcinoma(PTC). In Korea where PTC comprises about 90-95% of reported thyroid cancers, the prevalence of BRAF^{V600E} mutation in papillary thyroid cancer(PTC) is over 80%.

Objective : We investigated a usefulness of preoperative the BRAF^{V600E} mutation analysis as an adjunctive diagnostic tool with routine FNAB and analyzed the surgical result according to management guideline based on the BRAF^{V600E} mutation status of thyroid nodules

Methods : From March 2007 to February 2009, total 865 FNAB cases of thyroid nodule were all analysed prospectively for BRAF^{V600E} mutation status by pyrosequencing method. For the patients who had a diagnosis of indeterminate cytology, we recommended surgery when BRAF^{V600E} mutation was positive or the nodules were clinically suspicious. **Results :** Among 865 cases, 504(58.5%), 141(16.3%), 54(6.2%), 140(16.2%) 10(1.2%), and 16(1.8%) cases were cytologically diagnosed as benign, indeterminate, suspicious for PTC, PTC, follicular neoplasm(FN), and inadequate(25.5%), respectively.

None of the 540 benign nodules showed BRAF^{V600E} mutation. Forty five(31.9%) of 141 indeterminate cases, 46(85.2%) of 54 cases suspicious for PTC, and 129(92.1%) of 140 PTC cases showed positive BRAF^{V600E} mutation. Of 10 follicular neoplasm, 9 turned out negative BRAF^{V600E} mutation and confirmed 3 NH, 1 FA, and 3 FTC. One BRAF^{V600E} mutation positive case lost.

All patients diagnosed as suspicious for PTC or PTC were recommended to have an operation and turned out to have PTC regardless of their BRAF^{V600E} mutation status. All 45 patients with BRAF^{V600E} mutation positive and indeterminate nodules were recommended to have an operation, among them 22 patients underwent surgery and 21 had PTC and one had nodular hyperplasia.

BRAF^{V600E} mutation analysis by pyrosequencing method showed a positive predictive value of 99.4%, negative predictive value of 51.3%, and diagnostic accuracy of 89.7%.

Conclusion : We found that BRAF^{V600E} mutation analysis in FNAB specimens provide a great help to make a therapeutic decision in thyroid nodules when the FNAB results are equivocal.

Nothing to Disclose: SKK, DLK, KHS, EJL, TSH

P1-548

Prognostic Features in Well-Differentiated Thyroid Carcinoma: Comparison of BRAF Mutational Analysis, Immunohistochemistry and Morphology in 410 Cases.

Sonia Cheng¹, Shereen Ezzat¹, Moises Mercado² and Sylvia Asa¹.

¹Ontario Cancer Inst Toronto, Canada and ²IMSS Mexico City, Mexico.

Introduction: Thyroid cancer is the most frequent form of endocrine neoplasia. The incidence of thyroid cancer is increasing, and there is a need to identify tumor features that predict aggressive disease behavior to justify rational therapy stratification.

Material and Methods: A series of 410 cases of well differentiated thyroid carcinoma was collected and characterized for the following features: morphologic classification, extra-thyroidal extension, lymph node metastases, and vascular invasion. Tissues were retrieved and all tumors were analyzed for BRAF mutation. A tissue microarray (TMA) was constructed with triplicate cores of every tumor and corresponding normal thyroid tissue. TMA slides were stained for a large number of immunohistochemical markers including recognized and novel biomarkers and analyzed with the Aperio Scanscope and Spectrum software. Statistical analysis was performed with SPSS software to determine markers that predict invasive and metastatic behavior.

Results: BRAF mutant tumors showed increased risk for all types of invasion compared to tumors with wild type BRAF. However, when classified according to morphologic appearance, classic variant papillary thyroid carcinoma (PTC) showed much higher risk for these outcomes compared with follicular variant PTC independent of BRAF status. In classic variant PTC, NIS, Cyclin D1, p27 and ER- β were associated with aggressive behavior. Galectin-3, HBME-1 and CK19 over-expression were statistically associated with aggressive behavior in follicular variant PTC. In contrast CEACAM-1 and PPAR γ reactivity failed to identify cases with invasive features. Interestingly, ER- β was significantly over-expressed in lesions with invasive behavior, particularly in classic variant PTC.

Conclusions: This study has identified the value of specific markers in identifying well differentiated thyroid carcinomas that have aggressive behavior. Importantly, our findings underscore the importance of accurate morphologic classification in the interpretation of genetic and tissue biomarker performance.

Nothing to Disclose: SC, SE, MM, SA

P1-549

Childhood Thyroid Nodular Disease and the Risk of Cancer: Long vs. Short Splice Forms of FLIP in Childhood Thyroid Nodular Disease.

SA Bluvshsteyn MD, PhD¹, J Maceluch PhD¹ and M Niedziela MD, PhD¹.

¹Poznan Univ of Med Scis Poznan, Poland.

Background

Autoimmune thyroiditis, because of its' destructive nature, may set a stage for neoplastic growth. The constant attack from the immune system may lead to abnormal proliferative gene expression. Expression of certain genes decide the pathway towards proliferation (a cancer risk increase) or towards apoptosis (usually with an increase in inflammation). Incorporation of FLIP-L or FLIP-S into DISC (death inducing signaling complex) inhibits the autocatalytic cleavage of caspase-8/caspase-10. FLIP-L acts as apoptotic inhibitor at high ectopic expression levels but usually promotes activation of caspase-8 and therefore mediator of apoptosis. FLIP-S isoform, when in increased concentration relative to FLIP-L, decreases apoptosis leading to proliferation. In this project we hoped to show expression of the FLIP-L to FLIP-S within 7 cytologically determined classes of patients, and to determine whether the cells from these patients follow a pro-apoptotic or anti-apoptotic pathway. This increases the chances of identifying patients and targeting their treatment as future cancer risks. Also, it would differentiate between papillary carcinoma and autoimmune thyroiditis, thereby decreasing the necessity for surgical intervention.

Materials and methods

The study contains samples from 119 patients less than 18 years of age with numerous types of thyroid disease/lesions. These patients were divided into 7 cytological classes from benign to malignant. After complete clinical, hormonal, cytological and histological evaluation DNA and RNA was extracted from the biological remnants of the cytological screening. The RNA from each patient was then analyzed using QPCR method using β -2-microglobulin housekeeping gene as a control of expression. Thirty-five samples were then further studied for gene promoter mutation via gene sequencing.

Results

The results have shown us that in the pediatric population of this study, the levels of FLIP-L, FLIP-S, or the ratio between L:S did not correlate with a specific disease state as seen in the adult population studies in current and past literature. This study also showed that there is an intact promoter with little to no aberrations, none of which directly correlate to a disease state in this pediatric study.

The results revealed no association between the levels of TSH, ATPO, or TgAb and the expression of FLIP-L/FLIP-S. This shows a contradiction to much of the data seen in the adult cancer population.

Nothing to Disclose: SAB, JM, MN

P1-550

Anti-WDR1 Antibody: A Novel Biochemical Marker for the Differential Diagnosis of Thyroid Neoplasm.

S Izawa MD¹, S Taniguchi MD & PhD¹, K Matsuzawa MD¹, T Ohkura MD & PhD¹, H Ohkura MD & PhD¹, T Okamura MD & PhD², K Ishiguro MD & PhD¹, JY Noh MD³, K Kamijo MD & PhD⁴, A Yoshida MD & PhD¹ and C Shigemasa MD & PhD¹.

¹Tottori Univ Fac of Med Yonago, Japan ; ²Graduate Sch of Med, Univ of Tokyo Bunkyo-ku, Japan ; ³Ito Hosp Shibuya-ku, Japan and ⁴Kamijo Thyroid/Pituitary Clin Sapporo, Japan.

Background:

Thyroid nodules are common in adults, but only a small fraction of them are malignant. Cytological evaluation by fine needle aspiration is the most reliable tool, though 10-40% of them are diagnosed as intermediate. More reliable markers are expected.

Serological identification of antigens by recombinant expression cloning (SEREX) is established for detection of molecular targets of therapy and diagnosis. We focused on the serological diagnostic markers for anaplastic carcinoma, and prepared expression cDNA library from the tissue. Using SEREX strategy, we finally isolated WD repeat domain 1 (WDR1) and its specific antibody in patients' sera.

Patients and Methods:

We studied the titer of anti-WDR1 antibody (AWA) in 3 chronic thyroiditis (CT), 4 Graves' disease (GD), 10 anaplastic or poorly differentiated thyroid carcinoma (AC), 21 papillary thyroid carcinoma (PC), 12 follicular thyroid tumor (FT), 9 adenomatous goiter (AG), and 38 controls (CR). WDR1 recombinant protein was obtained as Glutathione-S transferase (GST) fusion WDR1 protein (WDR1/GST). Titration of AWA in patients' sera, obtained from OD_{490nm} value calculated by subtracting the value of anti-GST from that of anti-WDR1/GST, was examined by indirect ELISA.

Results:

The titer of AC (1.31±0.17) and PC (1.22±0.15) were significantly higher than that of AG (0.52±0.30), GD (0.53±0.21), CT (0.46±0.23), and NR (0.44±0.22) (p<0.001). FT (0.95±0.36) was significantly higher than that of AG (p=0.001), GD (p=0.038), CT (p=0.025), and NR (p<0.001), though no significant difference was observed between adenoma and carcinoma. The titer of AWA correlated weakly with age (R=0.36) and estimated thyroid volume (R=0.59). No significant correlation was observed between thyroid function, serum thyroglobulin, and tumor diameter. The cut-off estimated by ROC for differentiating AC or PC from CR was 0.91 (sensitivity 96.8%, specificity 96.3%).

Discussions:

WDR1 is associated with actin cytoskeletal dynamics in mammalian cells, although its biological function in human still remains unclear. Thyroid neoplasm with higher proliferating activity can over-express WDR1. AC or PC with histological invasion possibly interacts with immune system, and results in the automatic generation of AWA, which indirectly correlated with the proliferation and invasion of malignancies. In conclusion, anti-WDR1 antibody could be a novel biochemical marker for the early diagnosis of papillary and anaplastic thyroid carcinoma.

Nothing to Disclose: SI, ST, KM, TO, HO, TO, KI, JYN, KK, AY, CS

P1-551

Preoperative Serum Thyroid Stimulating Hormone Level in Well Differentiated Thyroid Carcinoma Is Predictive Factor for Lymph Node Involvement.

JE Huh MD¹, MR Kim MD¹, JY Mok MD¹, YK Jeon MD¹, SS Kim MD¹, BY Kim MD¹, YK Kim MD² and IJ Kim MD¹.

¹Pusan Natl Univ Hosp Busan, Korea and ²Kim Yong Ki Internal Med Clin Busan, Korea.

Background: Several growth factors and oncogenes are involved in the development and growth of well differentiated thyroid cancers, but the role of thyroid stimulating hormone (TSH) in this field has been undervalued. The purpose of our study is to determine the role of preoperative serum TSH level in the growth of differentiated thyroid cancer.

Methods: We conducted a retrospective study of 618 Korean patients who underwent thyroidectomy for thyroid cancer during a 3-year period at our hospital. To determine the relationship between TSH and the clinicopathological features of differentiated thyroid cancers, 554 patients with differentiated thyroid cancer were analyzed.

Results: The preoperative TSH level was significantly higher in differentiated thyroid cancer with extrathyroid extension ($p=0.002$) and lateral lymph node metastasis ($p=0.007$). Moreover, higher level of preoperative TSH (≥ 2.5 mIU/L) was associated with extrathyroid extension and nodal metastases ($p=0.006$, $p=0.024$ respectively). Patients ≥ 45 years of age exhibited a significant trend for higher TSH in those with stage III and IV disease vs. stage I and II disease ($p<0.001$). Hashimoto's thyroiditis itself was not associated with the status of extrathyroid extension and regional lymph node metastasis. Using multiple logistic regression, TSH was predictive for the presence of extrathyroid extension ($p = 0.008$) and lateral lymph node metastasis ($p=0.025$).

Conclusion: Preoperative TSH level correlated with several prognostic factors for differentiated thyroid cancer and may serve as a preoperative supplementary marker for determining the optimal extent of differentiated thyroid cancer surgery.

Nothing to Disclose: JEH, MRK, JYM, YKJ, SSK, BYK, YKK, IJK

P1-552

TSH Level as Predictor of Differentiated Thyroid Cancer.

Anne Dorange-Recton MD¹, Frédéric Illouz MD¹, Stéphane Triau MD¹, Stéphanie Mucci-Hennekinne MD¹, Alain Bizon MD¹, Sandrine Laboureau MD¹, Séverine Dubois MD¹, Vincent Rohmer MD¹ and Patrice Rodien PhD, MD¹.

¹CHU d'Angers Angers, France ; ²INSERM U694 Angers, France and ³Univ d'Angers Angers, France.

Introduction. The incidence of differentiated thyroid cancer (DTC) is increasing in Western countries. Studies have shown that TSH level could be a preoperative predictor of malignant thyroid nodule.

Material and methods. We retrospectively compared the TSH level of 2 groups of subjects: the first with an adenoma and the second with a more than 10 mm DTC, both based on histologic examination after thyroidectomy. The histological features of DTC were similar to literature. All subjects have a normal TSH level (0.1-4.5mU/l). The subjects were matched on sex and age. We performed univariate and multivariate analyses to identify potential markers of malignancy.

Results. Forty-seven subjects with an adenoma were compared to 47 subjects with a DTC. The tumors were papillary (78.7%), follicular (19.1%) or oxyphilic (2.2%) carcinomas. Sixty-two percent of cancers were at stage 1 according to the TNM. There was no significant difference between the 2 groups as far as age, sex, thyroid familial history, menopausal status or presence of thyroid antibodies were concerned. The final size of the nodule was significantly larger in the DTC group than in the benign group (24.1 vs. 18.6 mm, respectively p=0.03). Subjects with a DTC had a preoperative median TSH level significantly higher than subjects with a benign nodule (1.30mU/l vs. 0.84mU/l, respectively, p = 0.003). The patients who had a TSH over 1mU/l had a higher risk to have a malignant nodule compared to those with a TSH under 1mU/l (OR=4.3; 95%CI: 1.79-10.33). On the ROC curve, the TSH threshold of 1.15mU/l allowed to have a specificity of 66% and a sensitivity of 66%. The multivariate analysis showed that the TSH level over 1mU/l was an independent risk factor of developing DTC with a relative risk of 5.8. Moreover, the size of nodule of 20 mm or more, as well as central microcalcifications and nodular irregular margins established as suspicious ultrasonographic features of malignancy, were characteristics associated with malignancy with relative risks of 1.08, 4.93 and 6.77, respectively.

Conclusion. The TSH level seems to be higher in subjects with a DTC than in subjects with adenomas. This study emphasizes that the baseline values of TSH >1.15 mU/l could be another criteria to help differentiating benign from malignant thyroid tumors. However, a prospective study would be necessary to compare the TSH level with the well-defined clinical and ultrasonographic malignant characteristics.

Nothing to Disclose: AD-R, FI, ST, SM-H, AB, SL, SD, VR, PR

P1-553

Non-Clinical (Euthyroid) Hashimoto Thyroiditis as a Risk Factor for Thyroid Cancer.

K Todorova-Koteva MD^{1,2}, A Staii MD^{1,2} and J C Jaume MD^{1,2}.

¹Univ of Wisconsin-Madison Madison, WI and ²Veterans Affairs Med Ctr Madison, WI.

Background: Hashimoto thyroiditis (HT) was excluded as a probable risk factor for thyroid cancer 25 years ago; when in a classical paper, 829 patients with HT were followed over 22 years to find that none developed thyroid cancer (1). Recently, higher (but within normal range) TSH was found associated with a greater risk of differentiated thyroid cancer (DTC) (2). Since higher TSH usually accompanies HT, the observed increase risk of DTC with higher-but-within-normal TSH, may be a consequence of non-clinically manifested HT. This hypothesis helps unify the two observed outcomes. On one hand, clinical HT unlikely evolves into thyroid cancer (1). On the other, non-clinical HT, capable of increasing TSH but within normal range might do (2).

Objective: Using a prospectively developed database of patients with thyroid problems referred to the UW Thyroid Multidisciplinary Clinic, we tried to determine if an association exists between non-clinical (euthyroid) HT and DTC.

Design: We retrospectively studied 1577 consecutive patients who had undergone thyroid resection over 14.5 year period.

Results: The analysis of our database revealed that of the 1577 patients, 252 (16.0%) had the pathological diagnosis of HT. 69 (4.4%) of those patients were identified as having clinical disease (pre-operatively diagnosed hypothyroid, on thyroid hormone replacement) and 183 (11.6%) as having non-clinical disease (pre-operatively euthyroid, normal thyroid hormones and TSH). 72 (4.6%) had both DTC and HT, from which 59 (3.7%) were euthyroid and 13 (0.8%) were hypothyroid prior to surgery. There were 180 (11.4%) patients with HT without DTC, from which 124 (7.9%) were euthyroid and 56 (3.5%) were hypothyroid prior to surgery. We found the association between euthyroid HT and DTC to be statistically significant when compared with that of hypothyroid HT and DTC (Fisher's exact test, two-tailed p-value <0.05).

Conclusion: Our study shows for the first time that the prevalence of DTC in patients with euthyroid HT is significantly higher when compared to that of patients with hypothyroid HT. Strikingly, one third of the euthyroid HT cases had DTC. We also report an unexpected high prevalence (11.6%) of euthyroid HT in patients undergoing thyroid surgery. We concluded we have identified a major risk factor for thyroid cancer. Furthermore, our results provide an explanation for the previously described association of higher (but within normal) TSH and thyroid cancer risk (2,3).

(1)Holm LE et al., N Engl J Med 1985; 312:601

(2)Haymart MR et al., J Clin Endocrinol Metab 2008; 93:809

(3)Haymart MR et al., Clin Endocrinol (Oxf) 2009; 71:434

Nothing to Disclose: KT-K, AS, JCJ

P1-554

Thyroglobulin Antibody Is Associated with Increased Cancer Risk in Thyroid Nodules.

KH Baek MD¹, ES Kim MD¹, DJ Lim MD¹, MI Kang MD¹ and KW Lee MD¹.

¹The Catholic Univ of Korea Seoul, Korea.

Background: The association between autoimmune thyroiditis (AIT) and thyroid cancer is still not clear in spite of many previous reports. This study investigated whether Serologic thyroid antibodies are predictive of thyroid cancer in patients with thyroid nodules.

Method: We retrospectively reviewed records of patients with thyroid nodules evaluated by ultrasonography (US) guided-fine needle aspiration cytology (FNAC) at our institution between January 2006 and December 2008. Thyroid autoimmunity was assessed by measuring thyroglobulin antibody (TgAb) and thyroperoxidase antibody (TPOAb). The final outcome deciding a benign or malignant status involved a combination of cytology and histology.

Results: Of the 1638 patients, malignant nodules had a higher rate of positive TgAb (30.8 % vs. 19.6 %; $P < 0.001$) and elevated TSH levels (2.5 ± 2.8 mIU/L vs. 2.1 ± 2.0 mIU/L; $P = 0.021$) than benign nodules. The rate of positive TPOAb was not higher in malignant nodules, although both TPOAb and TgAb were well correlated with TSH levels and histological AIT. In the multivariate analysis, a positive TgAb was significantly associated with thyroid cancer [odds ratio (OR) = 1.61, 95% confidence interval (CI) 1.12-2.33] with upper tertile of normal range of TSH levels (OR = 1.72, 95 % CI 1.12-2.63) and above normal range of TSH levels (OR = 1.98, 95% CI 1.06-3.70).

Conclusion: We report for the first time that a positive serum TgAb test was an independent predictor for thyroid malignancy in thyroid nodules along with serum TSH levels regardless of the presence of AIT. Our results suggest that TgAb measurement could give additional information for predicting malignancy in cytologically indeterminate thyroid nodules in conjunction with clinical risk factors and TSH levels.

Nothing to Disclose: KHB, ESK, DJL, MIK, KWL

P1-556

Prediction of Malignancy in Nodular Thyroid Disease by Serum Thyroglobulin (Tg) Measurement.

S Singla MD¹, J Gogineni MD¹, L Wartofsky MD¹ and KD Burman MD¹.

¹Washington Hosp Ctr Washington, DC.

Introduction

Serum thyroglobulin (Tg) is a specific tissue marker manufactured by both normal thyroid tissue and differentiated thyroid cancer (DTC). Post operative Tg levels are used as a reliable tissue marker of recurrent disease or distant metastasis in patients with DTC. However, few studies have evaluated the importance of preoperative serum Tg levels as a predictor of cancer in nodular thyroid disease.

Objective

The purpose of the study is to determine whether preoperative Tg levels can predict malignancy of thyroid nodules.

Methods

A retrospective chart review was performed on patients diagnosed with nodular thyroid disease between January 2008 and June 2009. 158 patients met study criteria. We reviewed the preoperative serum Tg levels in these patients in conjunction with serum TSH, TT3, FT4 levels. Based on the preoperative FNA (fine needle aspiration) patients were classified as having either benign or malignant results. These results were compared with the surgical pathology in 64 patients in the benign group and 22 patients in the malignant group who underwent surgery. Patients with thyrotoxicosis and positive anti Tg antibodies were excluded. Serum Tg measurements were not obtained within 2 weeks following thyroid FNA.

Results

Thyroid function tests were normal in all patients. Total number of patients with benign and malignant nodular thyroid disease were 134 and 24, respectively. Mean and median Tg level in the benign group were 144 ng/ml and 54 ng/ml, respectively (SD 530) and in the malignant group were 164 ng/ml and 36 ng/ml, respectively (SD 298). The mean serum TSH concentration in the benign group was 1.8uU/ml (SD 3.1) and in the malignant group was 1.9 uU/ml (SD 1.8). The correlation coefficient for the relationship between serum Tg and TSH in the benign group was 0.04 with a p-value 0.6 (NS) and in the malignant group was 0.006 with a p-value 0.9 (NS). Because Tg and TSH variables were not normally distributed, Wilcoxon Two-Sample test was used to test whether the mean of Tg and TSH variables were significantly different between the two groups but were found to be not statistically significantly different (p=0.49 and p=0.28).

Conclusion

1.) In patients with nodular thyroid disease, Tg and TSH levels were not correlated. 2.) Because the mean and median serum Tg values were not statistically significantly different, preoperative serum Tg concentrations do not help discriminate between patients having benign or malignant thyroid nodules.

Nothing to Disclose: SS, JG, LW, KDB

P1-557

Comparison of the Vitamin D Levels in Patients with Differentiated Thyroid Carcinoma.

F Deniz MD^{1,2}, E Bolu MD¹, O Azal MD¹, A Corakci MD¹ and M Kutlu MD¹.

¹Gulhane Sch of Med Ankara, Turkey and ²Etimesgut Military Hosp Ankara, Turkey.

Patients with differentiated thyroid carcinoma (DTC) treated with thyroid hormone suppressive therapy are at the risk of cardiovascular disease. Low levels of vitamin D also associated with an increased risk of cardiovascular disease. Although routine oral calcium and vitamin D supplementation after bilateral thyroid resection may prevent hypocalcemic crisis and accelerate hospital discharge, there is so scarce data about vitamin D levels and calcium status in patients with differentiated thyroid carcinoma in the long term. We aimed to estimate serum 25 hydroxyvitamin D₃ (vitamin D₃, 25-OH vitamin D, Calcifediol) levels in patients with differentiated thyroid carcinoma.

A total of 142 patients who underwent total thyroidectomy for differentiated papillary thyroid carcinoma (PTC, n=112) or follicular thyroid carcinoma (FTC, n=30) at least 6 months ago were enrolled into this study. Serum was obtained during "dark" months. Serum 25-OH vitamin D levels were measured by direct radioimmunoassay method using commercially available kits (Diagnostic Systems Laboratories, Inc., Webster, TX, USA). Subjects had no previous history of kidney, liver, intestine, or metabolic bone disease. None of them were taking any additional supplements or medicine except L-thyroxine.

Although serum 25-OH vitamin D levels of both PTC and FTC patients were significantly lower than control group (21.53 ± 10.57 µg/L, 16.6 ± 4.12 µg/L and 38.23 ± 12.97 µg/L respectively), 25-OH vitamin D levels in patients with FTC were lower than in patients with PTC.

Mean Vitamin D3 Levels of Subjects

| VARIABLE | CONTROL | PTC | FTC |
|-----------|---------------|---------------|---------------|
| FT3 | 3,19 ± 0,34 | 3,12 ± 0,74 | 3,17 ± 0,68 |
| FT4 | 1,28 ± 0,17 | 1,88 ± 0,62 | 3,09 ± 0,42 |
| TSH | 1,54 ± 0,61 | 0,76 ± 1,25 | 0,23 ± 0,16 |
| PTH | 32,05 ± 12,74 | 40,99 ± 18,27 | 31,27 ± 19,10 |
| CALCIUM | 9,84 ± 0,29 | 8,88 ± 0,82 | 9,14 ± 1,27 |
| PHOSPHOR | 3,59 ± 0,42 | 3,85 ± 0,97 | 3,84 ± 0,90 |
| MAGNESIUM | 2,10 ± 0,12 | 1,97 ± 0,72 | 1,96 ± 0,15 |
| VITAMIN-D | 38,23 ± 12,97 | 21,53 ± 10,57 | 16,6 ± 4,12 |

Vitamin D deficiency is less serious in patients with PTC than with FTC. Thus our findings suggest that vitamin D levels should be measured during dark months both in patients with PTC and especially in with FTC. Additionally, deficiency of vitamin D should be replaced efficiently.

Nothing to Disclose: FD, EB, OA, AC, MK

P1-558

Prevalence of Thyroid Papillary Carcinoma Is Very High in Korean Acromegalic Patients.

HC Kang PhD, MD¹ and HK Kim MD¹.

¹Chonnam Natl Univ Med Sch Gwangju, Korea.

Background/Aims: Acromegaly is characterized by chronic excess of growth hormone (GH) with increased mortality from cardiovascular diseases. Furthermore, an increased risk of cancer contributes to mortality in acromegaly, which has been best studied for colon cancer (1). Thyroid abnormalities such as goiter and thyroid nodules are common findings in acromegaly, and some researchers suggested increased incidence of thyroid cancers in this population(2). We aimed to investigate the prevalence of thyroid cancers in acromegaly to clarify the controversial issue.

Methods: We studied, retrospectively, a series of 34 acromegalic patients referred to Chonnam National University Hwasun Hospital from April 2004 to December 2009. In all patients, thyroid morphology was assessed by high-resolution thyroid ultrasonography (US). When nodules were detected, US-guided FNA were selectively done on nodules with suspicious US findings regardless of the size.

Results: Among 34 patients, 28 patients (82.4%) had thyroid nodules. Six patients (17.6%) with malignant cytologic findings underwent thyroidectomy. Histologic examination revealed 5 thyroid papillary carcinomas (all female) and 1 mixed papillary-follicular cancer (male). Two patients had regional LN metastases and tumor stages were T1 in all patients. Although the follow-up period is limited, there has been no tumor recurrence so far.

Conclusions: The prevalence of thyroid cancer in Korean acromegalic patients is very high, although most patients have limited tumor stage. Screening thyroid US may be justified when the diagnosis of acromegaly is made. Further follow-up studies are needed to clarify whether the clinical behavior of thyroid cancers in acromegaly is more aggressive due to growth promoting effect of IGF-1.

(1)Melmed S., NEJM 2001;86:2929

(2)Siegel G et al., Endocr Res 2005;31:51

Nothing to Disclose: HCK, HKK

P1-559

Differential Diagnosis of Thyroid Carcinoma and Focal Lymphocytic Thyroiditis in Thyroid Nodule with Suspicious Sonographic Features.

Sena Hwang MD¹, Dong Yeob Shin MD¹, Eun-Kyung Kim MD² and Eun Jig Lee PhD¹.

¹Yonsei Univ Coll of Med Seoul, Republic of Korea.

BACKGROUND Thyroid carcinoma is suspected when suspicious ultrasonography (US) features including microcalcification; hypoechoic; infiltrative margin; and taller than wide on transverse view. However, it is often difficult to discriminate focal lymphocytic thyroiditis (FLT) because of similar US findings.

METHOD We retrospectively analyzed 180 patients with suspicious featured- thyroid nodules who underwent FNAB from 2007 to 2008. Clinical and biochemical findings, and US features were evaluated to discriminate each disease.

RESULT One hundred and five (58.3%) were papillary thyroid carcinoma (PTC), 42 (23.3%) FLT (FLT), and 33 (18.3%) benign adenomatous hyperplasia (AH). PTC was confirmed by surgery. Clinically Hashimoto's thyroiditis was found in 59 (32.8%) and diffuse thyroid disease (DTD) pattern in US in 35 (19.4%). There is no difference in age, sex ratio, and serum FT4 and TSH. "Taller than wide" in US features is more common in PTC (55.2%) than AH (30.3%, $p=0.012$), but not so common as FLT (64.3%, $p=0.316$). "DTD" is more common in FLT (54.8%) than either PTC (10.5%, $p=0.000$) or AH (3.0%, $p<0.001$). Other US features were not different among diseases. Positivity for thyroid peroxidase antibody (TPO Ab) in FLT (50%) is more common than in PTC (21.1%, $p=0.16$) and AH (4.6%, $p=0.000$). Hashimoto's thyroiditis is less commonly associated with AH (9.1%) than PTC (31.4%, $p=0.004$) or FLT (54.8%, $p=0.011$).

CONCLUSION FLT can be more suspected than PTC when DTD pattern in US and presence of TPO antibody (Hashimoto's thyroiditis) are shown in patients with suspicious US features.

Nothing to Disclose: SH, DYS, E-KK, EJJ

P1-560

Thyroid Volume and Nodularity in Dyslipidemic Patients on Statin Treatment.

JH Suk M.D.¹, MG Chon M.D.¹, KI Cho M.D.¹, HG Lee M.D.¹, SM Kim M.D.¹, MK Kim M.D.¹ and TI Kim M.D.¹.

¹Maryknoll Med Ctr Busan, Korea.

Background and aims: Routine health check-up using high resolution ultrasonography has resulted in explosively increased detection of thyroid nodules in our country. Statins have marked beneficial effects on lipid profile, but also have pleiotrophic actions. Some observational studies suggested that the overall cancer incidence might be reduced in patients treated with statins. A recent report suggested an association between statin treatment, and reduced thyroid volume and nodularity. But, little information has been available concerning the possible antiproliferative effects of statins on thyroid cells.

Subjects and Methods: We recruited elderly euthyroid patients who were affected by type 2 diabetes mellitus, hypertension, or ischemic heart disease on long-term treatment with statins. We also recruited euthyroid patients without previous history of statin treatment as a control group. We performed high resolution ultrasonography to all the subjects and evaluated the thyroid volume, and number and size of thyroid nodule.

Results: Ninety four patients treated on statins and 88 untreated controls were enrolled. There was no statistical difference of thyroid volume and prevalence, number, volume of thyroid nodules between patients treated with statins and control group.

Conclusion: Our data did not show an association between statin treatment and reduced thyroid volume and nodularity, and did not support a possible antiproliferative effect of long-term statin treatment on thyroid cells. But the limitation of number and study design necessitate further confirmation of the results.

Nothing to Disclose: JHS, MGC, KIC, HGL, SMK, MKK, TIK

P1-561

The Value of Fine Needle Aspiration Cytologies in Thyroid Swellings.

k Hafeez M.B.B.S¹, N Sopher¹, J Jiva¹, K Ranat¹, R George¹ and A Norris¹.

¹North Manchester Gen Hosp Manchester, UK.

Introduction: Fine needle cytology are done for solitary nodule or a dominant nodule in multinodular goitre. We have studied the fine needle aspiration cytologies over the period of six years and compare them with tissue diagnosis. They are done in clinical settings done by physicians, surgeons or radiologist.

Aim: to assess the diagnostic yield of fine needle aspiration cytologies.

Methods: Data were collected from pathology laboratory and medical records to determine whether cytologies were accurate. There were 484 FNAs of which 195 had subsequent histologies and 261 had normal follow up providing 456 patients for analysis. All cytologies and histologies were reviewed with no significant difference in diagnosis. The FNAs were classified into four groups: benign, suspicious, inadequate and malignant.

Results: there were 271 benign FNAs, 262 had non malignant (74/262) histologies or normal follow up (188/262), and 9 had malignancies (six carcinoma, two lymphoma, one follicular carcinoma) There were 142 inadequate FNAs, 130 were benign, 78 had non malignant histologies, 52 had normal follow up, and 12 were malignant. Out of 78 patients only 33 had repeat FNAs. There were 38 suspicious FNAs, 26 were benign (13 colloid nodular goitre, 10 follicular adenoma, 3 nodular hyperplasia) and 12 (either follicular or papillary carcinoma) were malignant. There were 5 malignant FNAs, 4 were malignant and 1 was benign. There were 262 true negative, 28 true positive, 9 false negative and 27 false positive. This gives sensitivity of 75%, a specificity of 90%, a positive predictive value of 50%, a negative predictive value of 96%, and an accuracy of 88%.

Conclusion: FNA cytology of a thyroid has a high negative predictive value which is very helpful to reassure the patients who are presented with thyroid swelling. However a negative FNA should never exclude malignancy if there is a clinical suspicion. If this rule is used large number of patients will be spared from unnecessary surgery.

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Nothing to Disclose: KH, NS, JJ, KR, RG, AN

P1-562

Unsuspected Papillary Thyroid Carcinoma in Graves Disease Surgical Specimens.

Noreen T Nazir MD¹, Aaron Hoschar MD¹ and Leann Olansky MD¹.

¹Cleveland Clin Cleveland, OH.

Introduction: The relationship between Graves Disease (GD) and Papillary Thyroid Carcinoma (PTC) is unclear. It was suspected that suppressed TSH might be preventative for tumor development but thyroid stimulating immunoglobulin (TSI) seems to cause thyrocyte proliferation and could increase risk for PCT. We have examined the thyroid pathology database at the Cleveland Clinic for GD and PTC.

Results: Review of hospital pathology database revealed 12 patients with GD and PTC. Ten of the 12 patients (83%) were female. Average age was 43.08 years (F 43.3 years and M 42 years). Average duration of GD before going for surgery was 38.3 months (range 1-120 months). A bimodal distribution was noted with 7 patients having a shorter duration of GD, ranging from 1 to 18 months (average 7 months). The remaining patients had GD on average 81.5 months (range 60-120 months).

Eight of the 12 patients taken to surgery for goiter, 4 of 12 taken for ophthalmopathy, and 2 taken for both goiter and ophthalmopathy. One had elective thyroidectomy due to concern of possible hepatotoxicity of anti thyroid medications as she was awaiting liver transplantation. All had PTC and one had associated follicular thyroid carcinoma on final pathology. Two subjects had tall cell variant (TCV) of PTC (one male, one female, average age 43.5). Average size of carcinoma was 1.22cm (range 0.1-2.2cm). Nine patients had carcinoma <1.0cm and 6 had tumors <0.5cm. Three had tumor >1.0 cm. Four of 12 patients had lymph node metastasis (LNM).

One third received RAI therapy but 66% had not prior surgery. Most recent lab values prior to surgery were evaluated (1-63 days before surgery, mean 15.9 days). Average TSH 0.722 (undetectable to 3.56, reference range 0.400-5.500 uU/mL), and mean T4 value 1.51 (0.4-3.5, reference range 0.7-1.8 ng/dL).

Conclusion: Although the relationship between GD and PTC remains not fully understood, there seems to be certain common characteristics in patients with both GD and PTC. More patients are women, with average age being similar in both males and females. The size of carcinoma was noted to be smaller in more patients. Carcinomas were under 1 cm in most subjects but aggressive variants were seen in about 17% and 1/3 had LNM.

From this review it is clear that GD and PTC can occur not uncommonly in patients treated with thyroidectomy. A similar percentage likely exists in those treated by other methods. PTC <1 cm could be missed of scans done prior to RAI.

Cross GA, et al., *Arq Bras Endocrinol Metabol.* 2008;52:1194.

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Nothing to Disclose: NTN, AH, LO

P1-563

Acute Hematologic Consequences of High-Dose I-131 Therapy for Aggressive Thyroid Carcinoma.

JE Lagrew¹, HM Bush PhD¹ and KB Ain MD^{1,2}.

¹Univ of Kentucky Lexington, KY and ²Veterans Affairs Med Ctr Lexington, KY.

I-131 is the only tumoricidal systemic therapy for thyroid cancer. Most patients receive empiric doses, but high doses (using Dosimetry for maximal "safe" doses) can be used for aggressive & distantly metastatic tumors. Benua et al {Am J Roentgenol Rad Ther Nucl Med 87:171, 1962} first described acute hematologic responses to high-dose I-131 to define safe dose guidelines; but few studies assessed responses of blood counts. To define acute hematologic effects, we studied blood counts (baseline & weekly x 8 wks) after high-dose I-131, ~200 rads (2 Gy) marrow exposure, in 155 patients in the Thyroid Oncology Program, Univ. of KY (1991-2008).

Methods Patients with invasive or distant metastatic thyroid cancer had Dosimetry (Benua 1962; modified using body counts instead of urine) then I-131 for a first high-dose therapy with weekly blood counts (baseline to 8 wks post-therapy). Measures of leukocytes (WBC), red cells (RBC), reticulocytes (RC), platelets (PLT), & hemoglobin (HB) were normalized to baseline for percentage changes. We made comparisons using multiple linear regressions (age, sex, administered I-131 dose (AD), marrow dose (MD), nadir week & magnitude), analyzed recovery with log-rank test for categorical variables (sex & bone metastases), and used Cox Regression for continuous variables (age, AD, MD).

Results WBC nadir averaged 49% of baseline at 5 wks from therapy & recovered to 67% baseline by 8 wks (RTB); neutrophil (NP) nadir 44% at 5 wks & 67% RTB; lymphocyte nadir 39% at 1 wk & 64% RTB; monocyte nadir 54% at 4 wks & increased to 37% above baseline (IAB) by 8 wks; eosinophil (EO) nadir 19% at 5 wks & 41% RTB; RBC nadir 76% by 5 wks & 81% RTB; RC nadir 51% at 3 wks & 37% IAB by 8 wks; & PLT nadir 49% at 4 wks & 86% RTB. Significant correlations ($p < 0.05$) were noted as: increased MD lowered the monocyte nadir and shortened the HB nadir timing; increased AD lowered the nadirs of WBC, NP, EO & PLT without altering nadirs timings; increased age lowered the basophil nadir & raised the RBC nadir, but did not affect other blood components; bone metastases significantly lowered the NP nadir and delayed the PLT nadir; and female sex lowered the nadirs of WBC, NP, & PLT, while delaying recovery of RBC.

Conclusion High-dose I-131 (~2 Gy MD) changes all components of peripheral blood at predictable times, proportionate to AD but not MD, and was well tolerated. The presence of bone metastases and female sex increased sensitivity to I-131 exposure.

Sources of Research Support: VA Merit Review, NIH Grant R01CA125166, Gift from John and Judy Gardetto: all awarded to KBA.

Nothing to Disclose: JEL, HMB, KBA

P1-564

Radioactive Iodine (I-131) Therapy for Thyroglobulin Positive, Scan Negative Differentiated Thyroid Cancer.

Ali S. Alzahrani MD¹, Omalkhaire Alshaikh MD¹, Sameerah Al-Shehri MD¹ and Rafif Farhat MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

The impact of radioactive iodine (RAI) on Tg+ scan- differentiated thyroid cancer (DTC) is controversial. Most previous studies were observational with heterogeneous groups of patients (pts) and rarely included control groups. In this study, we compared unselected pts with Tg+ scan- DTC who were treated with RAI (group 1) with a similar group of pts who were followed up without intervention (group 2).

Patients and methods

We studied all new consecutive pts (353 pts) seen at our hospital in a 2-year period (1998-1999). At 6-12 months after RAI ablation, 151 pts were in remission (defined as negative DxWBS, stimulated Tg <2 ng/dl) while 188 pts had evidence of persistent disease (stimulated Tg ≥ 2 ng/dl and/or positive DxWBS). To ensure homogeneity, we excluded pts with distant metastases, high Tg antibodies, those who received other forms of Tx for Tg + scan- and those with unusually high stimulated Tg (>200 ng/dl) as those pts probably had high tumor burden or undiagnosed distant mets. The remaining 47 pts (33 F, 14 M, median age 37.9 yrs, range 8-68) were studied as cases of Tg+scan-. There were 23 pts in group 1 and 24 pts in group 2. There were no differences between the 2 groups in their age, original tumor size, TNM stage, lymph node mets, RAI ablation dose and stimulated Tg levels at 6-12 months after initial Tx.

Results

Group 1 received 1 dose of RAI (mean±SD was 153±43 mCi) while group 2 did not receive any Tx. The final outcome of the 2 groups was comparable with 12/23 (52%) pts achieving remission (as defined above) in group 1 compared with 8/24 (33%) pts in group 2 (P=0.192). Other pts continued to have persistent disease. Using Kaplan Meier analysis, there was also no difference in between the 2 groups in their median remission rate over time (9.05 yrs (95% CI, 8.45-9.65) for group 2 vs. 8.72 yrs (95% CI, 8.25-9.20) for group 1; P= 0.45 by log-rank test).

The post therapy WBS became positive in 16/23 pts. The median Tg level at baseline (6-12 months after RAI ablation) was 5.9 ng/dl (range, 2.30-84) in group 1 compared with 3.25 ng/dl (2.10-48) in group 2 (P =0.62). Over a median duration of about 5 yrs, it significantly declined to 1.4 ng/dl (0-80) compared with its baseline in group 1(P=0.020) and to a median of 1.5 ng/dl (0-49) at last visit in a group 2 (P=0.063). At the last visit, Tg was comparable in the 2 groups(P=0.79).

Conclusions

In pts with Tg+ scan- DTC, RAI induces Tg decline but does not affect the final outcome.

Nothing to Disclose: ASA, OA, SA-S, RF

P1-565

Long-Term Outcome of Patients with Persistent Differentiated Thyroid Cancer Following the Initial Management.

Ali S. Alzahrani MD¹, Rafif Farhat MD¹, Omalkhaire Al-Shaikh MD¹, Sameerah Al-Shehri MD¹, Mohammed Al-Harthy MD¹, Saud Al-Harthy MD¹, Ahmed Nazmi MD¹, Omar Al-Nozha MD¹ and Talal Dahhan MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Following the initial management (Tx), some patients (pts) with differentiated thyroid cancer (DTC) continue to have evidence of persistent disease (PD). The impact of therapeutic interventions and the long-term outcome of those pts have not been well studied and are the aims of this study.

Patients and methods:

Between Jan. 1998 and Dec. 1999, 353 consecutive new pts were seen at our hospital. After initial Tx (surgery and RAI remnant ablation), 165 pts (47%) were assessed to be in remission 6-12 months after RAI remnant ablation. The other 188 pts had persistent/metastatic disease. We excluded pts with distant mets (18 pts), grossly local recurrence (4 pts), lost for follow up (26 pts), died (1 pt) and those with unclear status (8 pts). The outcome of the remaining 131 pts with PD was studied (95 F, 36 M, median age 38 yrs, range 8-82). Their original tumors were classic PTC (88.5%), follicular variant PTC (7.6%), other variants of PTC (2.3%) and FTC (1.5%). Pts were in stage I in 64%, II in 7.6%, III in 6.1%, IVa in 12.2%, IVb in 3.1%, IVc in 2.3% and unstageable in 4.6%. We used the following definitions: remission; negative stimulated DxWBS and ultrasound of the neck and stimulated Tg <2 ng/dl, PD; positive DxWBS and/or serum Tg ≥2 ng/dl.

Results:

Management

Of the 131 pts with PD, 35 (26.7%) were followed up without intervention (observation group) and 96 pts (73.3%) received one or more forms of Tx (treated group). The latter group received RAI (75 Tx in 69 pts), surgery (63 surgeries in 51 pts), external radiotherapy (21 pts), alcohol injection (3 Tx in 2 pts). Fifty one pts received only one Tx while 45 pts received ≥ 2 modalities of Tx.

Outcome:

Over a median follow up of 8.1 yrs (range 0.6-10.34), 46 pts (35%) achieved remission, 69 (52.5%) continued to have PD, 6 (4.6%) developed distant mets, 5 (3.8%) developed locally invasive disease, 5 died (3.8%) with 3 deaths due to DTC (2.3%). In the untreated group, 9 (25.7%) achieved spontaneous remission, 24 pts (68.6%) continued to have stable PD, 1 pt developed distant mets and 1 pt had local invasion (5.8%). In the treated group, 37 (38.5%) achieved remission, 45 (46.9%) continued to have stable PD, 5 (5.2%) developed distant mets, 4 developed locally invasive disease, 5 died; 3 due to DTC.

Conclusion:

Despite multiple therapeutic interventions, the long-term remission in pts with PD occurs in only about 1/3 of pts. About 5% develop distant mets, 4% develop locally invasive disease and 2% die from DTC.

Nothing to Disclose: ASA, RF, OA-S, SA-S, MA-H, SA-H, AN, OA-N, TD

P1-566

Iodine Avid Whole Body Scans (WBS) and Undetectable Serum Thyroglobulin Levels (Tg) in Differentiated Papillary Thyroid Carcinoma (PTC).

D Katselnik MD¹ and JM Bruder MD¹.

¹UT Hlth Sci Ctr San Antonio San Antonio, TX.

Purpose: PTC is typically followed with serum Tg and WBS. Tg is known to be more sensitive than WBS, and a detectable Tg in the setting of a negative WBS is commonly encountered. Less common is an undetectable stimulated serum Tg with a positive WBS. The approach in this situation is not clear.

Materials and Methods: We evaluated 372 postoperative WBS in patients with PTC who underwent I-131 therapy between 2004-09. Negative Tg was defined as <2 ng/mL without antithyroglobulin antibodies in the setting of a stimulated TSH of >30 mIU/ml after LT4 withdrawal or rhTSH administration. Patients with iodine avid WBS were identified. Positive WBSs were confirmed by joint review with members of the Nuclear Medicine and Endocrinology departments. Charts were reviewed for common characteristics, treatment, follow-up and clinical course.

Results: 15 patients were found to have negative Tg and positive WBS (4%). M:F ratio was 5:10, 10 tumors were stage I, 2 stage 2, 2 stage 3 and 1 unknown. 5 patients were identified using rhTSH. Follicular variant PTC occurred in 6 of 15 patients (40%) with an expected rate of 12-24% (1-2). 5 patients were identified on first WBS, all of whom eventually became negative. The remainders were identified on second or third WBS. 7 of the 15 patients (47%) received additional I-131 therapy. 6 patients eventually became negative by both Tg and WBS with a mean time to negativity of 3.3 (1-10) years. WBSs remain positive in 9 patients after an average of 4 years of follow-up. Although the numbers are small, there were no significant differences in the outcome of patients who received additional treatment compared to those who did not. No deaths or significant morbidity occurred in any of the patients.

Conclusions: We identified 4% of patients followed for PTC with a negative Tg and positive WBS. This is less than the 6.3% described in similar studies (3). An increased frequency of follicular variant PTC was seen in this report. After a mean of 3.3 years approximately half are free of disease. Overall disease course, complications, morbidity and mortality were not different between patients receiving additional treatment and those that did not. The finding of a negative Tg and positive WBS does not appear to cause a worse prognosis. Additional I-131 dosing may be considered in these patients, but likely does not affect overall patient survival or complications.

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(2) Lang BH et al., World J Surg. 2006; 30(5):752-8

(3) Park EK et al., Eur J Nucl Med Mol Imaging 2009; 36:172-179

Nothing to Disclose: DK, JMB

P1-567

The Prevalence of Undetectable Thyroglobulin and Detectable Antithyroglobulin Antibody in Patients with Well-Differentiated Thyroid Cancer.

Efrem Gebremedhin MD^{1,2}, Rudruidee Karnchanasorn MD^{1,2} and Ken C. Chiu MD^{1,2}.

¹City of Hope Natl Med Ctr Duarte, CA and ²Harbor-UCLA Med Ctr Torrance, CA.

In the revised 2009 American Thyroid Association Management Guidelines for Differentiated Thyroid Cancer (DTC), thyroglobulin (Tg) measurement, along with ultrasound, is recommended in related to long-term management of DTC. However, various factors could affect the result of Tg, including anti-thyroglobulin antibody (TgAb). Moreover, some DTC do not produce Tg. Thus, Tg surveillance could be misleading on some occasions.

We reviewed the medical records from the patient with DTC treated at this institution, a tertiary referral center for thyroid cancer. Among them, Tg and TgAb were measured before initial I-131 ablation therapy in 131 patients (70% female; Age, 52±17 years old; age of diagnosis, 48±17 years old, mean±STD). The prevalence of either undetectable Tg or detectable TgAb in this population was determined. We compared the clinical characteristics of patients with or without detectable Tg or TgAb.

TgAb was present in 28 patients (25%) with DTC. Among them, 86% of patients were female while only 65% in those with negative TgAb ($P=0.04$). Age and age at diagnosis were marginally younger in the DTC patients with positive TgAb, as compared to those with negative TgAb (46±17 vs. 53±17 years old for age, $P=0.058$; 43±17 vs. 50±17 years old for age at diagnosis, $P=0.058$). However, no difference was seen in main tumor size, multifocality, lympho-vascular invasion, and capsular invasion between two groups.

Undetectable Tg was noted in 3 patients (3%) before initial I-131 ablation therapy. There was no difference in gender distribution, age, age of onset, main tumor size, multifocality, lympho-vascular invasion, and capsular invasion between two groups.

Our data indicated that the utility of Tg measurement is limited about 72% of patients with DTC. For those with initial undetectable Tg or with detectable TgAb, one should be very cautious in interpretation of followup Tg results and Tg measurement provides less useful information in those patients. Furthermore, Tg should be routinely measured before initial I-131 ablation to identify those with undetectable Tg. Additional modalities including cervical ultrasound, 18 FDG-PET scan, and follow up whole body scan are crucial among these patients. Further studies are required to define a better strategy in long-term management of the patients with DTC with initial undetectable Tg or detectable anti-Tg antibody.

Nothing to Disclose: EG, RK, KCC

P1-568

Utility of Low Concentrations of Serum Thyroglobulin in Predicting Residual or Recurrent Disease in Patients Treated for Well-Differentiated Thyroid Cancer.

K Iyengar MD¹, ME Shomali MD¹ and KD Burman MD².

¹Union Memorial Hosp Baltimore, MD and ²The Washington Hosp Ctr Washington, DC.

Serum thyroglobulin (Tg) is an important element in the surveillance of patients with well-differentiated thyroid cancer. A study by Mazzaferri *et al.* (JCEM 90: 5047-57, 2005) suggests using a TSH-stimulated Tg value of 2 ng/mL as a cutoff. However, the cutoff for optimizing the sensitivity and specificity of this test in predicting residual or recurrent disease is unclear. Our study aimed to evaluate the relevance of low serum Tg concentrations. We reviewed records of patients diagnosed with well-differentiated thyroid cancer treated at The Washington Hospital Center. Baseline and stimulated Tg values were collected in patients who were post-thyroidectomy and radioactive iodine ablation. Patients were classified as being disease-free or having residual or recurrent disease. Patients with evidence of metastatic disease at diagnosis, detectable anti-Tg antibodies, or a follow-up period of less than 1 year were excluded. 50 patients were studied.

The mean age was 43 years at the time of diagnosis; 84% of patients were female. 60% were Caucasian and 28% African-American. 86% of patients had stage I disease; 8% had stage II; 4% had stage III; and 2% had stage IV disease at diagnosis. Mean duration of follow-up was 8 years. 82% of patients had papillary cancer and 18% had follicular cancer. 38% of patients were found to have recurrent or residual disease at a mean of 2.5 years from the time of the original diagnosis. Of the 19 patients with recurrent or residual disease, 8 had either a baseline or a stimulated Tg < 0.2 ng/mL, 4 had a Tg between 0.2-2 ng/mL, and 8 had a Tg > 2 ng/mL.

The sensitivities and specificities for predicting disease as well as the positive and negative predictive values in this population appear in the following table. The analysis was performed in two ways: (1) Tg values > 0.2 ng/mL were considered a positive result and (2) only Tg values > 2 ng/mL were considered a positive result. Values are expressed as a percentage.

| | Thyroglobulin | Sensitivity | Specificity | PPV | NPV |
|-----------------------------------|---------------|-------------|-------------|-----|-----|
| Tg > 0.2ng/mL considered positive | Baseline | 32 | 100 | 100 | 70 |
| | Stimulated | 65 | 93 | 85 | 81 |
| Tg > 2ng/mL considered positive | Baseline | 16 | 100 | 100 | 66 |
| | Stimulated | 53 | 93 | 82 | 78 |

Conclusion: Utilizing serum Tg values of 2 ng/mL or lower improves the sensitivity of the thyroglobulin test without changing the specificity. Therefore, any detectable baseline Tg (even values = or < 2 ng/mL) strongly suggests recurrent or residual thyroid cancer.

Nothing to Disclose: KI, MES, KDB

P1-569

Clinical Practice Guidelines for Management of Differentiated Thyroid Cancer (DTC); a Tree Falling in the Woods? A Single Center Experience.

SE Chang Figueroa M.D.¹, A Dhillon M.D.¹ and U Khan M.D.¹.

¹Univ of Missouri Columbia, MO.

RATIONALE: The incidence of DTC is increasing in the United States. Despite significant efforts by the American Thyroid Association to establish consensus guidelines in 2006, recently updated in 2009, there is still significant controversy regarding how to approach and manage patients with DTC.

METHODS: Thereby, we utilized a retrospective cohort at a university setting of 28 patients with DTC diagnosed between February 2007 and February 2008 to assess implementation of the 2006 guidelines.

RESULTS: Of the 28 patients reviewed, 16 were initially evaluated by Ear, Nose and Throat Surgery, 6 by General Surgery and 6 by Endocrinology. We found no difference in pre-operative TSH determination, thyroid ultrasound (US) use or Fine Needle Aspiration (FNA) biopsy between subspecialties. An initial FNA of a thyroid nodule was done in 72% of cases. Total thyroidectomy was performed in 91% of patients with known papillary thyroid cancer (PTC), 50% of follicular neoplasm (FN), 67% of Hurthle cell (HC) neoplasm, and 50% of those with insufficient/indeterminate findings. Central neck lymph node (LN) dissection was performed in 64% of patients with PTC, 50% of FN, 33% of HC neoplasm and none of insufficient/indeterminate FNA. There was no difference in use of radioactive iodine (RAI) ablation after surgery. RAI was used in 50% of PTC <1cm, 50% of PTC 1-4 cm without LN involvement, 75% of PTC >4 cm, and in 81% of PTC cases with LN involvement. Cases with follicular cancer and HC cancer didn't undergo RAI ablation. No difference in the degree of post-operative TSH suppression, or suppressed thyroglobulin (Tg) determination at 6 months was noted. There was, however, a significant difference in 12 month stimulated Tg determination ($p=0.02$), and in cervical US at 6 and 12 mo ($p=0.02$).

DISCUSSION: Our findings highlight that even in an academic setting there is underutilization of thyroid FNA, preoperative thyroid US as well as during follow up, central neck LN dissection at initial surgery and use of Tg measurements during follow-up. RAI ablation may be over utilized in management of micro PTC. However, there may be underutilization of RAI ablation in DTC other than PTC. These findings suggest a lack of awareness of current guidelines on DTC among practitioners. Long term studies focusing on management of DTC are needed. A multidisciplinary approach involving education of all subspecialties involved in management of DTC as a chronic condition needs to be studied.

Nothing to Disclose: SECF, AD, UK

P1-570

Patients with Thyroid Cancer on TSH Suppression Have a High Risk of Atrial Fibrillation.

A Abonawara MD, FRCPC¹, A Quraishi MBBS, FCPS¹, J Sapp MD, FRCPC¹, M Al-Qamber MD, FRCPC¹, CM O'Connell Ph D¹, R Hart MD, FRCSC¹, M Rajaraman MD, FRCPC¹ and SA Imran MBBS, FRCP, FRCPC¹.

¹Dalhousie Univ Halifax, Canada.

Background: Long-term suppression of thyroid stimulating hormone (TSH) below the normal range has been shown to improve survival in patients with thyroid cancer (Thy ca). The effects of resultant iatrogenic thyrotoxicosis on cardiac rhythm remain unknown. We conducted a cross-sectional study to look for the prevalence of atrial fibrillation (AF) in Thy ca patients on TSH suppressive therapy.

Methods: All Thy ca patients in the province of Nova Scotia, Canada are followed through the multidisciplinary thyroid oncology clinic at the Queen Elizabeth II hospital in Halifax, Nova Scotia. For the purpose of this study, all consecutive follow-up patients with Thy ca who were on TSH suppressive therapy, defined as TSH level <0.35 (Normal = 0.35 - 5.5 mIU/L) between June 2009 and January 2010 were recruited. Each patient underwent an electrocardiogram and filled a questionnaire on causes and clinical features of paroxysmal AF. The prevalence of AF in this population was compared against the published prevalence of AF in general population as well as a control group comprising patients who underwent hemithyroidectomy for non-malignant thyroid nodules and were biochemically euthyroid with or without thyroxine replacement therapy.

Results: A total of 128 patients were recruited in the study group of which 87% were females. The mean age was 52 years (Range 18-86 years) and the mean follow-up was 11 years (range = 1-21 yrs). Of these, 13 patients (10%) were found to have AF (Twelve females and one male). Two of these patients had chronic while 11 patients had paroxysmal AF. In all but one of these patients AF was diagnosed after the initiation of the thyroid suppression therapy. The mean ages of patients with and without AF were 61.6 yrs and 51.4 yrs ($p = 0.02$). The control group comprised 52 euthyroid patients of which there were 84% females with a mean age of 52 yrs. The prevalence of AF in the study group (10%) was significantly higher than both general population (5%) and control group (4%) ($P = 0.004$). There was no significant difference between the mean ages of patients with AF in the study and control groups.

Conclusion: TSH suppression in patients with Thy ca is associated with a significant risk of AF particularly in older individuals .

Nothing to Disclose: AA, AQ, JS, MA-Q, CMO, RH, MR, SAI

P1-571

Malignant Pleural Metastases in Thyroid Cancer Patients.

M Ahmed MD¹, B Al-Amro MBBS¹, A AlAraj MBBS¹, H Raef MD¹, A R AlMahfouz MD¹ and F Al-Dayel MD¹

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Introduction:

The rank order frequency for organ-specific metastases for thyroid cancer (TC) consists of pulmonary (10%), followed by skeletal (3%). Pulmonary metastases compromises life expectancy, approaching 50% by 5 or even 10 yrs. However, there is limited information available regarding the incidence of pleural metastases & its impact on life expectancy. Accordingly, we studied 16 consecutive TC pts. w/ pleural metastases from among 3952 registered at our hospital between 1975 & 2007. We reported the first 6 pts. at the Endo. Mtgs. in 2000. Here, we report our experience of additional 10 w/ following aims: 1) To define their clinical, & histopathological findings 2) To help understand the natural hx of advanced TC complicated by malignant pleural metastases (MPM).

Clinical Cases:

Findings at initial presentation: Among 10 pts. 8 had papillary thyroid cancer (PTC), & 2 had poorly differentiated TC. Of 8 w/ PTC, 2 had tall-cell variant, one had focal anaplastic transformation, 4 had pulmonary/hilar metastases, cervical lymph node metastases were evident in 9, perithyroidal soft tissue involvement in 6, tracheoesophageal in 2. Four had MPM. Rx for primary TC consisted of near total thyroidx, high dose, I 131 Rx, XRT & LT4 suppression. They were followed for 29-288 mos (median 60). During FU 6 developed pulmonary mets between 24-132 mos (median 36), 4 transformed to anaplastic cancer. MPM occurred in 6 between 48-276 mos (median 48). Dx of MPM was based on detection of malignant cells in all 10 pts: either in pleural fluid cytology (n=9) or on pleural Bx (n=4) or on both (n=3), demonstrating anaplastic histology in 4. There were 6 females & 4 males, aged 34-84 yrs. (median 59). Five presented with acute respiratory distress. Pleural effusion was unilateral in 5, hemorrhagic in 3, & pleural deposits were evident on CT in 5. Serum supp. thyroglobulin (TG) was 2.5-51.5 ug/l TSH 0.13-0.16 mU/l & unsupp. TG 3.7->5000 (median TG 3867/TSH 3.7-87). Pleural fluid TG was tested in 2 & was detectable in both (upto 1746 Ug/l). Pts. underwent thoracentesis & chest tube drainage with instillation of sclerosing agents. One underwent thorocotomy, resection of malignant pleural mass, 9x8x2 cm, & decortication. 5 are dead 24-250 mos (median 46) following Dx TC & 1-36 mos (median 6) after Dx of MPM.

Conclusion:

All our pts. presented with advanced TC. Malignant pleural metastases occurred in 0.4%. It portends poor prognosis with a median survival of 6 months in 50%.

Nothing to Disclose: MA, BA-A, AA, HR, ARA, FA-D

P1-572

Advanced Stage Presentation of Thyroid Cancer at a Tertiary Care Public Hospital: Demographic, Clinical, and Pathologic Implications.

J Panergo M.D.¹, S Patel M.D.^{1,2} and L Fogelfeld M.D.^{1,2}.

¹John H Stroger Cook County Hosp Chicago, IL and ²Rush Univ Med Ctr Chicago, IL.

Introduction: Cancer patients treated in public hospitals have been shown to present with advanced stage disease. We evaluated the clinical and tumor characteristics of patients treated for thyroid cancer at a public hospital and examined whether there were differences in disease presentation across ethnic groups.

Methods: A retrospective analysis of 305 thyroid cancer patients treated at a large metropolitan public hospital between the periods of January 1978 to August 2006 was performed. Demographic, clinical, and pathological data were obtained from a department maintained database. Patients were staged according to the Ohio State University (OSU) classification.

Results: Of the 305 patients included in this study 10% were Caucasians, 31% African-Americans, 35% Hispanics, 16% Asians and 5% others. Mean age at presentation was 44 years (13-88) and the male to female ratio was 1 to 4.5. The most common histologic variant was papillary (82%) with a mean tumor size of 3.1 cm (0.1-10). Multicentric disease and lymph node involvement was present in 45% and 22% of patients respectively. Tumor size \geq 4.5 cm or local invasion (OSU Stage 3 disease) was present in 41% of patients. Across ethnic groups, Hispanic patients compared to non-Hispanic patients were younger (40 vs. 46 years old, $p < 0.001$), more likely to have lymph node involvement (34.3% vs. 17%, $p < 0.001$), and trended towards having extra capsular invasion (33% vs. 24%, $p = 0.114$). There was no difference in tumor size between the two groups. African-American patients were the least likely to have lymph node involvement (14%) in comparison to other ethnic groups ($p = 0.005$). The prevalence of risk factors associated with thyroid cancer (i.e. radiation exposure, family history) was low across all ethnic groups.

Conclusions: The percentage of patients presenting with advanced disease (Stage 3 or 4) at our public hospital was more than two fold higher than that of published general population based series. Comparisons across ethnic groups illustrated that Hispanic patients were younger and more likely to have locoregional invasion without increased tumor size. These differences in presentation are not entirely explained by limited access to healthcare services. Other explanations including differences in environmental, cultural, and genetic factors should be investigated.

Nothing to Disclose: TAM-Y, JP, SP, LF

P1-573

18F-FDG PET in the Management of Differentiated Thyroid Cancer in Patients with High Serum Thyroglobulin and Negative I-131 Whole Body Scan.

R G Martins¹, A P Santos¹, J Couto¹, A P Barbosa¹, J Teixeira¹, I Lucena¹, L Bastos¹ and I Torres¹.

¹Portuguese Inst of Oncology Oporto, Portugal.

INTRODUCTION: In the follow-up of differentiated thyroid cancer (DTC), approximately 15 to 20% of patients with high serum thyroglobulin (Tg) have negative diagnostic I-131 whole body scan (WBS). In such patients, who cannot benefit from I-131 therapy, precise localization of tumor site is essential to define the therapeutic strategy. Several recent studies have shown that 18F-FDG PET (PET) can be used to detect local recurrences and distant metastases of thyroid cancer in these patients.

DESIGN: Single institution retrospective study.

METHODS: Consecutive patients who underwent PET imaging for suspected recurrent DTC were identified and their charts reviewed. Tg was measured during thyroxine withdrawal at the time of I-131 WBS.

RESULTS: We studied 21 DTC (18 papillary, 3 follicular) patients with elevated serum Tg (mean 762ng/mL; minimum 49, maximum 7608) and negative WBS treated with total thyroidectomy and 131I ablation, who were submitted to PET scans (n=23). Five patients revealed negative PET findings. In two of them, PET was repeated later (16 and 17 months), revealing distant metastases. PET identified lesions in 16 out of 21 patients, giving a sensitivity of 76%. Ten of the 16 patients revealed limited loco-regional disease. Remaining 6 patients showed lung metastases. We didn't find a statistically significant difference between Tg levels of the PET positive patient group and the PET negative patient group.

CONCLUSION: Our results suggest that 18F-FDG PET is useful for the detection of loco-regional recurrences and distant metastases of DTC in patients with elevated serum Tg but negative I-131 WBS, although we had a significant proportion of false negatives. In some cases, it may be important to repeat PET scan. There were not statistically significant correlation between Tg levels and PET results.

Nothing to Disclose: RGM, APS, JC, APB, JT, IL, LB, IT

P1-574

Analysis of Inflammatory Cytokines Associated Leukocytosis for Anaplastic Thyroid Carcinoma.

Y Tomisawa MD¹, S Ogasawara PhD¹, M Kashiwaba PhD¹, S Nishizuka PhD¹, T Inaba PhD¹, Y Takeda MD¹, T Komatu PhD¹, T Tanimura MD¹, R Kawagishi MD¹ and G Wakabayashi PhD¹.

¹Iwate Med Univ Morioka, Japan.

[Objective] Human anaplastic thyroid carcinoma (ATCs) is one of the most aggressive neoplasms with marked inflammatory manifestations. We experience leukocytosis with patients of ATCs. In the present study, we retrospectively analyzed treatment outcomes for patients with anaplastic thyroid cancer at our department, and investigated white blood cell count and cytokines.

[Subjects and methods] Subjects were 23 patients who were diagnosed with ATCs and treated at the Department of Surgery at Iwate Medical University between January 2002 and December 2009. And Control group were 16 patients with Papillary thyroid carcinoma (PTCs). We measured the serum cytokines such as IL -1 β , IL-2, IL-4, IL -5, IL -6, IL -7, IL -8, IL -10, IL -12, IL -13, IL -17, IFN- γ , MCP-1, MIP-1 β , TNF α , G-CSF and GM-CSF by ELISA. We examined G-CSF and G-CSFR protein expression of the tumor by immunohistological staining. Univariate analysis was performed for the relationship between survival time and leukocyte count (<10,000/mm³ and \geq 10000/mm³).

[Results](1) Subjects included 10 men and 13 women with a mean age of 72.2 years (range, 58-90 years). Survival time was between 33 and 520 days (median, 150 days). (2) There were significant differences White blood cell count (p=0.0002),IL-6(p=0.0018),IL-7(p=0.004),IL-8(p=0.0022),IL-12(p=0.0457),IL-17(p=0.0339),MCP-1(p=0.0104),TNF- α (p=0.0073),G-CSF(p=0.0271) between ATCs and PTCs. (3) In multivariate analysis, G-CSF (p=0.0004) was independent factor for leukocytosis. (4) The results of immunohistochemistry showed that the G-CSF expression (45%) and G-CSFR expression (64%) were positive. (5) Univariate analysis revealed significant differences in survival time with regard to G-CSF \geq 100pg/ml (p=0.0457) and leukocytosis(white blood cell count \geq 10000/m³)(p=0.0001).

[Discussion] ATCs was hypercytokinemia in comparison with the PTCs. This suggests that increases in white blood cell count was caused by G-CSF production, and it was identified as a prognostic factor.

Nothing to Disclose: YT, SO, MK, SN, TI, YT, TK, TT, RK, GW

P1-575

Value of Calcitonin Measurement in Washout Fluid from Fine Needle Aspiration in the Diagnosis of Recurrent or Metastatic Medullary Thyroid Carcinoma.

M Martinho MD¹, N Cunha¹, T Azevedo MD¹, T Martins MD¹, MJ Rascao MD¹, O Ilheu MD¹, P Gilde MD¹, C Cruz MD¹, I Gomes MD¹, P Figueiredo MD¹, F Valido MD¹, B Campos MD¹ and F Rodrigues MD¹.

¹Inst Português de Oncologia de Coimbra FG- EPE Coimbra, Portugal.

Background: The sensitivity of fine needle aspiration cytology in the pre-surgical diagnosis of medullary thyroid carcinoma is lower than the serum calcitonin measurement (63% and 98%, respectively). Some studies have suggested that the calcitonin (CT) measurement in washout fluid from fine needle aspiration (FNA-CT) is useful in the diagnosis of primary and metastatic MTC.

Aims: To evaluate the clinical usefulness of FNA-CT measurement in the diagnosis of cervical lymph nodes (CLN) metastasis or local recurrence (LR) from medullary thyroid cancer (MTC).

Methods: The results from FNA cytology and FNA-CT of suspicious CLN or LR were retrospectively analysed. The CT measurement in the wash-out was obtained using an immunometric chemiluminescent assay (IMMULITE 2000; functional sensitivity of 2.0 pg/ mL).

Results: Thirty ultrasound or palpation-guided FNA were performed between 2004 and 2009, comprising fourteen patients with suspected CLN metastasis (n=27) or LR (n=3); with a mean age of 62.86±12.68 years. Seven were women (50%). The FNA-CT was clearly positive (31-201396 pg/mL) in 13 cases (10 CLN and 3 LR); the FNA cytology was positive in 6 CLN, negative in 2 cases (1 CLN and 1 LR) and inconclusive in 5 cases (3 CLN and 2 LR). The surgical excision of 10 CLN and 2 LR confirmed the diagnosis of MTC metastasis suggested by the elevated FNA-CT. One patient refused surgery. The seventeen CLN with a negative FNA-CT (<2,9pg/mL), had either benign or inconclusive cytology (70.6 and 29.4%, respectively). Five of these were excised and the pathological result was reactive adenitis (n=4) and papillary thyroid cancer metastasis in the other. Twelve were not removed and there was no evidence of persistent disease during follow-up. The FNA-CT reached 100% sensitivity and specificity while FNA-cytology had 55% and 100%, respectively.

Conclusions: The FNA-CT was the most sensitive method for the diagnosis of lymph node metastasis and local recurrence further supporting its value in the follow-up of patients with medullary thyroid cancer.

Nothing to Disclose: MM, NC, TA, TM, MJR, OI, PG, CC, IG, PF, FV, BC, FR

P1-576

Role of Calcitonin (Ct) in Wash-Out Fluid from Fine-Needle Aspiration (FNAB) in the Diagnosis of Medullary Thyroid Cancer (MTC): Comparison with Basal Calcitonin, Cytology and Pentagastrin Stimulation Test (Pg-Test).

V Rochira¹, C Diazzì¹, L Zirilli¹, S Romano¹, C Carani¹ and B Madeo¹.

¹Univ of Modena and Reggio Emilia Modena, Italy.

INTRODUCTION: Serum Ct is considered the most sensitive tool for MTC screening, but with poor specificity. Pg-test sensitivity and specificity are high, but doubtful for 30-to-100pg/mL of Ct peak. FNA-B is not routinely used due to its very low sensitivity and specificity. Only few studies from a small number of patients are available on the role of Ct measurement in the wash-out fluid (Ct-FNAB) for MTC diagnosis, providing conflicting results.

AIM OF THE STUDY: To assess clinical usefulness of Ct-FNAB in the identification of primary MTC in thyroid nodules.

SUBJECTS AND METHODS: 33 patients (10M, 23F, mean+SD age: 55.5+11years), with thyroid nodules and concomitant increased basal serum Ct. All subjects underwent a Pg-stimulated Ct evaluation and a total of 54 thyroid nodules underwent US-guided FNA-B for both cytology and Ct-FNAB.

RESULTS: Ct-FNAB levels in nodules with MTC or C-Cells Hyperplasia (CCH) was significantly ($p<0.0001$) higher than in hyperplastic nodules and than serum Ct ($p=0.02$). The sensitivity and the specificity for basal Ct were 100% and 18.2% respectively. The sensitivity for Pg-test resulted 100%, and the specificity 95.4%. Cytology sensitivity was very low (10%) with a specificity of 95.4%. The ROC curves showed that both Ct-FNAB and Pg-test to correctly distinguish MTC or CCH nodules from hyperplastic nodules better than basal Ct. Regarding Ct-FNAB alone the best pair of values for highest sensitivity (90%) and specificity (100%) was found using a cut-off of 100 pg/ml or of 36 pg/ml, with the best values at 100 pg/ml.

DISCUSSION: The results of this study suggest that using both the cut-offs of 36 and 100pg/mL, Ct-FNAB may be an highly reliable marker with a high sensitivity and specificity for MTC early detection in skilled hands. In clinical practice, only Ct-FNAB allows to know the exact localization and size of MTC already before thyroidectomy.

Nothing to Disclose: VR, CD, LZ, SR, CC, BM

P1-577

Localization of Medullary Thyroid Carcinoma after Surgery Using ^{11}C -Methionine PET/CT: Comparison with ^{18}F -FDG PET/CT.

Hye Won Jang MD¹, Ji In Lee MD¹, Hee Kyung Kim MD¹, Seo Young Sohn MD¹, Sun Wook Kim MD, PhD¹ and Jae Hoon Chung MD, PhD¹.

¹Samsung Med Ctr, Sungkyunkwan Univ Sch of Med Seoul, Korea.

Objective: Tumor localization is difficult in patients with medullary thyroid carcinoma (MTC) who have persistent hypercalcitoninemia after thyroidectomy. We compared ^{11}C -methionine positron emission tomography/computed tomography (PET/CT) with conventional nuclear imaging modalities and ^{18}F -FDG PET/CT for diagnostic sensitivity in detecting residual or metastatic disease.

Methods: ^{11}C -methionine PET/CT and ^{18}F -FDG PET/CT were performed on 16 consecutive MTC patients with persistent hypercalcitoninemia after surgery, in this prospective, single-center study. Patient- and lesion-based analyses were performed using a composite reference standard which was the sum of the lesions confirmed by all combined modalities, including ultrasonography with or without fine needle aspiration cytology, CT, bone scan, magnetic resonance imaging (MRI), follow-up PET/CT and surgery.

Results: By patient-based analysis, the sensitivities of ^{11}C -methionine PET/CT and ^{18}F -FDG PET/CT were both 83.3%. By lesion-based analysis, the sensitivity of ^{11}C -methionine PET/CT was similar to ^{18}F -FDG PET/CT (74.0% vs. 76.0%). Excluding hepatic lesions, which could not be detected because of physiological uptake of methionine ^{11}C -methionine PET/CT showed higher sensitivity than did ^{18}F -FDG PET/CT especially in detecting cervical lymph node lesions. No significant differences were observed in the SUVmax of detected lesions measured by ^{11}C -methionine PET or ^{18}F -FDG PET. All patients with serum calcitonin levels higher than 370.0 pg/ml were considered positive by ^{11}C -methionine PET/CT and ^{18}F -FDG PET/CT.

Conclusion: Through our preliminary data, ^{11}C -methionine PET/CT showed similar sensitivity to ^{18}F -FDG PET/CT in detecting residual or metastatic disease of medullary thyroid carcinoma. Despite its limitation at liver, ^{11}C -methionine PET/CT might be complementary to ^{18}F -FDG PET/CT in localizing cervical lymph nodes metastases.

Nothing to Disclose: HWJ, JIL, HKK, SYS, SWK, JHC

P1-578

High Penetrance of Pheochromocytoma Associated with the Novel C634Y/Y791F Double Germline Mutation in the RET Protooncogene.

RA Toledo PhD¹, SM Wagner PhD³, FL Coutinho MD¹, DM Lourenco, Jr MD¹, JA Azevedo PhD¹, VC Longuini PhD¹, MCBV Fragoso MD¹, PL Dahia MD, PhD², LM Mulligan PhD³ and SP Toledo MD¹.

¹Univ of Sao Paulo Sao Paulo, Brazil ; ²Univ of Texas Hlth Sci Ctr San Antonio, TX and ³Cancer Res Inst, Queen's Univ Kingston, Canada.

Context: Previous studies have shown that double RET mutations may be associated with unusual multiple endocrine neoplasia type 2 (MEN 2) phenotypes.

Objective: Our objective was to report the clinical features of patients harboring a previously unreported double mutation of the RET gene and to characterize this mutation in vitro.

Patients: Sixteen patients from four unrelated families and harboring the C634Y/Y791F double RET germline mutation were included in the study.

Results: Large pheochromocytomas measuring 6.0–14 cm and weighing up to 640 g were identified in the four index cases. Three of the four tumors were bilateral. High penetrance of pheochromocytoma was also seen in the C634Y/Y791F-mutation-positive relatives (seven of nine, 77.7%). Of these, two cases had bilateral tumors, one presented with multifocal tumors, two cases had large tumors (>5 cm), and one case, which was diagnosed with a large (5.5 x 4.5 x 4.0 cm) pheochromocytoma, reported early onset of symptoms of the disease (14 yr old). The overall penetrance of pheochromocytoma was 84.6% (11 of 13). Development of medullary thyroid carcinoma in our patients seemed similar to that observed in patients with codon 634 mutations. Haplotype analysis demonstrated that the mutation did not arise from a common ancestor. In vitro studies showed the double C634Y/Y791F RET receptor was significantly more phosphorylated than either activated wild-type receptor or single C634Y and Y791F RET mutants.

Conclusions: Our data suggest that the natural history of the novel C634Y/Y791F double mutation carries a codon 634-like pattern of medullary thyroid carcinoma development, is associated with increased susceptibility to unusually large bilateral pheochromocytomas, and is likely more biologically active than each individual mutation.

Sources of Research Support: Sao Paulo State Research Foundation (FAPESP), by a Canadian Institutes of Health Research operating grant (L.M.M.). R.A.T. holds a Ph.D. fellowship from Coordenacao de Aperfeicoamento de Pessoal de Nível Superior (CAPES); D.M.L. and S.P.A.T. are partially supported by Fundacao Faculdade de Medicina. P.L.M.D. is a Kimmel Foundation Scholar.

Nothing to Disclose: RAT, SMW, FLC, DML, JAA, VCL, MCBVF, PLD, LMM, SPT

P1-579

High Frequency of Hirschprung Disease in a Large C620R-RET Mutated Family.

EPS Quedas PhD¹, RA Toledo PhD¹ and SP Toledo MD¹.

¹Univ of Sao Paulo Sao Paulo, Brazil.

BACKGROUND: Multiple Endocrine Neoplasia type 2 (MEN2) syndrome is caused by activating mutations in the RET protooncogene. MEN2-related MTC is a very aggressive cancer and usually may develop at early ages. Mutation screening in MEN2 kindreds is an essential tool to identify genetically predisposed family members and thus recommend early and preventive total thyroidectomy (TT). Importantly, patients harboring RET activating mutations in codons 609, 618 and 620 may also develop Hirschsprung disease (HSCR), which is characterized by an enlargement of the colon due to an aganglionic section of intestine. If not diagnosed and treated in time, HSCR is a life-threatening disorder.

AIMS: To report high frequency of Hirschsprung disease in a large family carrying the C620R mutation in the RET protooncogene.

RESULTS: The mutational hot spots of the RET proto-oncogene (exons 10,11,13-16) were studied by sequencing analysis. The index-case harbor the known Cys620Arg RET mutation in exon 10, classified as risk 2 by the Consensus for Multiple Endocrine Neoplasia. The at-risk family members who live in Sao Paulo were invited to a screening program at the Hospital das Clinicas, Sao Paulo. As the majority of the relatives live in the Northeast of Brazil a filed travel was undertook. A four generation family with 91 at-risk family members was localized. Molecular diagnosis allowed us to recommend early TT to disease-causing RET mutation carriers. Importantly, at least seven patients had HSCR and at least two family members have died due to the Hirschsprung disease.

DISCUSSION: We identified a large MEN2 family harboring the C620R RET mutation and presenting high frequency of HSCR. Our results indicated the need of a very early surveillance in newborns of C620R mutation carriers regarding Hirschsprung disease. The molecular mechanisms leading to MTC involves phosphorylation of proteins that ultimately will trigger oncogenic pathways. On contrary, the molecular mechanism leading to Hirschsprung is RET-induced apoptosis via its own cleavage by caspases. We are currently investigating whether RET polymorphisms may play a role in the Hirschsprung pathogenesis.

Nothing to Disclose: EPSQ, RAT, SPT

P1-580

Early Diagnosis of Medullary Thyroid Cancer: A Case for Early Prophylactic Thyroidectomy.

Mohammed Ahmed MD¹ and Hindi Al-Hindi MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Introduction:

Detection of RET proto-oncogene mutation is a predictor of medullary thyroid cancer (MTC)/MEN 2 prior to clinical or biochemical evidence of the disease providing a platform for prophylactic total thyroidectomy (TT) since there is no effective Rx once metastases emerge. Malignancy occurs in all subject who inherit the mutation. MTC is the most common cause of death in pts. with familial MTC/MEN 2A. Young pts. harboring the mutation are known to be free of persistent/recurrent MTC 5 or more yrs. after TT. Prophylactic TT is generally recommended for FMTC/MEN2A gene carriers at the age of 4-6 yrs. Since codon analysis appears to be an important prognostic factor, further refinement for optimum timing of TT continues. Here we present a study of kindred members who have inherited the RET mutation in whom preventive TT was done at early ages without complications.

Case Series: We have screened 31 members of a family with familial medullary thyroidcancer (MTC)/MEN 2A syndrome through 4 generations. Fourteen (45%) had RET mutation in exon 10: Codon 618. Seven had surgeries done for MEN 2A, 4 for MTC, 2 for C Cell hyperplasia and one is awaiting surgery. Here we report our experience in 3 members, ages 1- 5 yrs. with positive mutations. Two underwent uneventful prophylactic TT & lymph node sampling with histopathological evidence of C-cell hyperplasia only, confirmed by immunostaining for calcitonin, chromogranin and synaptophysin and there was no evidence of lymph node metastases. Pts. A & B are sibs whose father had surgeries for MEN 2A. Pt A: had the mutation detected at age one; however surgery was postponed till age 5 yrs. Serum calcitonin at surgery was 2.2 pmol/l (RR: 0.1-5.5), CEA 3.1 ug/l (RR: up to 3.4), had negative US neck. Pt. B: a 2 yrs. old sister of Pt. A has increased calcitonin (8.9), CEA 1.8 & is awaiting surgery soon. Pt C: age 3 yrs. is an off spring of a male who has surgeries for MEN 2A. She had TT with findings of C cell hyperplasia and no lymph node metastases. Her calcitonin was 4.8, CEA 1.6. None of these 3 had evidence of pheochromocytoma at their early ages.

Conclusion: Early detection of RET mutation followed by total thyroidectomy offers the best chance to prevent development of MET/MEN syndrome, especially in affected families. This strategy avoids the otherwise poor outcome of delayed diagnosis.

Nothing to Disclose: MA, HA-H

P1-581

Papillary Thyroid Cancer Showing High-Grade Transformation (Dedifferentiation) into Anaplastic Squamous Cell Lines.

LZ Khan MD¹, LM Giroux MD, PhD¹, U Duvvuri MD, PhD¹, EL Barnes MD¹ and RH Rao MD¹.

¹Univ of Pittsburgh Pittsburgh, PA.

Anaplastic thyroid cancers are extremely aggressive tumors that rank with the most lethal human malignancies. They are difficult to both treat and study, given a short life expectancy and their relative rarity, but consensus opinion holds that they arise from transformation of pre-existing differentiated thyroid cancers. We present a case of papillary thyroid cancer with anaplastic squamous histologic features possessing shared molecular markers that suggest a single clonal origin.

An 85 year old female presented with 3 weeks of worsening dysphagia, 70lb weight loss over 4 months, and a 50 gm multinodular goiter. CT scan showed an infiltrative thyroid mass invading the strap muscles anteriorly and encasing the esophagus posteriorly, cervical lymphadenopathy, and multiple pulmonary nodules. FNA revealed papillary thyroid cancer, and esophagoscopy showed that the cancer had not yet penetrated the lumen. In order to relieve dysphagia, the patient underwent palliative surgery consisting of a total thyroidectomy with a limited central neck dissection. Pathology revealed two morphologically distinct histological types, namely, anaplastic squamous cell carcinoma, and papillary carcinoma, tall cell variant, with angioinvasion and metastases to multiple cervical nodes. The BRAF V600E mutation was identified in both histologic components, suggesting that a previously differentiated papillary thyroid carcinoma with tall cell features had transformed (de-differentiated) into cell lines with anaplastic squamous features. The histologic appearance of the tumor with two distinct areas showing tall cell variant papillary and anaplastic squamous features is unique. The molecular mechanisms responsible for such a pattern of transformation are unknown. As a general rule, though, anaplastic transformation is thought to occur in most cases from mutations involving tumor suppressor genes, which are conserved across morphologically distinct areas⁽¹⁾. The presence of the BRAF mutation in all areas of the tumor is consistent with this postulate, and suggests a common (single clonal) origin from a previously differentiated papillary thyroid carcinoma.

1. Hunt JL, Tometsko M, LiVolsi VA, Swalsky P, Finkelstein SD, Barnes EL. Molecular evidence of anaplastic transformation in coexisting well-differentiated and anaplastic carcinomas of the thyroid. *Am J Surg Pathol.* 2003; 12: 1559-64.

Nothing to Disclose: LZK, LMG, UD, ELB, RHR

P1-582

PET-Associated Incidental Finding of the Thyroid in a 60-Year-Old Patient with Multiple Myeloma.

D.B. Burrows M.D.¹ and V. Kantorovich M.D.¹.

¹Univ of Arkansas for Med Scis Little Rock, AR.

Introduction:

Since the introduction of PET imaging, physicians and patients have struggled with decisions regarding its incidental findings, especially in the setting of a concurrent primary malignancy. Second malignancies can be discovered on PET imaging in up to 6% of patients. Here, we present a patient with multiple myeloma and an incidental finding of diffusely increased thyroid FDG uptake on PET imaging.

Clinical Case:

A 60-year-old man was diagnosed with multiple myeloma following 4 months of lower back pain; vertebral biopsy revealed a B-cell proliferative disorder with plasmacytic differentiation. He was treated only with focal radiation at that time. PET imaging was obtained to survey the extent of his disease, revealing several lesions related to multiple myeloma and diffusely increased FDG uptake in both lobes of the thyroid (SUV measurements of 2.6 and 3.0), suggesting thyroiditis or Grave's disease. He had no symptoms of thyroidal illness. TSH value was 0.96 uIU/mL (normal 0.34-5.6); free T4 was 0.58 ng/dL (0.58-1.64); total T3 value was elevated at 3.6 ng/mL (0.6-1.8). TPO antibody levels were elevated at 34.7 IU/mL (0.0-3.9) while TSH receptor antibody levels were normal at 9% (0-9). Thyroid U/S revealed findings consistent with multinodular goiter with multiple large nodules. A thyroid uptake and scan revealed 4- and 24-hour uptake values of 9% and 7%, respectively and two hot nodules in the left inferior pole. Thyroid biopsy was not done because of preexisting malignancy. Follow-up thyroid function testing was variable. He is currently receiving treatment for multiple myeloma.

Conclusion/Discussion:

PET-associated incidental thyroidal findings pose difficult questions to physicians and patients regarding future diagnostic workup. The pattern of FDG uptake should be highly considered as well as autoimmune antibodies. Unlike Hashimoto's thyroiditis, Grave's disease is often associated with extra thyroidal FDG uptake in skeletal muscle¹ due to upregulation of glucose transporters; the exact mechanism of why both entities are associated with increased thyroidal FDG uptake is unknown. Thyroidal gallium scanning also fails to differentiate these diseases². Incidental findings of increased FDG uptake in the thyroid in a patient with equivocal symptoms and biochemical findings should prompt evaluation with thyroid uptake and scan; clinical benefit of this has yet to be determined. Biopsy should be undertaken if clinically indicated.

(1) Chen et al., Clin Nucl Med 2007; 32: 816-817

(2) Lincke et al., Thyroid 2009; 19:4: 381-389

Nothing to Disclose: DBB, VK

P1-583

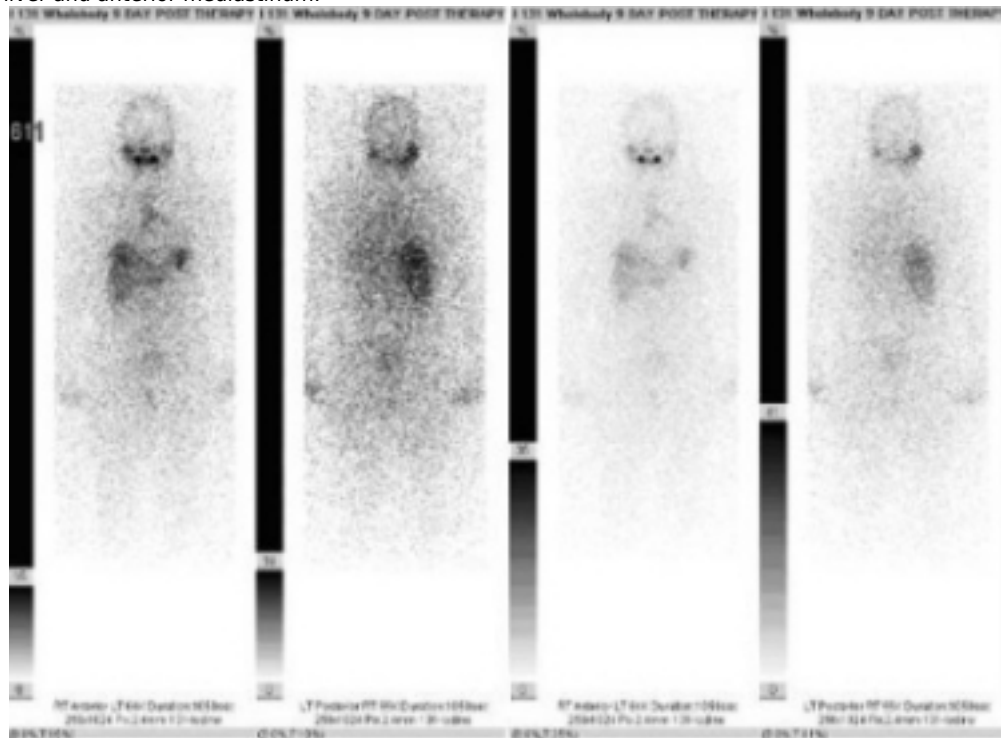
A Rare Case of a False Positive Radioactive Iodine Uptake in Liver and Mediastinum in a Patient with Papillary Thyroid Cancer.

V Mundra MD¹ and CV Villabona MD¹.

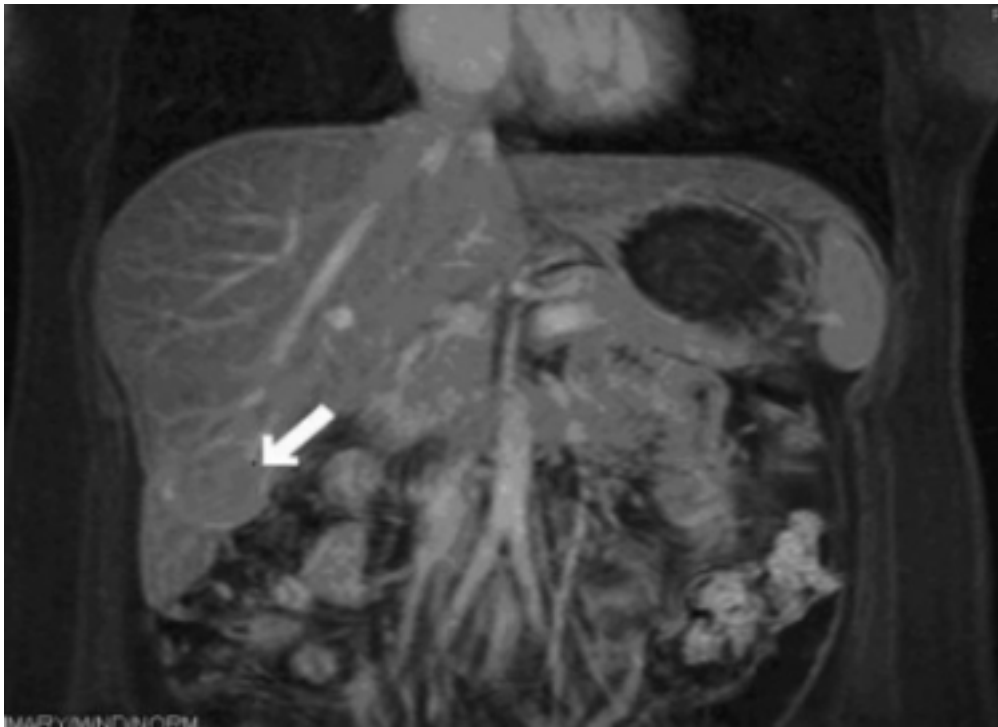
¹Cleveland Clin Florida Weston, FL.

Introduction: Papillary Thyroid cancer (PTC) is one of the most common endocrine neoplasms. I-131 whole body imaging is used to detect metastasis and recurrence of the disease. We report a case of a young female with "false positive" uptake in the liver and mediastinum.

Case: 27-year-old female with PTC underwent total thyroidectomy in 2004 and radical neck dissection surgery in 2009 for recurrence, followed by administration of 200 mCi of I-131. Medications included synthroid 137 mcg/d . Physical exam was unremarkable. Her last TSH was 5.38 mIU/mL (0.4-4.5 mIU/mL), Thyroglobulin (TG) was undetectable and TG antibodies were negative. Her post-therapy I-131 whole body scan revealed a diffuse uptake of radiotracer within the liver and anterior mediastinum.



A Chest CT scan showed a triangular density in the anterior mediastinum suggesting residual thymus. An abdominal MRI showed a vascular lesion in the dome of the right lobe of the liver consistent with a hemangioma.



She had a "false positive" uptake of I-131 in thymic tissue and in a liver hemangioma.

Conclusion: I-131 uptake by non thyroidal tissue is rare. However, it has been described in: thymus, hepatic cysts, papillary meningioma, disseminated gastric adenocarcinoma and vertebral hemangiomas¹⁻⁹. Scientists have confirmed the presence of sodium iodide symporter in non-thyroid cells¹⁰. Other proposed mechanisms explaining I-131 uptake in some tissues especially in highly vascular tumors include: pooling of blood in dilated vessels and transcapillary escape of iodine within the interstitium¹¹. In patients with PTC with positive I-131 uptake in unusual anatomical locations other tests as: TG levels, MRI, CT scans and tissue biopsies can assist the physician in making a correct clinical diagnosis and adequate treatment.

- (1) Khan S et al., Nucl Med Rev Cent East Eur. 2008; 11(1):30-3.
- (2) Lin DS. et al., Semin Nucl Med 1983; 13:395-396.
- (3) Caplan RH et al., Clin Nucl Med 1987; 12:760-762.
- (4) Zwas ST et al., Semin Nucl Med 1989; 19:340-342.
- (5) Preisman RA et al., AJR Am J Roentgenol 1977; 129:349-350.
- (6) Burt RW. Clin Nucl Med 1978; 3:155-156.
- (7) Fernandez-Ulloa M, et al., JAMA 1976; 236:857-858.
- (8) Acosta J, et al., Clin Nucl Med 1982; 7:368-369.
- (9) Wu SY, et al., J Nucl Med 1984; 25:1204-1209.
- (10) Riedel, C et al., Trends in biochem sciences 2001; 26: 490-426
- (11) Ishak KG, et al., Med Clin North Am 1975; 59:995-1013

Nothing to Disclose: VM, CVV

P1-584

Differentiated Thyroid Cancer Transformation to Poorly Differentiated Carcinoma after 23 Years Follow Up.

M Ahmed MD¹, A Al-Araj MD¹, A Al-Faraj MD¹ and F Al-Dayel MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Introduction:

Poorly differentiated thyroid cancer (PDTC) is justifiably recognized as an aggressive distinct clinicopathological entity based on large series of tumors sharing structural & histopathological criteria. It falls between classical differentiated thyroid cancer (DTC) & anaplastic CA with regards to the clinicopathological behavior & aggressiveness. It was included in the WHO classification of thyroid tumours in 2004. A diagnostic algorithm based on the presence of a solid/trabecular/insular growth pattern was suggested. However, others considered high grade features such as atypia, high mitotic count, & necrosis as specific markers. A confounding factor has been the recognition of geographical differences. A recent study (1) utilizing strictly classified PDTC demonstrated genetically homogeneous feature in that Ras mutations were almost exclusive genetic markers. Ras mutation analysis in PDTC has an important role in assessing risk stratification & the need for novel Rx strategies such as targeting Akt or mTOR pathways. Here we describe a case of DTC that transformed to PDTC after 23 years follow-up.

Clinical Case:

An 11-yr old girl had undergone total thyroidectomy in 1986 for papillary thyroid cancer (PTC), followed by 8 sessions of I-131 RX for a cumulative dose of 980 mCi. for recurrence & progressive diffuse bilateral macronodular pulmonary metastases/mediastinal & hilar metastatic lesions. Last I 131 Rx was given 13 yrs ago for bilateral lung uptake of 7%, serum TG of 130 ug/l, negative TG antibodies & serum TSH 370 mU/l. She remained in stable status. At age 34 yrs. she presented recently with left pleural effusion. At thoracentesis 4.5 L fluid was removed followed by installation of sclerosing agent. Pleural fluid cytology revealed metastatic PTC cells. Current labs: TG 27, TSH 0.45, anti TG 470. CT chest/abdomen: Diffuse bilateral progressive pulmonary macronodular, mediastinal, hilar & new lesions in T9, T11, L4 vertebrae & pelvic bones. Bx of a left axillary lymph node revealed PDTC. Sorafenib 400 mg BID po was started. A week later she developed diffuse maculopapular rash & the drug had to be stopped.

Conclusions:

Our case provides answer to a fundamental question regarding tumorigenesis of PDTC that it originates from PTC lineage rather than denovo. A FU of such cases can substantiate the notion that PTC can progress into PDTC and finally to anaplastic cancer.

Marco Volante, et al: RAS Mutations are the predominant Molecular Alterations in Poorly differentiated Thyroid Carcinomas and Bear Prognostic Impact. JCE&M 2009;94;4735-4741.

Nothing to Disclose: MA, AA-A, AA-F, FA-D

P1-585

Papillary Thyroid Cancer (PTC) Presenting as Extensive Radioiodine-Resistant Bone Metastases in a Young Woman a Decade after Neck Irradiation for Hodgkin's Lymphoma.

H Gharwan MD¹, ML Pratt MD¹ and HG Fein MD^{2,3}.

¹Johns Hopkins-Sinai Program in Internal Med, Sinai Hosp of Baltimore Baltimore, MD ; ²Sinai Hosp of Baltimore Baltimore, MD and ³Johns Hopkins Univ Sch of Med Baltimore, MD.

Background: Therapeutic external irradiation of the neck during childhood can result in subsequent hypothyroidism and in both benign and malignant thyroid neoplasms.

Clinical Case: A 21-year-old woman presented with progressive severe pain in her upper thoracic spine and chest. Ten years earlier, she had received chemotherapy (CHOP) and mantle radiation (2100 cGy) as treatment of Hodgkin's Lymphoma and had since been in remission and given birth to a child, who was two years old. Thyromegaly and tenderness of the cervical and thoracic spine and of paraspinal muscles were noted. Imaging showed lytic lesions in all vertebrae from C6 to T8 and in the manubrium sterni, compression fractures of T1 and T3 and retropulsion of bone fragments causing spinal stenosis with probable cord compression.

The patient was euthyroid with FT4 0.78 ng/dL and TSH 1.61 mIU/mL but thyroglobulin was 5339 ng/mL (normal: 1.6–59.9). Multiple thyroid nodules seen on ultrasonography were aspirated and PTC was found. The patient underwent total thyroidectomy. Her pre-operative β -hCG was positive and she eventually elected to terminate the pregnancy for medical reasons. Pathologic examination of the thyroid revealed multifocal well-differentiated PTC. Multiple lymph nodes were adherent to the thyroid and all contained PTC. A withdrawal iodine-123 scan showed intense uptake in the thyroid bed but no uptake in the vertebral bones or the sternum. Subsequent biopsy of the T7 lesion found metastatic PTC. The skeletal metastases were treated with dexamethasone and external beam radiation and thyroid bed disease was ablated with iodine-131.

Conclusion: The presence of radioiodine uptake in the thyroid bed and its concomitant absence in distant bony metastases of PTC bolsters recent data suggesting that neck-mantle irradiation may have an even greater carcinogenic potential than previously thought, especially in girls (1). This case supports a recent study that PTC may be more aggressive during and shortly after pregnancy.(2).

(1) Constine LS et al.. *Int J Radiat Oncol Biol Phys* 2008; 72:24

(2) Vannucchi G et al.. *Eur J Endocrinol* 2010; 162:145

Nothing to Disclose: HG, MLP, HGF

P1-586

Renal Cell Carcinoma Masquerading as Hurthle Cells.

Jennifer J Miranda MD¹, Latha R Pisharodi MD¹ and Marc J Laufgraben MD¹.

¹Brown Univ/Rhode Island Hosp Providence, RI.

Background: Renal cell carcinoma is one of the most common malignancies that metastasize to the thyroid. However, this diagnosis may not be readily apparent on cytology (1).

Clinical Case: A 50 year-old woman with a history of renal cell carcinoma s/p right nephrectomy 7 years earlier was incidentally noted to have thyroid nodules on a carotid ultrasound. Subsequently, a thyroid ultrasound demonstrated a right lower pole nodule measuring 1.5 x 2.9 x 1 cm, as well as a nodule in the isthmus measuring 0.3 x 0.3 cm. Fine needle aspiration of the right thyroid nodule was interpreted as "suggestive of follicular (Hurthle cell type) neoplasm." Contemporaneous to the thyroid evaluation, the patient was noted to have multiple pulmonary nodules on a CT scan done to exclude pulmonary embolism. Lung biopsy demonstrated metastatic renal cell carcinoma.

The patient underwent a right thyroid lobectomy for suspicion of Hurthle cell neoplasm. Surgical pathology revealed metastatic renal cell cancer measuring 1.9 x 1.7 x 1.5 cm. Incidentally noted was a 0.4 x 0.3 cm papillary microcarcinoma.

The patient is to start Sutent for treatment of metastatic renal cell cancer. Completion thyroidectomy for papillary microcarcinoma was not recommended.

Conclusion: The cytological appearance of Hurthle cells—polygonal cells with abundant cytoplasm and round nuclei containing a prominent central nucleolus—is similar to the appearance of renal cell carcinoma. Careful morphologic analysis and immunohistochemical stains are necessary to make the correct diagnosis. Physicians submitting thyroid cytology specimens should note any history of nonthyroidal malignancy, and cytologists should actively assess for evidence of metastatic disease in specimens from such patients. Such an approach will increase the likelihood of the correct diagnosis and management of these patients.

(1) Kim, T., Kim, W., Gong, G., Hong, S., Shong, Y. Metastasis to the thyroid diagnosed by fine-needle aspiration biopsy. *Clinical Endocrinology* (2005) 62, 236-241.

Nothing to Disclose: JJM, LRP, MJL

P1-587

A Case of Rapid Progression from Papillary to Poorly Differentiated Thyroid Cancer.

PN Surampudi MD¹, E Kandil MD¹, K Moroz MD¹, J Waddadar MD¹, S Ali MD¹, T Thethi MD¹ and J John-Kalarickal MD¹.

¹Tulane Sch of Med New Orleans, LA.

Introduction: We present a case of recurrent thyroid cancer with rapid progression from papillary thyroid cancer (PTC) to poorly differentiated thyroid cancer.

Clinical Case: A 67 year old female presented with a left thyroid nodule in January 2009. She had a history of non-toxic multinodular goiter (MNG) with right lobectomy in 1995, toxic MNG in 2003 [treated with radioactive iodine (RAI)] & subsequent hypothyroidism. The biopsy of the thyroid nodule revealed PTC. Completion thyroidectomy in July 2009 noted invasion to the constrictor muscle & left recurrent laryngeal nerve. The mass was a multifocal PTC with some poorly differentiated areas and positive margins. Two months after RAI ablation, the patient presented with dysphagia, dyspnea and hoarseness. CT of the neck & chest noted a right neck mass abutting the esophagus with compression of trachea, mediastinal extension, and enlarged lymph nodes. The patient subsequently underwent mass resection with modified neck dissection that involved the cricoid cartilage, trachea, upper mediastinum, prevertebral fascia, laryngeal nerve & carotid and jugular vessels without invasion in December 2009.

Pathology showed a mass with mainly poorly differentiated (diffuse sclerosing & tall variant) areas. In comparison, the July 2009 pathology report revealed a multifocal PTC with some areas of poorly differentiated (diffuse sclerosing & tall variant) type. The recent immunohistochemistry showed positive thyroglobulin (luminal), weakly positive for ER and GCDFP-15, with loss of thyroid transcription factor 1 (TTF-1). This was different from the initial sample that had TTF-1, thyroglobulin (cytoplasmic & luminal), and weakly positive for estrogen receptor (ER).

Discussion: The rapid progression from PTC to poorly differentiated cancer, with change in TTF-1 status, ER +ve and GCDFP-15 +ve, prompted a work-up for another source for the recurrent mass. Mammogram was -ve for a breast mass and no other source could be found by CT chest, abdomen & pelvis, EGD and colonoscopy. Review of literature reveals there are reports of PTC that can be ER +ve and thus it is felt that the recurrent mass is thyroid in origin. The +ve GCDFP-15 may be reflective of divergent differentiation. This case illustrates the degree of variance that can exist in markers due to dedifferentiation of thyroid cancer and importance of close follow-up in patients with PTC with areas of poorly differentiated cancer.

Nothing to Disclose: PNS, EK, KM, JW, SA, TT, JJ-K

P1-588

Papillary Thyroid Microcarcinoma with Distant Metastasis at the Time of Surgery: Report of Four Cases.

MHC Lopes MD, PhD¹, MNCL Vidal MD¹, LB Santana MD¹, GN Cortes MD¹, CV Parente MD¹, JAC Lopes MD¹ and MS Faria MD, PhD¹.

¹Hosp Univ Presidente Dutra of Fed Univ of Maranhao Sao Luis, Brazil.

INTRODUCTION: The recent development and spread of ultrasonography-guided fine needle aspiration biopsy (FNAB) has facilitated the detection of small papillary microcarcinomas of the thyroid measuring 1 cm or less (PTMC). Most of these patients have benign clinical courses and receive less aggressive therapeutic procedures in most medical centers. However, distant metastasis caused by papillary thyroid microcarcinoma has been reported (1 - 4). We report four cases of papillary thyroid microcarcinoma with distant metastasis at the time of surgery, as follows:

Table 1

| Case | Age/sex | Tumor size | Metastasis | Thyroid surgery | Radioiodine Ablation | Follow-up |
|------|---------|------------------------------|-----------------------|---|----------------------|-----------|
| 1 | 67/F | 1mm(smaller) 3mm(greater) | Bone | Total Thyroidectomy | 600 mCi | 46 months |
| 2 | 46/F | 3mm | Cervical lymphnode | Total Thyroidectomy + Modified Neck Dissection | 100 mCi | 35 months |
| 3 | 69/F | 10mm | Cervical lymphnode | Total Thyroidectomy + Modified Neck Dissection | 250 mCi | 17 months |
| 4 | 47/M | 8mm | Cervical lymphnode | Total Thyroidectomy + Modified Neck Dissection | 100 mCi | 4 months |

CONCLUSION: Despite the excellent prognosis of PTMC, a subset of these tumours shows aggressive biological and clinical features. In these cases, since predictive cytogenetic markers are still missing, their treatment should then be the same as for conventional PTC according to risk stratification and cancer stage.

(1)Lin KD, Lin JD, Huang HS, Jeng LB, Ho YS. Skull metastasis with brain invasion from thyroid papillary microcarcinoma. J Formos Med Assoc. 1997; 96(4): 2890-2.

(2)Mazeh H, Divino C, Nagi C, Bleiweiss JJ, Weber K. Incidental metastatic microcarcinoma of the thyroid identified after total parathyroidectomy. Thyroid 2007; 17(7): 685-7.

(3)Davies R, Pring M, Aw J, Hughes C, Thomas S. Isolated submandibular metastasis from a contralateral thyroid papillary microcarcinoma: an unusual case. Dentomaxillofac Radiol. 2009; 38(8): 546-9.

(4)Liou MJ, Lin JD, Chung MH, Liao CT, Hsueh C. Renal metastasis from papillary thyroid microcarcinoma. Acta Otolaryngol. 2005; 125(4): 438-42.

Nothing to Disclose: MHCL, MNCLV, LBS, GNC, CVP, JAACL, MSF

P1-589

Kikuchi Disease and Thyroid Follicular Adenoma - A Case Report.

SA Ali MD¹, J Waddadar MD¹, P Surampudi MD¹, T Thethi MD¹, E Kandil MD¹, A Khan MD¹ and J Kalarickal MD¹.

¹Louisiana State Univ New Orleans, LA.

INTRODUCTION;

Kikuchi's disease is also known as Kikuchi-Fujimoto disease and Kikuchi's histiocytic necrotizing lymphadenitis. It is a rare, benign condition of unknown etiology characterized by cervical lymphadenopathy and fever. It has been reported to be associated with other diseases including B cell lymphoma, Still's disease, and systemic lupus erythematosus.

CASE

Thirty three year old female admitted to the ICU with worsening shortness of breath and dysphagia. She was diagnosed with Kikuchi's disease by an excisional biopsy of a retropharyngeal lymph node at an outside institution, one month prior to this presentation. Past medical history included multiple episodes of venous thrombosis which lead to amputation of toes on her right foot at the metatarsal two months ago. She was on anticoagulation. There has been no family history of lupus or any other autoimmune disease.

On physical exam, there was prominent right thyroid lobe enlargement was noted causing tracheal deviation. Chest x-ray reveals bilateral pleural effusions and leftward deviation of the trachea. Ultrasound revealed a right-sided thyroid mass which displaced the trachea and carotid vessels. CT of the neck without contrast showed diffuse enlargement of the thyroid, particularly of the right lobe. There was left tracheal deviation with marked narrowing with the smallest diameter being 0.5cm. There were multiple densities in the mediastinum consistent with lymphadenopathy associated with Kikuchi's disease. Liver function tests were normal. And she was biochemically euthyroid. Anti-Smith antibody and anti-double stranded DNA were positive, and the patient was subsequently diagnosed with lupus. IgG to EBV viral capsid and early antigens were positive. Serum parvovirus B19 PCR was negative. Fine needle aspiration and biopsy of the nodule in the right lobe showed colloid and follicular cells, consistent with a benign nodule. She underwent subtotal thyroidectomy. Pathology showed the mass to be a follicular adenoma. The patient's shortness of breath and dysphagia resolved.

Conclusion.

This case report is the first record demonstrating an association between Kikuchi's disease and thyroid follicular adenoma that can cause significant tracheal compression and deviation. Thyroidectomy is safe and effective treatment for these patients.

Nothing to Disclose: SAA, JW, PS, TT, EK, AK, JK

P1-590

PTEN Germline Mutation and Multiple Thyroid Follicular Adenomas in a 62 Year Old Man with a History of Renal Cancer and Multiple Gastrointestinal Hamartomatous Polyps - Cowden Syndrome.

J Udayasankar MBBS¹ and K Foster-Schubert MD¹.

¹Univ of Washington Seattle, WA.

A 62 year old male had an age-appropriate screening colonoscopy which revealed > 70 polyps though out his entire colon. The preliminary pathology report indicated colonic ganglioneuromatosis. This finding prompted a referral to our Endocrine Clinic for evaluation of possible Multiple Endocrine Neoplasia - Type 2B (MEN-2B) Syndrome. His past medical history was significant for left nephrectomy for renal cancer 14 years ago. There was a strong family history of cancers of the breast, thyroid, testes, liver, skin and prostate.

On clinical exam, the patient was macrocephalic, (head circumference was 62 centimeters, > 97th percentile). Multiple facial papules, cobble-stoning of buccal mucosa, and several trichilemmomas on face and trunk were noted. No thyromegaly was appreciated on exam. Serum TSH, calcium, calcitonin, and plasma metanephrines were all normal, and hence not supportive of a diagnosis of MEN-2B.

The patient then underwent Upper GI endoscopy, which revealed several gastric and duodenal polyps. Subsequent immunohistochemistry of the duodenal and colon polyps showed negative staining for S-100 (neuron marker) and markedly positive staining for actin (smooth muscle marker) indicating the diagnosis of multiple gastrointestinal hamartomatous polyps.

Clinical diagnosis of Cowden syndrome was made and genetic testing was positive for a mutation in the phosphatase and tensin homolog (PTEN) gene, notably a non-sense mutation denoted as E307X (glutamic acid at position 307 in the PTEN protein resulting in premature truncation), confirming this rare autosomal dominant condition.

After breast cancer, thyroid cancer is the second most common cancer in patients with Cowden syndrome ¹. Therefore, he underwent ultrasound imaging of the thyroid, which revealed multiple bilateral hypoechoic nodules. Given the high predilection for thyroid cancer, the patient underwent total thyroidectomy. Pathology revealed several follicular adenomas without any evidence of cancer. The patient is receiving thyroid hormone replacement and he remains well two years post-surgery. His follow-up includes periodic imaging of the remaining kidney, colonoscopy, and mammography. Genetic screening of his siblings and children after genetic counseling was discussed.

Conclusion: Cowden Syndrome is a rare cause of familial thyroid cancer. Clinicians need to be aware of this condition as patients and their families will benefit from genetic testing and screening for other cancers.

1. Hobert JA et al., Genet Med 2009; 11(10):687-94.

Nothing to Disclose: JU, KF-S

P1-591

Mixed Medullary-Follicular Thyroid Carcinoma: A Case Report.

S Teixeira MD¹, C Freitas MD¹, P Farrajota MD¹ and MF Borges MD¹.

¹Ctr Hosp do Porto Porto, Portugal.

Background: The term mixed medullary-follicular thyroid carcinoma comprises a rare group of tumors exhibiting morphological and immunophenotypical aspects of both medullary and follicular components within the same lesion. **Clinical Case:** We report a case of a 43 year-old-woman with a past history of a right thyroidectomy for a follicular adenoma in 1990. She was referred to our clinic in 1999 because of a thyroid nodule in the left thyroid lobe with 17mm. The fine-needle aspiration biopsy revealed colloid hyperplasia. She was reevaluated in 2009 because growing of this nodule (45mm). The cytology revealed again hyperplasia. Lab study showed a high value of serum calcitonin (1393pg/mL; n <20pg/mL) and carcinoembryonic antigen (CEA) (564.9µg/L; n <5,4µg/L). A left thyroidectomy was performed. Histopathological examination of the tumor specimens revealed a mixed medullary-follicular carcinoma with positive immunohistochemical staining to calcitonin, chromogranin A, CEA and thyroglobulin in the dominant nodule and a papillary microcarcinoma in a smaller nodule localized in the periphery. The serum level of calcitonin decreased to normal after surgery.

Conclusion: The authors present this case not only for its rarity but also to illustrate the importance of calcitonin in the evaluation of a thyroid nodule.

Nothing to Disclose: ST, CF, PF, MFB

P1-592

A Case of Medullary Thyroid Carcinoma with Multiple Pulmonary and Liver Metastases.

N Hiroi¹, N Masai¹, R Iga¹, A Yoshihara¹, S Mariko¹, Y Ando¹ and G Yoshino¹.

¹Toho Univ Tokyo, Japan.

A 50 year-old man with a family history of multiple endocrine neoplasia type 2A (MEN2A) was admitted to our hospital for evaluation of diffuse goiter and neck lymph-node swelling. An endocrinological examination detected hypothyroidism. There was no elevation of adrenocortical hormones, catecholamines or parathyroid hormone. Levels of carcinoembryonic antigen and calcitonin were 2,108 ng/ml and 39,900 pg/ml, respectively. Ultrasound sonography and CT scan of the neck showed an irregular hypoechoic diffuse large thyroid gland containing calcification with bilateral lymph-node swelling. Multiple metastatic lesions in the lung and liver, and a left adrenal mass were observed in an abdominal CT scan. Positron emission tomography showed bilateral enlargement of thyroid glands and lymph-nodes, and significant uptake in lung and liver. ¹³¹I-MIBG scintigraphy was negative. Fine needle aspiration cytology of thyroid glands revealed poorly differentiated carcinoma. A final diagnosis of MEN2A with medullary thyroid carcinoma (MTC) stage T4N1M1 (Stage IV) was made. No advanced therapy was given because of advanced carcinoma with multiple metastases. A tracheal stent was inserted for severe compression due to MTC. DNA sequence analysis of the RET gene showed a TGC to TAC mutation at codon 634 of exon 11.

MTC is a tumor that originates from thyroid parafollicular cells, and it accounts for 1.4% of total cases of thyroid carcinomas in Japan [1]. Inherited MTC syndromes including MEN2A, 2B and familial MTC, affect approximately 1 in 30,000 individuals [2]. The most common RET gene mutation which causes MEN2A is at codon 634 [3], and is associated with early onset of MTC and frequent development of pheochromocytoma [2]. The 10 year survival rate for stage IV of MTC has been reported as 21% [4]. This case is rare because the MTC had a late-onset despite the codon 634 mutation.

- (1)Ezaki H et al., Cancer 1992;70:808
- (2)Kloos RT et al., Thyroid 2009;19:565
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Nothing to Disclose: NH, NM, RI, AY, SM, YA, GY

P1-593

Multinodular Goiter as the Initial Presentation of Systemic Sarcoidosis: Limitation of Fine Needle Biopsy.

Thanh D. Hoang D.O.¹, Vinh Q. Mai D.O.¹, Huong D. Nguyen M.D.¹, Patrick W. Clyde M.D.¹, Babette C. Glister M.D.¹ and K.M. Mohamed Shakir M.D.¹.

¹Natl Naval Med Ctr Bethesda, MD.

Background: In systemic sarcoidosis, involvement of the thyroid gland occurs in 4.4% of the cases. We report a case of a 65-year-old woman presenting with a nontoxic multinodular goiter as the initial manifestation of systemic sarcoidosis. **Clinical case:** A 65 yo woman presented with a left thyroid mass, dyspnea predominantly in the supine position, and rightward tracheal deviation. Thyroid sonogram revealed a multinodular goiter with a 3.4cm x 2.5cm x 3.8 cm ill-defined mass in the left lobe. Fine needle biopsy of this mass under ultrasound guidance showed cytological features consistent with benign nodular goiter. Despite a benign cytology result, patient underwent total thyroidectomy because of her compressive symptoms. Histopathological examination revealed non-caseating granulomata diffusely distributed throughout both thyroid lobes suggesting sarcoidosis. Grocott's Methenamine Silver and Ziehl-Neelsen stains were negative for fungi and mycobacteria, respectively. Subsequent chest CT scan confirmed extensive bulky mediastinal and hilar adenopathy and multiple small pulmonary nodules, consistent with pulmonary sarcoidosis. Pulmonary function tests demonstrated normal baseline spirometry and diffusing capacity for carbon monoxide (DLCO). Laboratory results are shown in Table1.

LABORATORY VALUES AT INITIAL DIAGNOSIS

| TESTS | VALUES | REFERENCE |
|--|--------|-----------|
| Serum calcium (mg/dL) | 10.0 | 8.7-10.4 |
| Ionized calcium (mmol/L) | 1.23 | 1.12-1.32 |
| Albumin (g/dL) | 4.9 | 3.4-5.0 |
| 1,25 dihydroxyvitamin D (pg/mL) | 66 | 18-72 |
| 25 hydroxyvitamin D (ng/mL) | 37 | 30-80 |
| Serum PTH (pg/mL) | 46 | 13-75 |
| Angiotensin converting enzyme, ACE (U/L) | 30 | 9-67 |
| TSH (mIU/mL) | 2.1 | 0.35-5.5 |
| Thyroperoxidase Ab (IU/mL) | 10 | 0-20 |

Conclusion: A nontoxic multinodular goiter can herald systemic sarcoidosis. Of note, the fine needle biopsy did not diagnose thyroidal involvement by sarcoidosis in our patient. When histology reveals non-caseating granulomata in the thyroid, further evaluation for systemic sarcoidosis, including chest X-ray and/or CT scan of the chest should be obtained. In patients with a history of systemic sarcoidosis, careful thyroid palpation and thyroid function tests should be performed. We believe that further studies should be conducted to determine the accuracy of fine needle biopsy in diagnosing thyroid involvement by sarcoidosis in patients with systemic sarcoidosis and a coexisting multinodular goiter.

Vailati A., et al. Sarcoidosis of the Thyroid: Report of a Case and a Review of the Literature. *Sarcoidosis* 1993; 10:66-68.

Nothing to Disclose: TDH, VQM, HDN, PWC, BCG, KMMS

P1-594

Rare Case of Subacute Thyroiditis Related to Infectious Mononucleosis.

A Korman MD¹, N Needleman MS⁴*, D Beyda MD¹ and L Newman MD¹.

¹Beth Israel Med Ctr Manhattan, NY and ²Albert Einstein Coll of Med Bronx, NY.

Background: Subacute thyroiditis has been associated with viral syndromes but with only a rare report in the setting of infectious mononucleosis.

Case: An 18 year-old male presented with 3 weeks of fevers, night sweats, a 5lb weight loss and moderate cervical and axillary adenopathy. Past medical history was significant for asthma. The PMD felt that he had infectious mononucleosis however his heterophile Ab screen was negative. AST and ALT were slightly elevated at 50U/L(15-46U/L) and 83U/L(13-69U/L) respectively. WBC was normal but with 25% atypical lymphocytes and a general health screen revealed TSH was low at 0.01mIU/L(0.50-4.30mIU/L). The patient was referred to Endocrinology.

Physical exam was significant for tachycardia of 108bpm, warm and diaphoretic skin, cervical and axillary lymphadenopathy, tremulousness, and a palpable 30 gram smooth thyroid without bruit. TSH was 0.01 mIU/L, Free T4 1.6ng/dL(0.9-1.4), Total T3 293ng/dL(97-186ng/dL), Thyroglobulin 72.4ng/mL(2-35 ng/mL), and thyroglobulin and TPO antibodies were negative. AST and ALT remained elevated. Since infectious mononucleosis was suspected and the heterophile antibody screen was negative, EBV titers were obtained and found to be elevated with IgG 0.12(0-0.9) and EBV IgM >7(0-0.9). Further thyroid evaluation with ultrasound revealed borderline goiter without thyroid nodules with surrounding reactive lymphadenopathy. 24-hr RAIU and thyroid scan showed low uptake of 5%(normal-10-30%) with poor visualization of the thyroid gland. A diagnosis of subacute thyroiditis thought to be secondary to infectious mononucleosis was made given the time frame of illnesses and positive EBV IgM antibodies. The patient was treated with supportive care and Verapamil ER 120 mg daily. Approximately two months later the patient returned to the office with a mild abatement of symptoms. Repeat laboratory testing showed resolution of the atypical lymphocytes and transaminitis. TSH was now elevated to 7.39mIU/L(0.38-5.50mIU/L) with normal Total T3 127ng/dL(60-181ng/dL) and Free T4 0.8ng/dL(0.7-1.7ng/dL). The patient was diagnosed as being in the hypothyroid phase of subacute thyroiditis and prescribed Synthroid 25mcg daily.

Conclusion: Subacute thyroiditis may be caused by infectious mononucleosis. Concurrent diagnoses of these illnesses must be considered in patients with chemical hyperthyroidism in the setting of significant cervical adenopathy and other signs/symptoms of infectious mononucleosis.

Nothing to Disclose: AK, NN, DB, LN

P1-595

Is Black Thyroid a Benign Disease?.

S A Ali MD¹, A Khan MD¹, E Kandil MD¹, V Fonseca MD¹ and J J Kalariaichal MD¹.

¹Tulane Univ New Orleans, LA.

Background: Black thyroid is a rare entity and has been considered pathognomonic of chronic minocycline ingestion. An association of "black" thyroid with thyroid cancer has been speculated but no direct causal relationship has been established. Hence, we sought to identify the malignant potential of such glands which, to our knowledge, has not been documented.

Methods: A retrospective chart review of 433 patients was performed. Patients were grouped based on pathology: 1) benign, 2) papillary carcinoma (PTC) and 3) non-papillary thyroid carcinoma tumors (non-PTC).

Results: 63 patients (15%) with "black" thyroid gland were identified. Among these, 22 (34.92%) were benign, 21 (33.33%) had PTC and 20 (31.75%)

had non-PTC. Hence, more of the "black" thyroids were malignant than benign

($p = 0.0001$). Further analysis of PTC patients revealed that 25% of those with "black" thyroid ($n = 5$) had macro-adenoma vs. 44.44% ($n = 24$) in the non-black PTC group ($p = 0.1281$). Similarly 45% ($n = 9$) had multifocal vs. 59% ($n = 32$) in black vs. non-black PTC group respectively. ($p = 0.27$).

Conclusion: The finding of "black" thyroid gland is not rare as thought before. To our knowledge, this is the first study aimed at documenting the malignant potential of such glands with this finding. Our study shows that the risk of malignancy is higher in such glands. Furthermore, amongst those with PTC, presence of pigment did not impact the size or focality of the tumor.

Disclosures: VF: Investigator, Novo Nordisk; Consultant, Novo Nordisk.

Nothing to Disclose: SAA, AK, EK, JJK

P1-596

Rapidly Expanding Neck Mass in a 60 Year Old Female Causing Compressive Symptoms.

J Waddadar MD¹, H Safah MD¹, M Goswami MD¹, A Khan MD¹, B Jaffe MD¹, O Abdullah MD¹, T Thethi MD¹ and E Kandil MD¹.

¹Tulane Hlth Scis Ctr New Orleans, LA.

Presentation: The patient, a 60 year old white female with past medical history of primary hypothyroidism being treated with levothyroxine presented with a right-sided non tender neck mass rapidly expanding over 3 weeks causing airway compression and difficulty in swallowing. There were no constitutional symptoms. A CT scan of the neck revealed a right-sided thyroid mass measuring 8.7×7.1×7.0 cm. Fine needle aspiration and flow cytometry studies established the diagnosis of lymphoma. The patient then underwent an incisional neck biopsy, which showed the findings of a lymphoma consisting of small to medium sized cells. Immunohistochemistry and flow cytometry confirmed a B cell lymphoma. The final diagnosis according to latest WHO criteria was "B cell lymphoma, unclassifiable with features intermediate between diffuse large B cell lymphoma (DLBCL) and Burkitt like", an entity previously known as Burkitt like lymphoma, a variant of Burkitt's lymphoma (BL).

The patient was started on the chemotherapeutic regimen for Burkitt lymphoma, which caused rapid shrinkage of the neck mass over one week. A CT-PET scan performed three months later showed no evidence of residual disease.

Discussion: Burkitt like lymphoma, are aggressive lymphomas that have morphological and genetic features intermediate between DLBCL and BL. It is a subtype of non-Hodgkin lymphoma that most commonly affects lymph nodes and the reticuloendothelial system. It rarely affects the thyroid. Epidemiological studies have shown that patients with chronic lymphocytic thyroiditis have an increased risk of developing thyroid lymphomas when compared to age and gender matched individuals. The female predominance of Hashimoto's thyroiditis explains the 3 to1 female to male gender ratio found in primary thyroid lymphoma.

The rarity of this tumor, in conjunction with the lack of specific imaging findings, can be diagnostically challenging. Clinicians should keep thyroid lymphomas in mind when encountering a rapidly expanding compressive neck swelling, especially in a patient with chronic lymphocytic thyroiditis, as the treatment results can be dramatic.

Nothing to Disclose: JW, HS, MG, AK, BJ, OA, TT, EK

P1-597

A Rapidly Enlarging Thyroid Mass.

LC Dinkins Lagonowicz M.D.¹ and B Miranda-Palma¹.

¹Univ of Miami, Miller Sch of Med, Jackson Memorial Hosp Miami, FL.

Background: Thyroid lymphoma is a rare malignancy similar to anaplastic thyroid cancer that can present as a rapidly enlarging neck mass fixed to surrounding tissues. It usually presents in the seventh decade of life and is often associated with Hashimoto's thyroiditis. This case illustrates how thyroid lymphoma can present earlier in life and is curable when diagnosed promptly.

Case: A 47 year old female presented to the ER with a four-week history of a rapidly growing left neck mass. She developed a sore throat two weeks prior to presentation that resolved but reports worsening hoarseness. She admits to fatigue, constipation, cold intolerance, but denies fever, weight loss/gain, shortness of breath, dysphagia, night sweats, a family history of thyroid cancer nor neck radiation. On exam, she had an enlarged thyroid, left > right, extending below the sternocleidomastoid associated with lymphadenopathy. It is firm, nontender, immobile with swallowing with no bruits appreciated. No masses were seen with laryngoscope but left vocal cord paralysis was noted. CT scan of the neck revealed bilateral thyroid masses, largest on the left measuring 4.8 cm with enlarged lymph nodes. The trachea is deviated to the right. Thyroid function tests revealed: TSH 11.13 uIU/ml (ref: 0.2-4.2 uIU/ml), free T4 0.9 ng/dl (ref: 0.9-1.7 ng/dl), T3 102.3 ng/dl (ref: 80-200 ng/dl), and microsomal/TPO antibodies 16 IU/mL (ref: <20 IU/mL). Large bore needle FNA revealed lymphoma versus anaplastic thyroid cancer. Core biopsy confirmed thyroid large B-cell lymphoma. PET scan showed metabolically-active tumor only in the neck. She was started on synthroid 75mcg for her hypothyroidism and symptoms improved. She underwent 8 cycles of chemotherapy with R-CHOP and received radiation with complete response.

Conclusions: Thyroid lymphoma can be misdiagnosed as anaplastic thyroid cancer because both malignancies present as a rapidly growing neck mass and can be difficult to distinguish on FNA. One distinguishing feature is that Hashimoto's thyroiditis may be a predisposing factor for thyroid lymphoma but not for anaplastic thyroid cancer. However, this case illustrates that thyroid lymphoma can be associated with hypothyroidism not secondary to Hashimoto's thyroiditis in a patient with a rapidly growing neck mass. Because large cell thyroid lymphomas have an aggressive course, a rapid diagnosis is important to increase the likelihood of a complete remission, which is illustrated in this case.

Nothing to Disclose: LCDL, BM-P

P1-598

Resistance to Thyroid Hormone Complicated with Graves' Disease in a Patient with Thyroid Hormone Receptor beta Gene Mutation (G251R).

T Shiwa¹, K Oki¹, M Hirano¹, S Maeda¹, T Asao¹, T Sugihiro¹, T Awaya¹, S Nakanishi¹, K Yamane¹ and N Kohno¹.

¹Hiroshima Univ Hiroshima, Japan.

Background: Resistance to thyroid hormone (RTH), which is syndrome involving reduced tissues responsiveness to thyroid hormone, is characterized by elevated serum levels of thyroid hormone and normal or slightly increased serum thyrotropin (TSH) level. Also, Graves's disease shows elevated serum thyroid hormones, but suppressed serum TSH in Graves's disease is different from RTH. Therefore, when a patient has RTH complicated with Graves' disease, the clinical course would be extremely comprehensive.

Clinical Case: A 33-year-old female patient had the elevation of serum FT4 and FT3 levels (3.0 ng/dl and 9.3 pg/ml, restrictively) and suppressed serum TSH level (0.007 μ U/ml). Since anti thyroid receptor antibodies was positive (TBII 80.7% and TSAb 1832%), she was diagnosed as having Graves' disease and thiamazole (MMI) was administered. Interestingly, TSH elevated gradually, even though thyroid hormones never decreased. These findings absolutely expressed syndrome of inappropriate secretion of TSH (SITSH), and thus both RTH and TSH producing adenoma were considered as possible diagnoses. Three years after the initiation of MMI therapy, thyroid receptor antibody (TRAb) showed negative. There was no pituitary tumor in pituitary gland by Magnetic resonance imaging. The TRH-stimulation tests after administration of graded doses of liothyronine (L-T3) were performed. Before and after L-T3 administration of 50 μ g, 100 μ g, or 200 μ g per day for 3 consecutive days, the TSH responses to TRH stimulation were examined. TSH showed normal response before administration of L-T3, and the peak of TSH responses to TRH after graded doses of L-T3 reduced dependent of L-T3 doses. On the basis of these findings, we diagnosed her as RTH clinically. Direct sequence analysis of the THRB gene revealed a mutation causing replacement of a glycine (G) with arginine (R) at codon 251.

Conclusion: We demonstrated the patient who was diagnosed as having RTH during therapy of Graves' disease. In this case, it was difficult to assess the remission of Graves' disease only by thyroid hormone levels. The elevation of TSH without the decrease of thyroid hormone level and negative for TRAb would be useful to evaluate the remission of Graves' disease in patients with RTH.

Nothing to Disclose: TS, KO, MH, SM, TA, TS, TA, SN, KY, NK

P1-599

Thyroidectomized Patients with Resistance to Thyroid Hormone (RTH): Is It a Model of Altered Deiodinase Activity?.

RM Paragliola Dr¹, RM Lovicu Dr¹, MP Ricciato Dr¹, F Ianni Dr¹, A De Rosa Dr¹, F Gallo Dr¹, P Locantore Dr¹, P Senes Dr¹, A Pontecorvi Prof¹ and SM Corsello Prof¹.

¹Catholic Univ Sch of Med Rome, Italy.

RTH is a genetic disease caused by point mutations in the THRB gene that encodes thyroid hormone receptor β , characterized by a reduced responsiveness of target tissues to thyroid hormone. Therefore, the biochemical feature is a persistent elevation of circulating FT4 and FT3 associated with non-suppressed TSH levels. We observed 3 patients in which RTH was unequivocally diagnosed on the basis of familiar history, laboratory data and genetic testing. Thyroid function evaluation confirmed hyperthyroidism with inappropriate TSH secretion (mean TSH values $\sim 10 \mu\text{U/ml}$). Patients underwent total thyroidectomy for multinodular goiter and after surgery they were started on LT4 therapy. Laboratory evaluations performed on substitutive LT4 therapy were: (patient 1) FT3 4.01 pg/ml; FT4 18.4 pg/ml; TSH 69 $\mu\text{U/ml}$; (patient 2) FT3 3.4 pg/ml; FT4 15.6 pg/ml; TSH 53 $\mu\text{U/ml}$; (patient 3) FT3 3.9 pg/ml; FT4 16.2 pg/ml; TSH 72 $\mu\text{U/ml}$. We observed that, on LT4 $\sim 2.5 \mu\text{g/kg/day}$, despite a clinical euthyroid status and a normal-high FT3 and FT4 similar to the preoperative values, TSH was persistently much higher than pre-surgical levels.

We suppose that this phenomenon can be explained with the different biochemical features between exogenous LT4 and endogenous thyroid hormone, as well with the deiodinase activity that could be impaired in RTH patients. As well known, synthetic LT4 is the standard replacement therapy in patients with hypothyroidism while T3 is due to peripheral conversion of LT4 to T3 by deiodinase activity. In particular, type 2 deiodinase (D2) generates intracellular T3 in several human tissues, including hypothalamus and pituitary, playing a central role in the negative feedback regulation of TSH secretion.

It is possible that synthetic LT4, uniquely in RTH patients, presents a reduced affinity for D2. Decreased hypothalamic and pituitary D2-dependent deiodination could further worsen, after total thyroidectomy, the inability of thyroid hormones to determine an adequate negative feedback and could explain the further sudden increase of TSH values despite high-normal peripheral values of FT3 and FT4.

Nothing to Disclose: RMP, RML, MPR, FI, ADR, FG, PL, PS, AP, SMC

P1-600

A Case of Resistance to Thyroid Hormone (RTH) Accompanying Hyperthyroidism.

K Inagaki M.D.¹, F Otsuka M.D., Ph.D.¹, J Suzuki M.D.¹, E Nakamura M.D.¹, N Tsukamoto M.D.¹, M Takeda M.D.¹, T Miyoshi M.D.¹, Y Mimura M.D.¹, T Ogura M.D.¹ and H Makino M.D., Ph.D.¹.

¹Okayama Univ Graduate Sch of Med, Dentistry and Pharma Scis Okayama, Japan.

Introduction: Coincidence of RTH and hyperthyroidism is clinically very rare. We here report an interesting case with RTH complicated with hyperthyroidism. **Case presentation:** A 40-year-old male having palpitation, body weight loss and diffuse goiter was primarily diagnosed as Graves' disease complicated with atrial fibrillation (Af). Serum free thyroxine (FT4) and free triiodothyronine (FT3) levels were elevated (5.1 ng/dl and 11.9 pg/ml, respectively), and serum thyrotropin (TSH) was lowered (<0.03 μ U/ml). Since TSH-receptor antibody (TRAb) was positive, thiamazole had been transiently administered for 1 year as diagnosis of Graves' disease. Thyrotoxic symptoms had been gradually ameliorated in accordance with lowering thyroid hormones although Af persisted. However, at the age of 44, hyperthyroidism recurred and both FT4 and FT3 were found to be high (4.3 and 17.9, respectively) with lowering TSH (<0.01). Treatment with thiamazole was then resumed and the patient was referred to our hospital. At the first examinations of thyroid function in our clinic, serum TSH level was found to be inappropriately high (5.24), i.e. SITSH, regardless of hyperthyroidism (FT4 2.18 and FT3 6.20) under thiamazole (10mg) therapy. Ultrasonography showed diffuse hypoechoic goiter (~70 g) with inhomogeneous pattern. Technetium scintigraphy exhibited diffuse-high uptake of tracer in the thyroid (17.2%, normal <4.0). Anti-thyroid peroxidase antibody and anti-thyroglobulin antibody were positive, and TRAb and thyroid stimulating antibody (TSAb) levels were slightly high above the normal ranges. Considering the absence of pituitary tumor by MRI and the presence of TSH hyperresponse to TRH stimulation, RTH status was suspected as a cause of SITSH. Direct sequencing analysis of the thyroid hormone receptor- β gene revealed heterozygous replacement of arginine with histidine at amino acid position 383. The patient was diagnosed as sporadic RTH associated with autoimmune thyroiditis. Administration of thiamazole was gradually reduced and discontinued eventually. Hyperthyroidism has not been recurred and the size of thyroid was gradually reduced. **Conclusion:** In the present case, the preceding autoimmune-thyroiditis was considered to be a cause of his hyperthyroidism. It is clinically difficult to diagnose RTH especially when patients suffer hyperthyroidism. Careful observation is necessary to distinguish RTH and to avoid prolonged therapy with antithyroid drugs.

Nothing to Disclose: KI, FO, JS, EN, NT, MT, TM, YM, TO, HM

P1-601

Factitious TSH Elevation Due to Interfering IgG Secreted by an Effusion Lymphoma.

TL Thompson MD², G Bahtiyar MD^{1,3,4}, R Batra MD^{1,3}, G Hidalgo MD^{1,3}, RM Flores MD^{1,3} and A Sacerdote MD^{1,3,4}.

¹Woodhull Med Ctr Brooklyn, NY ; ²SUNY Downstate Med Ctr Brooklyn, NY ; ³New York Univ New York, NY and ⁴St George's Univ Sch of Med St George's, Grenada.

Introduction/Background

Effusion lymphomas are extremely rare, especially in HIV negative patients. They have been reported to secrete IgG, as in this patient.

Clinical Case

A 67 y/o Hispanic man with type 2 DM, hypertension, and effusion lymphoma was admitted for pneumonia with pleural effusions and edema, complicated during admission by sepsis and adrenal crisis. The patient was evaluated for abnormal TFTs. Thyroid function tests during the hospital course and IgG levels are shown in the table below. TSH and free T4 were done by chemiluminescence, T4 and T3 by immunoassay, T3RU by spectrophotometry, and IgG by immunoturbidimetry.

Lab Values

| TSH (0.35-5.50 mIU/L) | T4 (4.5-10.9 mcg/dl) | T3RU (22.5-37.0 %) | Free T4 index (4.5-10.9) | FT4 (0.89-1.76 ng/dl) | T3 (60.0-181.0 ng/dl) | IgG (700-1600 mg/dl) | |
|-----------------------|----------------------|--------------------|--------------------------|-----------------------|-----------------------|----------------------|----------------------------|
| 6.58 | 7.2 | 48.8 | 11.7 | | 60.8 | 2542 | Baseline 1 |
| 10.84 | 4.7 | 48.8 | 7.5 | 0.86 | 45.0 | 2880 | Baseline 2 |
| 2.83 | 2.4 | 52.5 | 4.2 | | | 1598 | On hydrocortisone 50mg q6h |

Reverse T3 by RIA was 74 mg/dl (11-32) concomitant with the last set of TFTs. Thyroid autoantibodies were negative.

Conclusion

Although glucocorticoids suppress TSH secretion, the precipitous normalization of serum TSH simultaneously with the approximate halving of the IgG level, indicates that the neoplastic IgG caused factitious TSH elevation. This is the first reported case of a tumoral IgG interfering with TSH measurement.

Nothing to Disclose: TL, GB, RB, GH, RMF, AS

P1-602

Hypokalemic Periodic Paralysis: A Rare Complication of Hashimoto's Thyroiditis.

J Singh M.D.¹, K Soe M.D.², YL Chen M.D.¹, MA Michel-Vincent M.D.¹, R Pant M.D.¹, A Sacerdote M.D.^{1,2} and G Bahtiyar M.D.^{1,2}.

¹Woodhull Med Ctr, NYU Sch of Med Brooklyn, NY and ²State Univ of New York, Hlth Sci Ctr at Brooklyn Brooklyn, NY.

Background: Periodic paralysis (PP) is a heterogeneous group of muscle disorders characterized by episodes of flaccid weakness occurring at irregular intervals associated with hypokalemia. It is usually due to hyperthyroidism and rarely seen as a consequence of hypothyroidism. The incidence is less than 0.1%. A proposed mechanism of PP due to hypothyroidism is the destruction of proximal renal tubular cell by antibodies against thyroid gland resulting type II renal tubular acidosis which is characterized by an inability to reabsorb bicarbonate and excessive loss of potassium in urine. The initial treatment is potassium replacement to acutely reverse the paralytic attack. However the main goal of therapy should be to establish a euthyroid state to prevent recurrences.

Clinical Case: A 22-year-old man from Ecuador presented with acute paralysis of all extremities. He had a similar episode previously after which he had been suffering paroxysms of muscle weakness and paralysis. There is no family history of any neuromuscular diseases. His vital signs were within normal limits. Physical exam was unremarkable except for symmetrical proximal muscle weakness (1/5) in all extremities with no atrophy nor fasciculation and bilateral delayed deep tendon reflexes. EKG revealed first degree AV block and wide QRS. Serum electrolyte analysis was notable for potassium: 2.0 mEq/L (3.5-5.3 mEq/L), chloride: 110 mmol/L (95-108 mmol/L), bicarbonate: 18 mmol/L (24-31 mmol/L) and normal anion gap indicating hyperchloremic non-anion gap metabolic acidosis. Thyroid panel showed, TSH: 130.2 mU/L (0.45-4.5 mU/L), total T4: 1.5 mcg/dl (4.5-10.9 mcg/dl), T3 uptake: 26.2% (25-32%), free T4: 0.42 ng/dl (0.89-1.76 ng/dl) and thyroid microsomal antibody: 393 IU/ml (<60 IU/ml) and was consistent with Hashimoto's thyroiditis. In the view of elevated TSH, hypokalemia and episodic muscle weakness, a diagnosis of hypothyroid hypokalemic PP was made. He was treated with potassium and thyroid hormone supplement resulting in a full recovery within two days. After achieving a clinical euthyroid state, he had normal potassium without supplementation and experienced no subsequent paralytic episodes.

Conclusion: Although the association of hypokalemic periodic paralysis with thyroid disorder is well recognized, it is most often described in the context of thyrotoxicosis however it is important to consider Hashimoto's thyroiditis as a rare but significant etiology when investigating of PP.

(1)Papa L et al., AJCM.2005; 2:20

(2)Bandhakavi M. Clin Pediatr.2009; 48:677

Nothing to Disclose: JS, KS, YLC, MAM-V, RP, AS, GB

P1-603

An Unlikely Presentation of Myxedema Coma.

VS Lagari DO, MPH¹ and B Miranda MD¹.

¹Univ of Miami Sch of Med Miami, FL.

Background: Myxedema coma is rare and may not be considered as occurring as a consequence of hypothyroidism resulting from partial thyroidectomy. This case illustrates how myxedema coma may occur as a result of deficiency of thyroid hormone from partial thyroidectomy.

Case: A 71 year old woman presented because of progressive altered mental status over 2 weeks. One day before presentation, she complained of shortness of breath and her primary care physician prescribed a steroid taper and albuterol. Five months prior, she underwent right thyroidectomy for a follicular neoplasm. Final pathology revealed nodular hyperplasia. She never received thyroid hormone following surgery. On presentation, physical exam revealed hypothermia, periorbital edema, macroglossia, hypoactive bowel sounds, delayed deep tendon reflexes, and cold, dry skin. She was intubated emergently for respiratory depression (ABG: pH 7.11, CO₂ 150, O₂ 90, HCO₃ 47). Thyroid function tests revealed: TSH 45.7 uIU/ml (ref: 0.2-4.2 uIU/ml), free T₄ 0.2 ng/dl (ref: 0.9-1.7 ng/dl), and T₃ 26.9 ng/dl (ref: 80-200 ng/dl). She received a loading dose of levothyroxine 200 mcg IV and maintenance dose 88 mcg IV daily. She also received liothyronine 25 mcg BID. Prior to starting the thyroid hormone she received hydrocortisone 50 mg Q8 hours. Within 4 hours of receiving liothyronine, she was awake and following commands. Hydrocortisone was discontinued after co-syntropin stimulation test ruled out adrenal insufficiency. Her hospitalization was complicated by a pneumothorax and hospital acquired pneumonia. She remained intubated and did not recover. Follow-up free T₄ was 1.63 ng/dl.

Conclusions: Myxedema coma is rare and associated with a high mortality (60-70%). Predisposing factors include advanced age and underlying co-morbid conditions. Most cases of myxedema coma occur from severe hypothyroidism following total thyroidectomy. Hypothyroidism resulting from partial thyroidectomy is under appreciated and one recent review noted an incidence of 10.9%. This patient did not have a clear precipitating factor other than unrecognized hypothyroidism that caused myxedema coma. This case illustrates several important points. First, myxedema coma can result from hypothyroidism following partial thyroidectomy. She should have been monitored for the onset of hypothyroidism following her surgery. This case also highlights the morbidity associated with myxedema coma, as this patient was not able to make a full recovery.

Nothing to Disclose: VSL, BM

P1-604

Hypothyroidism Presenting as Cardiac Tamponade.

N Malhotra MD¹, SM Morduchowitz MD¹, AM Karnik MD^{1,2} and KH Hupart MD^{1,3}.

¹Nassau Univ Med Ctr East Meadow, NY ; ²SUNY Stony Brook Stony Brook, NY and ³Albert Einstein Coll of Medicine Bronx, NY.

Background:

Pericardial effusion has been reported to occur in 3-6%(1) hypothyroid patients when assessed by echocardiography and its occurrence appears to be related to the severity of thyroid dysfunction. However, cardiac tamponade rarely complicates hypothyroidism. We present the case of a young woman in whom hypothyroidism was discovered because of her presentation with cardiac tamponade.

Clinical Case:

MM is a 29 yo woman who presented with dyspnea on exertion, lightheadedness and menorrhagia. PEx demonstrated bradycardia (52bpm), hypotension (98/55), pallor, cool extremities, galactorrhea, doughy dry skin and delayed relaxation phase of deep tendon reflexes. EKG: low voltage. CXR: cardiomegaly. Labs: Hgb=5.8, TSH=190 uU/ml(0.27-4.2), FreeT4=0.11ng/dl(0.71-1.85) andT3<20ng/dl(85-202). She was admitted to the ICU and begun on T4 :75 mcg/d. Her course was complicated by syncope; ECHO displayed a large pericardial effusion, low EF(50%), right ventricular and atrial collapse, and other findings specific for cardiac tamponade(2). Pericardiocentesis drained 1L of clear fluid. Investigation for other causes of pericardial effusion was unrevealing. Within 24 hours pericardial fluid rapidly reaccumulated requiring pericardial window surgery. She evolved favorably in the post-operative period. At a recent outpatient visit she was euthyroid on therapy; repeat ECHO revealed EF 65% and no pericardial effusion.

Discussion:

Cardiac tamponade secondary to hypothyroidism was first reported in 1965(3). By 1992, fewer than 30 cases of hypothyroid cardiac tamponade had been reported in a literature review(4). Most of these were treated with pericardiocentesis or pericardial window placement; only one reported patient demonstrated reaccumulation mandating further intervention and this occurred one month after the initial surgery. Our patient's presentation is notable, both because of tamponade being the clinical event that lead to the diagnosis of hypothyroidism and because of the rapid accumulation of her effusion after successful drainage. It is interesting to note that the preponderance of clinical reports of hypothyroid cardiac tamponade have been from the developing world suggesting that limited access to health care may impair the diagnosis of hypothyroidism in its early stages. We hope to call attention to pericardial effusions complicating hypothyroidism and will present a review of the literature to suggest therapeutic approaches to these patients.

1 Kabadi UM and Kumar SP, Am Heart J 1990;120:1393

2 Armstrong WF et al.,Circulation 65:1491

3 Martin L et al., Br Med J 1965; 2:83

4 Jiménez-Nácher JJ et al., Rev Clin Esp 1993; 193:290

Nothing to Disclose: NM, SMM, AMK, KHH

P1-605

Primary Hypothyroidism and Thyroid Eye Disease: Two Common Disorders in an Uncommon Association.

CK Chew MRCP¹ and R Dalan MRCP¹.

¹Tan Tock Seng Hosp Singapore, Singapore.

Introduction : Thyroid eye disease (TED) is commonly seen in Graves' disease and associated with hyperthyroidism. We report two cases of thyroid eye disease associated with primary hypothyroidism.

Case Reports :

Case 1 : A 52 years old Chinese lady presented to the ophthalmologist with lacrimation, conjunctival congestion, exophthalmos and proptosis of the right eye. A mild abduction deficiency was detected on physical examination. A CT scan of the orbits showed right sided proptosis with thickening of the recti muscles suggestive of thyroid eye disease. Laboratory investigation showed free T4 = 9 pmol/L (RI:8-21) ; TSH = 9.26 mIU/L (RI:0.34-5.64); anti-TPO antibody = 249 mIU/L (RI<50), TSH receptor antibody (TRAb) = <0.4 (RI<0.4) ; TSI = 118% (RI:50-179). An ultrasound of the thyroid revealed a diffusely small hypoechoic thyroid gland consistent with chronic hashimoto's thyroiditis. She was started on thyroxine replacement and subsequently her thyroid function normalized with a significant improvement in eye symptoms and proptosis.

Case 2 : A 44 year-old Chinese gentleman presented to the neurologist for bilateral occipital headache. He complained of weight gain, hoarse voice and lethargy for past 2 months. On examination, he had a hoarse voice ,macroglossia, right eye proptosis with conjunctival congestion, and no goitre. A MRI of the brain showed right eye proptosis and excluded any significant space occupying lesions. Laboratory investigation included free T4 = 2 pmol/l (RI:8-21);TSH = 73.67mIU/L (RI:0.34-5.64), TRAb = 30 IU/L (RI <0.4), anti-TPO antibody = 138 IU/ml (RI <50), anti-thyroglobulin = 400 titre (RI < 100) and thyroid blocking antibody = 12 % (< 71% indicates blocking). He was started on thyroxine replacement with significant improvement in visual symptoms and hypothyroidism.

Discussion : Hashimoto's thyroiditis could be a possible trigger to autoreactive B cells to produce TRAb reactive to the TSH receptor on fibroblasts but is not reactive on the thyroid gland due to autoimmune destruction. In Graves' disease blocking antibodies may be reactive to TSH receptor on fibroblasts inducing TED and blocking the thyroidal TSH receptor inducing hypothyroidism.

Conclusion : TED can rarely occur in association with primary hypothyroidism. Initiation of replacement thyroxine results in a significant clinical improvement.

Nothing to Disclose: CKC, RD

P1-606

Thyroid Dysfunction in Oculoauriculovertеbral Spectrum (Goldenhar Syndrome): Report of 2 Cases.

AD Briscoe MD^{1,2}, TG Kelly MD^{1,2} and RS Newfield MD^{1,2}.

¹Univ of California San Diego San Diego, CA and ²Rady Children's Hosp San Diego San Diego, CA.

Goldenhar syndrome originates from defects in the first and second branchial arches and presents with a spectrum of clinical manifestations. The thyroid gland begins as an epithelial proliferation in the floor of the pharynx derived from the 1st, 2nd, 3rd and 4th branchial arches. It is thus plausible to have a malformed thyroid gland associated with Goldenhar syndrome, which has been reported once in a newborn with athyrosis.

Case 1: A 28 month old patient diagnosed with Goldenhar syndrome (right anotia, left microtia, right facial palsy and microcephaly) associated with pulmonary and cardiac defects developed hypothyroidism. He presented at 9 months with abnormal thyroid labs: T4 5.9 µg/dL (7.5-15.6), TSH 8.39 µIU/ml (0.35-5). Repeat labs were unremarkable, except lowish binding globulins: TSH 1.83, T3U 34.7% (25-35), T4 5.5 µg/dl(7.2-15.6), freeT4 1.1 ng/dl (0.8-2). He returned at 2 years with compensated acquired hypothyroidism: T4 6.9 µg/dl (6.4-13.3), TSH 21.2. He was started on Levothyroxine 50 mcg daily. A Tc99 thyroid scan suggested a right hemithyroid, confirmed via thyroid ultrasound.

Case 2: A 7 day old 31 week female born with dysmorphic features (vertebral segmentation anomalies, fusion of 2 sets of ribs, right microphthalmia, microtia and hypoplastic mandible, right sided facial skin tag, posterior cleft palate, right hand transverse palmar crease) and a presumptive diagnosis of Goldenhar syndrome, had a positive newborn screen for hypothyroidism (77hr of life): TSH 312.95 µIU/ml (<25). She was started on Levothyroxine 10mcg IV daily after confirmatory tests revealed TSH 608.77µIU/ml and T4 1.9 µg/dL (11-21.5). Tc99 thyroid scan done on DOL 20 revealed a lingual thyroid. Laboratory studies improved 2 weeks after initiation of treatment: fT4 1.0ng/dL (0.6-1.6), TSH 73.08 µIU/mL (0.34-5.6). The mother had poorly controlled A2 gestational diabetes. Gestational diabetes can cause many malformations, but this infant did not exhibit the more common diabetes-related dysmorphisms.

Conclusion: Given the potential embryologic increased risk for maldevelopment of the thyroid gland and possibility of late-onset hypothyroidism, it will be beneficial to annually screen young children with Goldenhar syndrome for thyroid function with a TSH level.

Nothing to Disclose: ADB, TGK, RSN

P1-607

A Case Report of Potentiated Acetaminophen Hepatotoxicity by Severe Hypothyroidism in a Patient on Therapeutic Doses of Acetaminophen.

WA Ayoub MD, PhD¹ and S Bindra MD¹.

¹Fairview Gen Hosp Cleveland Clin Hlth Syst Cleveland, OH.

Background: Data from animal studies suggest that hypothyroidism induced either chemically or by thyroidectomy has a protective effect against acetaminophen hepatotoxicity. The mechanism is attributed to the hypometabolism of liver cells in the setting of hypothyroid status (1). However, upon careful review of literature, those experimental data were not substantiated by evidence from any human based clinical studies or case reports. On the contrary, we detail the first case of acetaminophen hepatotoxicity and possible potentiation by severe hypothyroidism while on therapeutic doses of acetaminophen.

Clinical case: A 64 y.o. white, non alcoholic female admitted with 2 weeks of nausea, vomiting and abdominal pain. She had preexisting hypothyroidism and osteoarthritis on therapeutic doses of acetaminophen therapy and synthroid 100 mcg q.d. with baseline TSH of 0.2 ug/dl and synthroid therapy discontinued few weeks prior to admission because of nausea and vomiting. Physical examination showed normal blood pressure, heart rate and no findings suggestive of myxedema coma or encephalopathy. Biochemical workup admission was consistent with acute hepatitis with bilirubin of 4.7 mg/dL, AST of 14254 U/L (nl. 7-40), ALT of 5475 U/L (nl. 30-60) and alkaline phosphatase of 169 U/L (nl. 40-136). Thyroid profile showed severe hypothyroidism with a TSH of 92 mIU/mL (nl. 0.33-5.5), FT4 of 0.76 ng/dL (nl. 0.85-1.8) and FT3 of 0.52 pg/ml (nl. 1.8-4.6). Toxicology screen showed only a mildly elevated serum acetaminophen of 31 (nl. 4.5-20 ug/mL). Viral hepatitis panel and autoantibodies profile were negative. Abdominal ultrasound and computed tomography showed fatty infiltration with no intrahepatic or extrahepatic biliary dilatation. The patient was managed with IV acetylcysteine therapy, with resumption of her synthroid therapy with normal liver enzymes and TSH after 12 weeks. The temporal relation of the hepatotoxicity to cessation of synthroid therapy and severe hypothyroidism, in the absence of any other identifiable causes of acute hepatitis, strongly suggest a possible potentiation by severe hypothyroidism.

Clinical Lessons/Conclusion: We report a possible association between severe hypothyroidism and potentiated acetaminophen hepatotoxicity attributed to reduced clearance of toxic acetaminophen metabolites in the hypothyroid milieu. In any patient with severe hypothyroidism, cautious use of acetaminophen therapy is warranted to avoid possible potentiated hepatotoxicity.

1) Bruck R, Frenkel D, Shirin H et al: Hypothyroidism protects rat liver from acetaminophen hepatotoxicity. Dig Dis Sci. 1999 Jun; 44(6):1228-35.

Nothing to Disclose: WAA, SB

P1-608

Hypothyroidism Presenting as an Acute Coronary Syndrome.

Rola Zamel M.B.B.S¹, Merrill Edmonds MD, FRCPC¹, Gerard Shoemaker MD, FRCPC¹ and Tisha Joy MD, FRCPC¹.

¹Schulich Sch of Med and Dentistry, Univ of Western Ontario London, Canada.

Background:

Hypothyroidism is not typically included among the possible causes of acute coronary syndrome (ACS). We describe a case of severe hypothyroidism presenting as ACS.

Clinical Case:

This 43-year-old gentleman presented to the emergency department with a 15 minute history of ischemic type chest pain. Past medical history included depression, and hepatitis C from remote IV drug abuse. His only medications were venlafaxine and prn lorazepam. He denied any recent history of cocaine use. His physical examination including cardiovascular exam was normal. Initial creatine kinase (CK) and troponin I (TnI) were elevated at 371 U/L (normal 35-232) and 1.29 µg/L (normal 0.07 ≤µg/L) respectively. Despite ongoing chest pain for 1 hour, his initial and repeat 12-lead electrocardiogram demonstrated no ischemic changes. His peak CK and TnI values were 484 U/L and 10.14 µg/L. Consequently, a diagnosis of non-STEMI was established and the patient was admitted for appropriate management.

During hospitalization, a 2D echocardiogram demonstrated a mild pericardial effusion and LV dysfunction with an ejection fraction of 40% (normal ≥60%). He continued to have recurrent ischemic-type chest pain resolving each time with sublingual nitroglycerin, prompting further investigation with a coronary angiogram. Unexpectedly, the angiogram revealed normal coronary arteries, but there was evidence of global hypokinesia and mild LV dysfunction. Thyroid function tests (TFTs) were ordered as part of the evaluation into the cause of the cardiomyopathy. The serum TSH was 310.85 mU/L (normal 0.35-5.0), free T4 <5.0 pmol/L (normal 10-20), and free T3 2.8 pmol/L (normal 2.6-5.7). He did not exhibit any typical features of hypothyroidism on history or physical examination, apart from depression and some deepening of his voice.

In addition to usual anti-ischemic therapy, low-dose L-thyroxine was commenced. Angina resolved during levothyroxine treatment. Three weeks after starting levothyroxine, TFTs had improved with a TSH of 141.58, free T4 of 11, and free T3 of 3.8. CK and TnI had returned to normal at 221 and <0.04, respectively. Anti-TPO and anti-thyroglobulin antibodies were positive.

Conclusion: This interesting case of hypothyroidism presenting as ACS with normal coronary arteries serves as a reminder to include severe hypothyroidism in the differential diagnosis of a patient presenting with ACS.

Nothing to Disclose: RZ, ME, GS, TJ

P1-609

Unusually High Levothyroxine Replacement Dose Leads to Recognition of Subclinical Celiac Disease in 2 Patients.

N Speil MD¹, CM Fratianni MD¹, S Gupta MD¹ and R Khardori MD,PhD¹.

¹Southern Illinois Univ Sch of Med Springfield, IL.

Despite standard adult replacement doses of levothyroxine (LT4) ranging from 1.7-2.2 mcg/kg/day depending on the therapeutic TSH target, in practice very high doses of thyroid hormone replacement are sometimes required. We present 2 cases with unusually high LT4 requirements which led to recognition of celiac disease.

(A) An 83 year old female with metastatic papillary thyroid cancer presented with pulmonary metastases & left neck mass 11 years after initial presentation. At that time her LT4 dose was empirically increased to 2.2 mcg/kg to 137 mcg for 62.3 kg to target full TSH suppression. Over the next year she demonstrated a remarkable lack of TSH suppression despite progressive LT4 dose escalation to 5.3 mcg/kg/day. Notably her compliance with LT4 was fastidiously documented by family, including pill counts. Medications that could impair LT4 absorption were moved away from her LT4 dose by >4 hrs. During this time she remained clinically euthyroid & denied gastrointestinal complaints, although she was diagnosed with iron deficiency anemia. Tissue transglutaminase IgA was elevated at >250 (normal <20). Duodenal biopsy showed increased lymphocytes & focal villous blunting, c/w celiac sprue. After institution of gluten free diet x 6 weeks TSH became suppressed to 0.08 mIU/ml. Unfortunately, due to continued dietary intolerance, the patient took herself off of the gluten free diet shortly thereafter. TSH rose again despite no change in LT4 dose. It is notable that during her course we were able to document full TSH suppression only during the period of compliance with gluten free diet. Subsequently, despite compliance with LT4 dose escalation up to 5.3 mcg/kg/day, her TSH would not fully suppress while she remained noncompliant with a gluten free diet.

(B) A 42 year old woman with Hashimoto's thyroiditis remained clinically and biochemically hypothyroid over a year despite increasing doses of LT4 to 375 mcg for 130 kg, or 2.8 mcg LT4/kg/day. Free T4 was 0.6-0.8 ng/ml (normal 0.5-1.3) & TSH > 8 IU/ml (normal 0.34-5.6). The patient denied any gastrointestinal symptoms. A tissue transglutaminase IgA was 135 units (normal <20), endomysial antibodies IgA titer 1:40 (normal <1:10) & duodenal biopsy was c/w celiac disease.

Both cases demonstrate increased LT4 requirements due to impaired absorption in untreated celiac disease. The possibility of subclinical celiac disease should be considered in patients demonstrating unusually high LT4 requirements.

Nothing to Disclose: NS, CMF, SG, RK

P1-610

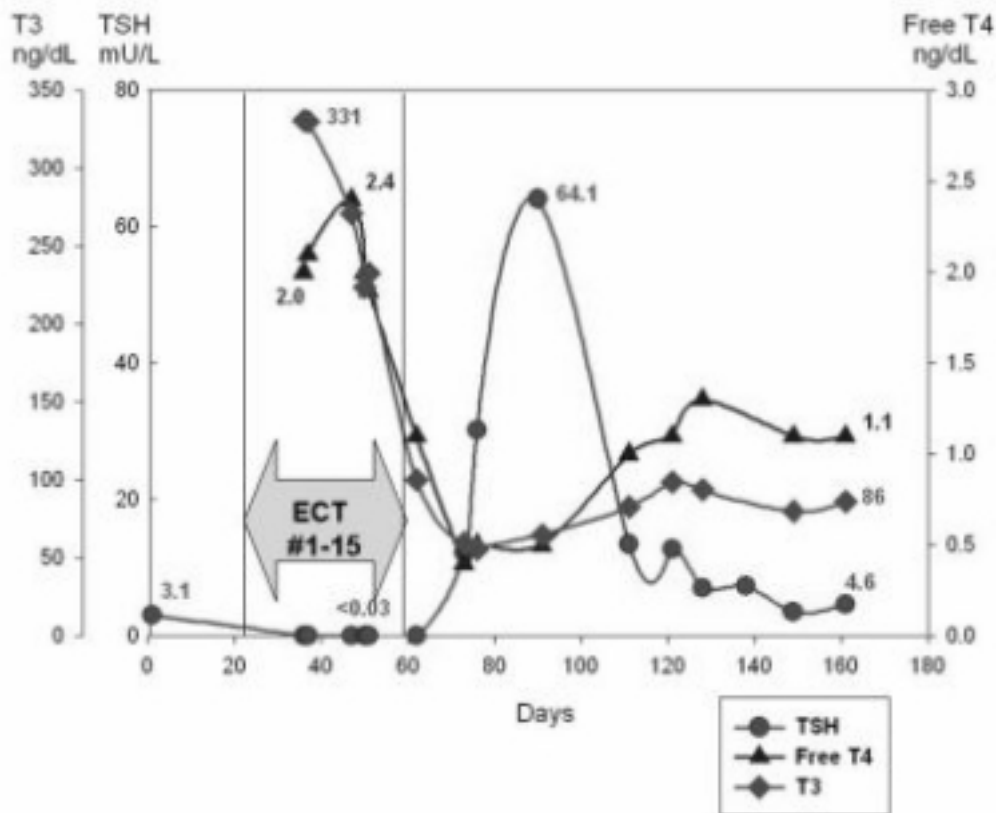
Electroconvulsive Therapy-Induced Thyroiditis?.

T Araki MD¹, A Ruminjo MD^{2,1} and A Gouller MD¹.

¹Beth Israel Med Ctr New York, NY.

Introduction: We report a unique case of painless thyroiditis most likely precipitated by electroconvulsive therapy (ECT).

Presentation: A 46 year old male was admitted to the inpatient psychiatric unit with major depressive episode. He was started on antidepressant and antipsychotic medications (Duloxetine, Trazodone, and Risperidone) with no clinical improvement. Twenty days after admission ECT therapy was initiated. It was standardized with 800mA for 8 seconds under general anesthesia 3 times per week. The patient had no prior history of thyroid disease and on presentation, his thyroid function tests were normal. After the sixth ECT session, he developed hyperthyroidism. No goiter or neck tenderness was appreciated on physical exam. ¹²³I thyroid uptake and scan revealed no uptake, consistent with thyroiditis. Antibodies to thyroglobulin were elevated, antibodies to thyroid peroxidase and to TSH receptor were within normal ranges. The patient received a total of fifteen ECT treatments with clinical improvement. Free thyroxine level peaked after the 10th session and normalized 5 days after completion of the ECT. Sixteen days post ECT, the patient became hypothyroid and was treated with Levothyroxine. He is currently euthyroid.



Discussion: The clinical course of hyperthyroidism followed by hypothyroidism and a lack of uptake on the thyroid scan is consistent with thyroiditis likely induced by ECT therapy. The pathophysiology is unknown. During ECT, the electric field is generated in proximity to the thyroid gland. Subsequently, a large electrical current can be transmitted through the gland and can cause destruction of the thyroid parenchyma.

The positivity of thyroglobulin antibody indicates that our patient likely had underlying chronic autoimmune thyroid disease and probably was more susceptible to the injury.

Conclusion: This is the first case describing ECT- induced thyroiditis. Case control studies are needed to clarify this association and to study the underlying pathophysiology.

Nothing to Disclose: TA, AR, AG

P1-611

Severe Hyponatremia Following Low Iodine Diet Prior to I-131 Therapy.

OM Al Nozha Alzoghaibi MD¹ and L Vautour MD¹.

¹McGill Univ Montreal, Canada.

In USA differentiated thyroid carcinoma accounts for 80-90% of the estimated 37,200 new cases of thyroid cancer for 2009(1) and most of the 4,700 annually estimated new Thyroid cancer patients in Canada(2). Postoperative treatment of differentiated thyroid carcinoma often consists of radioactive iodine (RAI), especially in high-risk patients(3;4). Thyroid tissue is able to concentrate iodine under the influence of thyroid stimulating hormone (TSH), which is achieved either by withholding thyroxin replacement for 4-6 weeks, or through the use of recombinant human TSH (rh-TSH)(5-7). Patients are typically placed on a low iodine diet (LID) for a minimum of two weeks to minimize dietary iodine interference, a practice important to optimize the uptake of RAI(8-11). However, it is not uncommon to see patients also restricting sodium chloride intake during the iodine restriction period in spite of available iodine free salt. Severe hyponatremia has been rarely observed and reported during LID(12;13). Most reported cases occurred in older patients who underwent thyroid hormone withdrawal (THW) and therefore had concurrent hypothyroidism(12;13). Severe hyponatremia (Na <125) has been shown in a prospective hospital-based study to have significantly higher mortality rates compared to controls(27% vs. 9%), especially in those who had an acute drop or a further decline during admission(14). This emphasizes the importance of preventing and managing such a problem; we report a patient who developed severe hyponatremia(107mmol/L) after 3 weeks of LID despite being euthyroid, having received rh-TSH. she had a full recovery.

| | |
|------------------------------|-------------|
| Plasma osm.(275-295 mosm/kg) | 232 |
| Uric Acid(147-353 umol/L) | 215 |
| Creatinine(48-85 umol/L) | 103 |
| Free T4(8.0-18.0 pmol/L) | 16.0 |
| R.Cortisol(120-535 nmol/L) | 366 |
| R.Glucose(3.9-11.0 mmol/L) | 11.5 |
| Urinary Na | 18 mmol/L |
| Urine osm. | 292 mmol/kg |

cases

| Publication | Age | Sex | Diagnosis | THW duration(days) | LID duration(days) | Na level(mmol/L) | Thiazide |
|---------------------|-----|-----|-----------------------------------|--------------------|--------------------|------------------|----------|
| Krishnamurthy et al | 70 | M | PTC | Y | 14 | 115 | N |
| Krishnamurthy et al | 81 | F | PTC, Breast Ca., Pulm nodule | * | NA | 116 | Y |
| Shakir et al | 87 | F | Follicular Thyroid ca. Pulm mets | 35 | 10/35 *2 | 118 | N |
| Shakir et al | 66 | F | PTC, Brain & Pulm mets | 42 | 42 | 114 | N |
| Shakir et al | 72 | F | PTC, diffuse Pulm mets | 42 | 7 | 121 | N |
| Shakir et al | 68 | M | PTC, Pulm mets | 28 | 10 | 115 | N |
| Shakir et al | 71 | F | Follicular Thyroid Ca. | 21 | 21 | 110 | N |
| our patient | 66 | F | Multifocal PTC, bilateral LN Mets | * | 28 | 107 | Y |

* used rh-TSH, *2 Prepared twice Y:yes, N:No

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Nothing to Disclose: OMANA, LV

P1-612

A Role for Steroidogenic Factor-1 (SF-1, NR5A1, Ad4BP) in Adrenal Angiogenesis.

B Ferraz-de-Souza MD¹, L Lin MD PhD¹ and JC Achermann MD¹.

¹UCL Inst of Child Hlth London, UK.

Background: Steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) is a nuclear receptor transcription factor that plays a central role in adrenal and gonadal development, steroidogenesis, lipid metabolism and tumorigenesis. To date, more than 40 SF-1 regulated genes have been identified in critical roles of human endocrine function. Angiogenesis is essential for the growth of the developing human fetal adrenal, and key angiogenic factors VEGF-A, FGF-2 and Angiopoietin 2 (ANGPT2) likely regulate this process in the definite zone of the fetal adrenal. Furthermore, somatic changes resulting in overexpression of SF-1 have been implicated in childhood adrenal cancer through mechanisms still poorly understood.

Aim: We aimed to identify a novel subset of SF-1 dependent target genes in the adrenal which themselves could represent novel regulators of adrenal development, steroid metabolism or tumorigenesis.

Methods: Chromatin immunoprecipitation microarrays (ChIP-on-chip) were used to detect SF-1 binding targets in NCI-H295R adrenocortical tumor cells using an anti-human SF-1 antibody and promoter arrays (Affymetrix Human Promoter 1.0R). ChIP-PCR and luciferase promoter constructs were used to validate results. Datasets were analyzed for network enrichment using GeneGo MetaCore systems biology software and integrated to publicly available adrenal tumor expression data.

Results: Analysis of microarray data with CisGenome (MA>3.5) identified 738 peaks corresponding to SF-1 binding sites, of which 365 were annotated to minimal promoter regions (-7.5kb to +2.5kb from transcription start). A peak was identified in the promoter region of ANGPT2 and confirmed by in silico and in vitro analysis with luciferase reporter assays. The identified subset of SF-1 targets was enriched for angiogenic factors, and integration with expression profiling of adrenal tumors revealed networks of newly identified targets clustering around ANGPT2 and IGFBP3.

Conclusions: Using ChIP-on-chip we have identified novel SF-1 targets in an adrenocortical cell line and detected enrichment for genes involved in angiogenesis. In particular, ANGPT2 emerged as an important potential SF-1 target and a highly expressed factor in different series of adrenal cancer expression profiling. Activation of ANGPT2 by SF-1 may represent a novel mechanism through which SF-1 orchestrates angiogenesis in the developing fetal adrenal and in cancer.

Nothing to Disclose: BF-d-S, LL, JCA

P1-613

Inhibition of EGF Receptor (EGFR) Signaling in Cushing's Disease: A Novel Pathway for Abrogating STAT3 Regulation of ACTH.

H Fukuoka M.D., Ph.D.¹, A Ben-Shlomo M.D.¹, A Mamelak M.D.¹, C Zhou Ph.D.¹, J Mizutani¹, S Ren M.D.¹, D Bruyette DVM² and S Melmed M.D.¹.

¹Cedars-Sinai Med Ctr Los Angeles, CA ; ²Cedars-Sinai Medcal Ctr Los Angeles, CA and ³VCA West Los Angeles Animal Hosp Los Angeles, CA.

Objective: As ACTH-secreting pituitary tumors express EGFR, we tested the role of EGFR in murine corticotroph tumor cell (AtT20) and canine Cushing's regulation as a potential target for therapy of Cushing's tumors.

Methods: We transfected AtT20 corticotroph cells with either empty or EGFR subcloned retroviral plasmid and generated AtT20 cells stably expressing the empty vector (control, EV-AtT20) or EGFR (EGFR-AtT20), and tested intracellular signal transduction, *pomc* gene expression, and ACTH secretion. We inoculated stable EGFR overexpressing hormone-secreting cells to Nu/J mice, and treated them with oral gefitinib, an EGFR tyrosine kinase inhibitor. We also treated primary cultured pituitary cells derived from resected canine ACTH secreting adenomas with gefitinib.

Results: *pomc* gene expression was enhanced 1.7 fold ($p < 0.01$) by EGF in EGFR-AtT20 but unchanged in EV-AtT20 which did not respond to EGF. ACTH levels increased 1.7 fold ($p < 0.01$) in EGFR-AtT20 transfectants, and EGF induction of phospho-STAT3 was dose dependently attenuated by gefitinib (0.01 – 10 mM). Furthermore, dominant negative STAT3 overexpression attenuated *pomc* promoter induction by EGF. Gefitinib (0.1 – 10 mM) dose dependently suppressed both ACTH levels (~ 50%, $p < 0.01$), and *pomc* promoter activity (~ 30%, $p < 0.01$), and also induced apoptosis with enhancing cleaved-caspase 3 levels. BrdU incorporation was suppressed by gefitinib (~ 60%, $p < 0.01$). Tumors in Nu/J mice inoculated with EGFR-AtT20 transfectants were larger than those injected with EV-AtT20 (76 ± 4 vs 45 ± 6 mg, $p < 0.05$). EGFR-AtT20 implanted mice treated with gefitinib (100 mg/kg) exhibited decreased tumor size (~ 40 %, $p < 0.01$) and decreased serum ACTH levels (640 ± 17 vs 378 ± 83 ng/ml, $p < 0.05$). Elevated plasma glucose levels in these hypercortisolemic mice were suppressed by gefitinib in mice with EGFR-AtT20 transfectants (392 ± 9 vs 351 ± 14 mg/dl, $p < 0.05$). Next, we treated primary canine corticotroph tumor cell cultures derived from resected canine Cushing's tumors with gefitinib. In 4 of 5 tumors, ACTH levels were suppressed by gefitinib (~50%, $p < 0.01$), and *pomc* gene expression levels were dose dependently suppressed by the drug (~70%, $p < 0.01$).

Conclusions: As corticotroph tumor EGFR signaling tumor induced ACTH expression via STAT3, and gefitinib blocked corticotroph growth & function *in vitro* and *in vivo*, inhibition of this receptor could be a promising targeted therapy for Cushing's disease.

Disclosures: SM: Principal Investigator, Novartis Pharmaceuticals; Consultant, Ipsen; Study Investigator, Ipsen.

Nothing to Disclose: HF, AB-S, AM, CZ, JM, SR, DB

P1-614

Variegated Zonation with Autonomous Expression of Aldosterone Synthase (CYP11B2) in Human Adrenal Cortex.

K Nishimoto^{1,2}, K Nakagawa PhD¹, M Oya PhD¹, H Shibata PhD¹, H Itoh PhD¹ and K Mukai PhD¹.

¹Sch of Med, Keio Univ Tokyo, Japan ; ²Sch of Med, Keio Univ Tokyo, Japan ; ³Sch of Med, Keio Univ Tokyo, Japan and ⁴ Federation of Natl Public Service Personnel Mutual Aid Associations, Tachikawa Hosp Tokyo, Japan.

INTRODUCTION AND OBJECTIVE: Adrenocortical production of aldosterone is believed to occur only in the zona glomerulosa (zG) of the morphologically concentric histology of the glands. However, localization of aldosterone-producing cells remains to be specified because immunohistochemistry (IHC) for aldosterone synthase (CYP11B2), which catalyzes the terminal steps of the synthesis, has not been established. The objective of this study is to describe functional zonation of human adrenal cortex by visualizing the aldosterone-producing cells immunohistochemically .

METHODS: Normal adrenal glands from patients with renal cell carcinoma and adrenal glands with an aldosterone-producing adenoma (APA) from patients with primary aldosteronism (PA) were immunohistochemically examined using anti-human CYP11B2 antibody. Simultaneously, IHC was performed using antibody specific to human CYP11B1 which produces cortisol.

RESULTS: Normal adrenal glands exhibited two types of distributions of the two enzymes; the conventional zonation and a novel variegated zonation. In the former, CYP11B2 was detected sporadically in zG, while CYP11B1 was detected entirely in the zona fasciculata (zF). In the latter, the adrenocortical tissues consisted of subcapsular cell clusters evidently expressing CYP11B2 and the remaining CYP11B1 expressing area. The novel cell clusters were termed aldosterone-producing cell clusters (APCCs), as we recently reported (1). APCCs were morphologically composed of zG-like cells and inner columnar zF-like cells. APAs contained varied population of cells expressing CYP11B2, consistent with previous observations. In zG of the non-tumor portions from patients with PA, CYP11B2 was hardly detected presumably due to suppressed renin-angiotensin system (RAS). Interestingly, the non-tumor portions frequently exhibited APCCs, which gave strong CYP11B2 signals similar to APCCs of normal glands, in spite of suppressed RAS. The results suggested that aldosterone synthesis is inducible in zG and autonomous in APCCs.

CONCLUSIONS: IHC for human CYP11B2 revealed novel cell clusters constitutively expressing CYP11B2 in the adrenal cortex. The results suggest that the adrenal cortex in humans exhibits the variegated zonation with cell clusters where aldosterone production is autonomous, in addition to the conventional zonation where the production is inducible in zG by RAS.

(1) Nishimoto K et al., J Clin Endocrinol Metab in press

Nothing to Disclose: KN, KN, MO, HS, HI, KM

P1-615

Antenatal Betamethasone (Beta) Exposure Enhances Leptin Induced Inhibition of Steroidogenic Acute Regulatory Protein (StAR) and ACTH-Receptor Expression in Adult Ovine Adrenocortical Cells.

Yixin Su M.D¹, Jorge P Figueroa M.D;PhD¹ and James C Rose Ph.D¹.

¹Wake Forest Univ Hlth Sci Winston-Salem, NC.

Objective: Prenatal exposure to elevated levels of glucocorticoids correlates with chronic cardiovascular and metabolic diseases and altered pituitary-adrenal function in adult life. We have shown that prenatal Beta upregulates leptin and leptin receptor mRNA levels in adrenal, and enhances leptin induced inhibition of basal and ACTH stimulated cortisol secretion. The aim of this study was to investigate the effect of leptin on ACTH-Receptor and StAR in adult adrenocortical cells from antenatal Beta exposed sheep.

Materials and Methods: Pregnant sheep were randomly treated with Beta [2 maternal IM doses (0.17 mg/kg with a maximum of 12 mg) or vehicle 24-h apart] at 80- 81 d of gestation, and allowed to deliver at term. Offspring were euthanized at 1-1.5 yr of age. Adrenal cortex was obtained and adrenocortical cells were cultured (Day1). On Day 3, cells (200,000/well) were preincubated with human leptin (10ng/ml, 100ng/ml) or media for 24 h then stimulated with ACTH (0.15nM). Cells from basal or ACTH-stimulation were then harvested for RNA extraction and relative mRNA expression of StAR and ACTH-R were measured by real time PCR. Data were analyzed by analysis of variance.

Results: 1. Leptin (100ng/ml) reduced basal StAR mRNA expression in adrenal cells 40% from vehicle and 64% from Beta exposed animals, $p < 0.05$; 2. The rise in StAR mRNA expression following ACTH stimulation in vitro was blunted by 39% in cells from vehicle and 56% in cells from in Beta animals by leptin, $p < 0.05$. 3. The rise in ACTH-R mRNA expression following ACTH stimulation was inhibited by 43% in cells from vehicle and 52% in cells from Beta animals by leptin. Basal ACTH-R mRNA expression was unaffected by leptin. 4. Leptin (10ng/ml) showed similar inhibitory effects on StAR and ACTH-R mRNA expression.

Conclusion: Our results suggested that leptin acts at the adrenal to inhibit basal and ACTH stimulated cortisol secretion possibly through down-regulating StAR expression and attenuating the ACTH induced upregulation of ACTH R and StAR. The more pronounced suppression of ACTH-R and StAR expression in cells from Beta may result from an increase in adrenal leptin receptor expression caused by antenatal Beta.

Sources of Research Support: NIH Grants HD47584 and HD11210.

Nothing to Disclose: YS, JPF, JCR

P1-616

Cooperation between Cyclin E Up-Regulation and p27^{Kip1} Loss in Corticotropinoma Development.

A Roussel-Gervais¹, S Bilodeau¹, S Vallette¹, F Berthelet², A Lacroix², D Figarella-Branger³, T Brue³ and J Drouin¹.

¹Inst de Recherches Clin de Montréal Montréal, Canada ; ²Ctr de Recherche du Ctr Hosp de l'Univ de Montréal Montréal, Canada and ³Univ de la Méditerranée Marseille, France.

Glucocorticoid-resistant corticotroph adenomas cause Cushing's disease through excessive and unrestrained production of ACTH. Investigation of transcriptional mechanisms required for trans-repression of the pro-opiomelanocortin (POMC) gene led us to identify new proteins that are essential for negative feedback regulation of POMC by glucocorticoids (Gc). Brg1, the ATPase sub-unit of the chromatin remodeling complex Swi/Snf, was found to be required to stabilize the trans-repression complex at the POMC promoter. The glucocorticoid-resistance of corticotroph adenomas may thus result from loss of Brg1. And indeed, we observed the loss of nuclear Brg1 in 33% of corticotroph adenomas.

Brg1 is also a tumor suppressor but its role in tumorigenesis remains elusive. We recently showed that loss of Brg1 in human pituitary corticotroph adenomas is correlated with up-regulation of cyclin E. Further, in the mouse corticotroph AtT-20 cells, Brg1 knockdown leads to up-regulation of cyclin E expression. In order to investigate the putative contribution of cyclin E to pituitary adenomas formation, we generated transgenic mice expressing cyclin E under control of the POMC promoter (Tg-PCE). In Tg-PCE mice, expression of cyclin E increased proliferation of differentiated corticotroph cells and resulted in delayed appearance of pituitary adenomas.

We also found a correlation between up-regulation of cyclin E and loss of p27^{Kip1} expression in human pituitary corticotroph adenomas, and investigated a putative cooperation between these mis-expressions. Over-expression of cyclin E associated with p27^{Kip1} loss in mice increased the incidence of pituitary adenomas formation, their size as well as their proliferation index. Cyclin E over-expression thus acts cooperatively with loss of p27^{Kip1} for pituitary tumor development.

Cyclin E over-expression may promote adenoma development by cell cycle re-entry of differentiated corticotrophs. We also observed increased centrosome instability in Tg-PCE mice. Further, centrosome instability was recently shown to promote genome instability. Up-regulation of cyclin E expression may thus predispose to pituitary adenoma formation through genome instability and aberrant gene expression.

Nothing to Disclose: AR-G, SB, SV, FB, AL, DF-B, TB, JD

P1-617

17 β -Hydroxysteroid Dehydrogenase 3 (17 β -HSD3), a Regulator of 11 β -Hydroxysteroid Dehydrogenase 1 (11 β -HSD1) Activity in Rat Leydig Cell.

S. A. Latif Ph. D.¹, R. Ge Ph.D.², C. M. Sottas², M. Shen¹ and D. J. Morris D. Phil¹.

¹Brown Univ Providence, RI and ²The Population Council/Rockefeller Univ New York, NY.

We have confirmed that Leydig cells display mainly (11 β -HSD1) oxidase in addition to reductase activity. We hypothesize (a) that 11 β -HSD1 oxidase directionality in Leydig cells serves as a NADPH-regenerating system that is tightly coupled in regulating 17 β -HSD3 which synthesizes testosterone (**Testo**) from androstenedione (**AD**) and (b) since co-factor NADP⁺ present in finite amounts is rate-limiting for 11 β -HSD1-oxidase activity, increased NADP⁺ supply by 17 β -HSD3 would up-regulate 11 β -HSD1 oxidase activity. Thus re-generation of NADP⁺ would stimulate 11 β HSD1 oxidase in a positive cycle.

To test 17 β -HSD3 for its effectiveness as a possible NADP⁺-regenerating system, Leydig cells were exposed to [³H]-corticosterone in the presence of increasing concentrations of corticosterone (**B**) plus or minus 30 mM **AD**. In the absence of **AD**, and with increasing concentrations (0.3 μ M to 6.0 μ M) of **B**, increasing amounts (3 - 44 picomoles) of 11-dehydro-**B** were produced. However, when 30 μ M **AD** was included in this incubation, the rate of 11-dehydro-**B** formation dramatically increased 1.3 to 5-fold, producing 4-210 picomoles, indicating the increase in the rate of 11 β -HSD1 reaction was most likely due to the increased supply of the co-factor NADP⁺ generated via 17 β -HSD3 reaction as evidenced by a concomitant large increase in **Testo** synthesis. In the presence of 30 μ M **AD**, and increasing concentrations of **B** (0.3 μ M to 6.0 μ M), 17 β -HSD3 reaction was enhanced dose dependently, 16-72-fold, producing 116-255 picomoles **Testo**. In contrast, in the absence of **AD**, synthesis of **Testo** was constant at approx. 4 picomoles and independent of **B** concentrations

These findings are consistent with the hypothesis that the two enzymes are coupled; NADPH produced by 11 β -HSD1 is utilized by 17 β -HSD3 for the synthesis of **Testo** re-generating NADP⁺ for the 11 β -HSD1 oxidase reaction. The effect of **AD** on 11 β -HSD1 is mainly due to an increase in Vmax (app) (from 35.6 to 91.4 picomoles of 11-dehydro-**B** synthesized/25000 cells/30 min) with no apparent change in the Km for the substrate (0.476 μ M without **AD** and 0.500 μ M in presence of 30 μ M **AD**). These findings also confirm the regulatory role played by 17 β -HSD3 in optimizing the levels of NADP⁺ necessary to maintain 11 β -HSD1 activity in oxidase direction.

Nothing to Disclose: SAL, RG, CMS, MS, DJM

P1-618

Krüppel-like Factor 9 Is a Direct Corticosteroid Receptor Target and Candidate Plasticity Gene in Mouse Hippocampal Neurons.

P Bagamasbad MS¹, T Ziera PhD², S Borden PhD² and RJ Denver PhD¹.

¹Univ of Michigan Ann Arbor, MI and ²Bayer Schering Pharma AG Berlin, Germany.

Glucocorticoids (GC) can have either facilitatory or inhibitory effects on hippocampal function that are highly dependent on the type and duration of the stressor. Chronic stress leads to atrophy of dendrites of hippocampal neurons and impairs performance in cognitive tasks, while acute stress can increase dendritic spine density and enhance memory consolidation and reconsolidation. The molecular mechanisms by which GCs influence hippocampal structure and function are poorly understood. Earlier we identified Krüppel-like factor 9 (KLF9/basic transcription element binding protein; BTEB1) as a direct thyroid hormone target gene that is highly expressed in the mouse hippocampus and functions as an intermediate in thyroid hormone action in neurite extension and branching. We also showed that KLF9 is induced by stress in *Xenopus*, and that this action is mediated by the glucocorticoid receptor (GR). Here we demonstrate similar GC regulation of KLF9 in mouse, and identify structural elements of the *Klf9* gene that mediate GR-dependent transactivation. Treatment of the mouse hippocampus-derived cell line HT-22 with corticosterone (CORT) caused a time and dose-dependent increase in KLF9 mRNA. In HT-22 cells that express both the GR and the mineralocorticoid receptor (MR), CORT-dependent expression of KLF9 mRNA was blocked by co-treatment with the GR receptor blocker RU486, suggesting that the gene is regulated by the GR in hippocampal neurons. Injection of CORT in postnatal day 7 and 30 mouse pups induced expression of KLF9 mRNA in hippocampus. Induction of KLF9 expression by CORT in HT22 cells was resistant to protein synthesis inhibition, suggesting that the *Klf9* gene is a direct GR target. Chromatin immunoprecipitation (ChIP) assay combined with hybridization to mouse promoter arrays identified *Klf9* as a corticosteroid receptor target gene. We identified a functional GC responsive element ~5.7 kb upstream of the transcription start site and verified its functionality by promoter-luciferase assays, mutational analysis and gel shift assays. Our results show that *Klf9* is a direct GR target gene that can integrate thyroid hormone and GC signals in hippocampal cells, thereby influencing neural development and plasticity.

Sources of Research Support: NSF grants IBN 0235401 and IOS 0922583, and NIH grant 1 R01 NS046690 to R.J.D.

Nothing to Disclose: PB, TZ, SB, RJD

P1-619

Differential Inhibitory Effects of Glucocorticoids on CRH and POMC Transcription.

Y Liu MD¹, A Kiss PhD², R MacGregor Mr¹ and G Aguilera MD¹.

¹Natl Inst of Child Hlth and Human Development Bethesda, MD and ²Slovak Academy of Sci Bratislava, Slovakia (Slovak Republic).

Glucocorticoid feedback inhibition of is critical for preventing pathological consequences due to excessive activation of the hypothalamic adrenal (HPA) axis. To determine the relative importance of pituitary and hypothalamus in the feedback mechanism, the effect of corticosterone administration on hypothalamic corticotrophin releasing hormone (CRH) and pituitary POMC heteronuclear RNA (hnRNA), was examined in intact rats subjected to the acute stressor of i.p. hypertonic saline injection (ipHS), or in primary cultures of hypothalamic neurons. IpHS caused rapid and transient increases in plasma ACTH (35 ± 6 to 340 ± 38 pg/ml) and corticosterone (25 ± 8 to 467 ± 54 ng/ml) at 30 min returning near to basal values by 2h. This was accompanied by marked increases in CRH hnRNA (45-fold) and POMC hnRNA (37-fold) by 15 min. Injection of corticosterone (2.5mg, s.c.) 30 min before stress increased corticosterone to 103 ± 24 ng/ml and decreased ACTH area under the curve (AUC) by 75%. Both CRH and POMC hnRNA AUCs decreased by about 60%. More prolonged elevations in plasma corticosterone (5 mg in oil twice at -16 and -3h before stress) inhibited CRH and POMC hnRNA by about 90%. On the other hand, sc corticosterone injected 5 min after stress reduced CRH hnRNA by only 20% and POMC hnRNA by 40%. The site of action of glucocorticoids was examined in primary cultures of hypothalamic neurons and anterior pituitary cells.

In hypothalamic cell cultures incubation with corticosterone (100nM) for 30min inhibited forskolin-stimulated CRH hnRNA by 50%, while preincubation for 16h had no significant effect. In contrast, corticosterone inhibited CRH-stimulated POMC hnRNA by 55% and 80% after 30min and 16h corticosterone, respectively. The data show direct inhibitory actions of glucocorticoids at both hypothalamic and pituitary levels. However, the marked inhibitory effect of prolonged glucocorticoid exposure on CRH transcription observed in vivo is likely to result from inhibition of afferent pathways to the PVN.

Sources of Research Support: IRP NICHD.

Nothing to Disclose: YL, AK, RM, GA

P1-620

Familial Alzheimer's Disease Mutations in APP and Presenilin1 Increase Anxiety-Related Behavior and Activate Neuroendocrine Stress Pathways.

NJ Justice Ph.D.¹, Q Guo¹ and H Zheng Ph.D.¹.

¹Baylor Coll of Med Houston, TX.

Stress symptoms are common to patients with Alzheimer's disease (AD) and increased cortisol levels predict more rapid progression of cognitive impairment in patients in the preliminary stages of AD, however, the changes occurring in AD that impact the function of stress responsive neuronal and endocrine pathways remain poorly understood. We have taken advantage of a new model of AD where Familial Alzheimer's Disease (FAD) mutations in Amyloid Precursor Protein (APP) and Presenilin (PS1) have been inserted at their endogenous loci (APP/hA β /PS1 mice), to study the relationship between stress and AD. Similar to the stress symptoms present in human cases of AD, APP/hA β /PS1 mice display increased anxiety-related behavior on the elevated plus maze, and increased freezing in the contextual fear test, suggesting that these mice have hyperactive stress responses even at relatively young ages before signs of AD pathologies are present. When we measured circulating corticosterone at rest and at peak levels of stress, we found that APP/hA β /PS1 mice display elevated corticosterone levels compared to wildtype animals, indicating hyperactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Consistent with elevated HPA axis responses, we find that Corticotropin Releasing Factor (CRF) levels are increased centrally in APP/hA β /PS1 mice. Elevated CRF and increased stress have been shown to cause decreased cognitive ability. We tested 4-6 month old APP/hA β /PS1 mice for cognitive performance on the Morris Water Maze and found that these mice display a deficit in memory acquisition. We are currently testing whether antagonizing elevated CRF signaling in APP/hA β /PS1 mice via genetic and pharmacologic strategies might alleviate this cognitive deficit. Together, these findings suggest that mutations in APP and PS1, which result in AD, perturb central and endocrine stress responses, and that over-activity in these systems may exacerbate the development and progression of the disease. Ongoing studies are being performed to determine the mechanism by which mutations in APP and PS1 activate stress responsive systems. Given stress and anxiety symptoms described in human AD patients and stress related phenotypes displayed by this AD model animal, we suggest that stress related symptoms that occur with AD might represent an early manifestation of AD that, if treated, may ameliorate cognitive decline in AD patients.

Nothing to Disclose: NJJ, QG, HZ

P1-621

Interactions between Melatonin and ACTH in the Human Adrenal Gland: Adrenal Clockwork Inhibition?.

C Campino BS¹, FJ Valenzuela BS², C Torres-Farfan PhD², L Abarzua-Catalan BS², HE Reynolds BS², E Arteaga MD¹, C Trucco MD¹, S Guzman MD¹, GJ Valenzuela MD,MBA³ and M Seron-Ferre PhD^{2,4}.

¹Pontificia Univ Católica Santiago, Chile ; ²Univ de Chile Santiago, Chile ; ³Arrowhead Regional Med Ctr Colton, CA and ⁴Univ de Tarapaca Arica, Chile.

Background. Recent data suggest the involvement of melatonin and adrenal clock genes in the glucocorticoid response to ACTH in several species. We postulate that in humans, melatonin inhibits ACTH stimulated StAR and 3 β -HSD expression and cortisol production by interacting with adrenal clock genes. **Aim.** To investigate: i.- Direct effects of melatonin on ACTH-induced StAR and 3 β -HSD expression and cortisol production; ii.-Expression of clock genes *ex vivo* and iii.- Direct effects of melatonin on BMAL1 protein and *PER1* mRNA expression in adrenal explants under ACTH stimulation. **Material and methods.** Adrenal tissue was obtained from six renal cancer patients undergoing unilateral nephrectomy-adrenalectomy. Five adrenals were diagnosed as normal and one was an adenoma. Expression of the clock genes *BMAL1*, *PER1*, *PER2*, *CRY2* and *CLOCK* was investigated by RT-PCR. Explants from four normal adrenals were pre-incubated in culture medium (6 h) followed by 12 hours in: medium alone (no-ACTH); ACTH (100 nM); ACTH plus melatonin (100 nM) and ACTH plus melatonin plus luzindole (MT1/MT2 melatonin antagonist, 1 μ M). Cortisol production (RIA), and the explants' content of StAR, 3 β -HSD, BMAL1 (immune slot-blot) and *PER1* mRNA (Real-time RT-PCR) were measured at the end of the incubation. **Results.** Adrenal gland expresses the clock genes *PER1*, *PER2*, *CRY2*, *CLOCK* and *BMAL1*. ACTH increased *PER1* mRNA and BMAL1, StAR and 3 β -HSD protein levels and cortisol production. These effects were inhibited by co-incubation with melatonin and reversed by luzindole. **Conclusion.** In the human adrenal gland, melatonin inhibition of the cortisol response to ACTH is possibly mediated through the adrenal clockwork.

Sources of Research Support: Grants from Department of Women's Health, Arrowhead Regional Medical Center, Colton, CA; Fondo Nacional de Ciencia y Tecnología (Fondecyt 1050833 and 1090381) and Departamento de Endocrinología, Facultad de Medicina, Pontificia Universidad Católica de Chile. F.J.V and L.A-C were supported by PhD fellowships from Comisión Nacional de Ciencia y Tecnología (Conicyt).

Nothing to Disclose: CC, FJV, CT-F, LA-C, HER, EA, CT, SG, GJV, MS-F

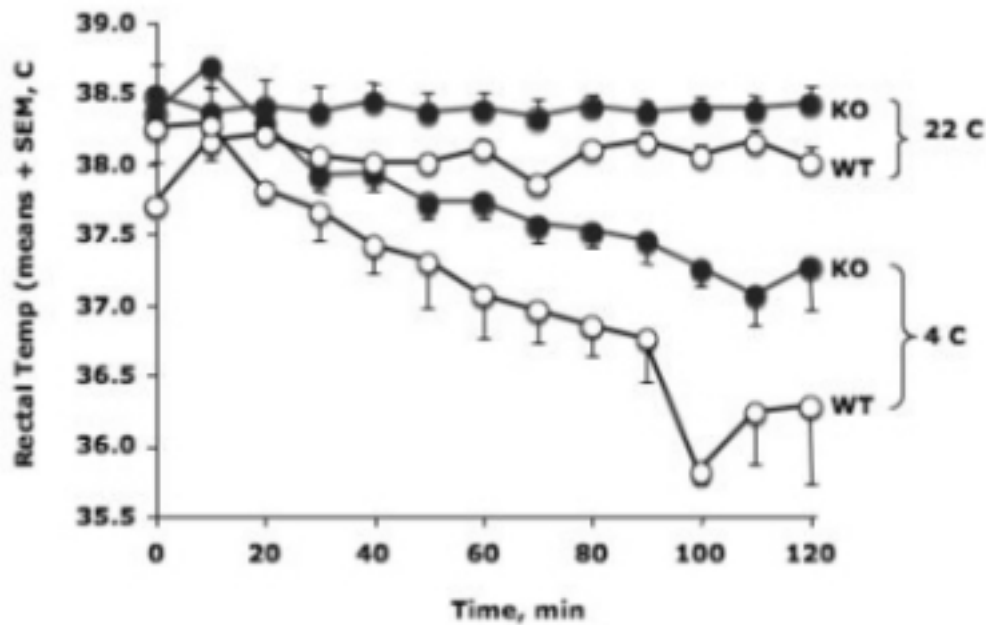
P1-622

Urocortin-1 Is Involved in Regulating Core Body Temperature.

Bayan Chaker BS¹, Tareq Samra¹, Basil Taha MD¹ and Abdul Abou-Samra MD/PhD¹.

¹Wayne State Univ Sch of Med Detroit, MI.

We have recently reported that Urocortin 1 knock out (UCN1-KO) mice have normal corticosterone response to 15 min restraint but a markedly decreased corticosterone response to 2 h cold stress (Am J Phys, Endo Metab 2007, 293, E259). Since UCN1-KO mice failed to increase corticosterone in response to a cold environment we raised the hypothesis that UCN1-KO mice tolerate low temperature better than wild type (WT) mice. To study the role of UCN1 in thermoregulation, adult (8-26 weeks of age) C57BL/6 wild type (WT) and UCN1-KO mice were exposed to 4°C or to ambient room temperature (22 C) for two hours between 9 AM and 5 PM. Internal body temperature was monitored by measuring rectal temperature at 10 minute intervals using an electronic rectal probe. The experiment was repeated 2 to 3 times for each mouse on different days and the data were averaged for each mouse. Both UCN1-KO (n=8) and WT (n=3) mice had a fairly stable internal body temperature at 22 C; however, the core body temperature of the UCN1-KO mice was on average 0.4 C higher than that of the WT mice (p<0.001). Moreover, the inner body temperature of the UCN1-KO mice was more stable during the 2 h exposure to the cold environment (4°C) than that of the WT mice. After the 2 h period at 4 C, the WT mice had a lower internal temperature than the UCN1-KO mice (37.3 ± 0.9 versus 36.3 ± 1.0 C, p<0.001); indicating that UCN1-KO mice are resistant to cold temperatures. Since corticosterone levels are normal in the UCN1-KO mice it is likely that the effects of UCN1 on core body temperature do not involve corticosterone. The data suggest a novel physiological role for UCN1 in the regulation of core body temperature.



Nothing to Disclose: BC, TS, BT, AA-S

P1-623

Effects of Hyperlipidic Diet and Adrenalectomy on Adiponectin and Adiponectin Receptors Gene Expression in Skeletal Muscle and Liver.

Cristiane de Oliveira¹, Ana Barbosa de Mattos¹, Carolina Biz¹, Lila Missae Oyama PhD² and Cláudia Oller do Nascimento PhD¹.

¹UNIFESP - Campus São Paulo São Paulo, Brazil and ²UNIFESP - Campus Baixada Santista Santos, Brazil.

Introduction: Adiponectin is an insulin-sensitive hormone (1, 2), playing a central role in glucose and lipid metabolism (3). Its increases fatty acid oxidation and glucose transport (4, 5) in the muscle and potentiates insulin inhibition of hepatic gluconeogenesis (6). Circulating adiponectin acts in muscle and liver via two specific receptors, AdipoR1 and AdipoR2. AdipoR1 is the major receptor expressed in skeletal muscle, whereas AdipoR2 is mainly expressed in liver (7, 8, 9). Consumption of high levels of dietary fat is thought to be a major factor in promoting obesity and insulin resistance (10, 11, 12, 13). Excess of cortisol is characterized by abdominal obesity, hypertension, glucose intolerance or diabetes and dyslipidemia, all these features share a state of insulin resistance. Adrenalectomy can reverse various metabolic defects, including hyperglycemia and hyperinsulinemia in many models of obesity (14, 15) and increase insulin sensitivity in obese mice (16).

Aim: Evaluate whether adiponectin and its receptors are regulated by factors that predispose to obesity and insulin resistance, as high-fat feeding and glucocorticoids.

Methods: 3 month-old male Wistar rats were fed with a hyperlipidic diet (15% of soybean oil) (H-group) or chow diet (C-group) for 21 days. After this period, the H and C were divided in 3 groups: sham-operated (SH and SC), adrenalectomized (AH and AC), and adrenalectomized treated with dexamethasone, 2 mg/1Kg twice daily by s.c. injection (ADH and ADC). 72 hours after surgery, the animals were sacrificed and muscle and liver were collected and total RNA was extracted. Adiponectin and adiponectin receptors gene expression were quantified using real time PCR. Serum corticosterone, glucose, insulin and adiponectin were analysed.

Results: The adiponectin receptors genes expressions, in liver, were similar among groups. However, the AdipoR1 and AdipoR2 gene expression was lower in SH than SC, the same result was observed when AC and ADC were compared to SC.

Conclusion: These results demonstrated that hyperlipidic diet promoted a decrease in adiponectin receptor gene expression in skeletal muscle which could contribute to the development of insulin resistance. Also, it demonstrated that the adrenalectomy effects on adiponectin receptors are not dependent of the decrease in corticosterone levels. These suggest that adrenal medullar catecholamines could be important for the adiponectin receptor gene expression in skeletal muscle.

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Sources of Research Support: Research relating to this abstract was funded by Fundação de Amparo à Pesquisa do

Estado de São Paulo (FAPESP); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES).

Nothing to Disclose: CdO, ABdM, CB, LMO, COdN

P1-624

Chronic Subordinate Colony (CSC) Housing Induces Adrenal Insufficiency in Mice - The Mechanisms behind.

NM Uschold PhD student¹, ID Neumann Prof. Dr.¹ and SO Reber Dr.¹.

¹Univ of Regensburg Regensburg, Germany.

The CSC paradigm has been established as clinically relevant model of chronic psychosocial stress for male mice and as such shown to cause behavioural, immunological as well as neuroendocrine changes. One of the most striking findings was that 19 days of CSC exposure affect proper hypothalamo-pituitary-adrenal (HPA) axis functionality, mainly at the level of the adrenal glands. This was indicated by the finding that although adrenal mass was increased, adrenal corticosterone response upon *in vivo* and *in vitro* ACTH stimulation was strongly dampened following CSC. Here, we investigated the mechanisms underlying the CSC-induced adrenal insufficiency, which was found to be body side specific.

As shown before, CSC resulted in a significant reduction in body weight gain as well as in an increase in absolute and relative adrenal mass, when compared with single housed control (SHC) mice. However, when distinguishing between left and right body side, adrenal mass was found to be significantly increased on the right side only. In the left adrenal there was only a slight trend towards an increased mass. Oil red/ nissel staining followed by computerized analysis revealed that increased mass of the right adrenal following CSC exposure was due to an enlargement of cortical areas, mainly of the glucocorticoid producing zona fasciculata. In contrast, the trend towards an increased mass in the left adrenal was due to an enlargement of the medulla. Furthermore, oil red staining followed by computerized analysis showed that the amount of cholesterol containing, cortical lipid vesicles per area in the left as well as in the right adrenal was not affected by CSC exposure. In contrast, *in situ* hybridization indicated that the ACTH receptor mRNA expression per cortical area/ cell in CSC mice was strongly reduced in the left as well as in the right adrenal. However, when performing an *in vitro* stimulation of isolated adrenal cells with different doses of ACTH we found decreased corticosterone levels compared with SHC mice only in the supernatants of cells isolated from the left adrenal gland. In summary, our data suggest that 19 days of CSC result in a decreased cellular sensitivity to ACTH in cells of both the left and right adrenal and that this gets probably compensated by an increase in the number of cortical zona fasciculata cells in the right adrenal.

Sources of Research Support: German Research Foundation.

Nothing to Disclose: NMU, IDN, SOR

P1-625

Knockdown of Syndecan-3 Expression Attenuates Adrenocortical Cell Proliferation Induced by Fibroblast Growth Factor-2: Implication for Human Fetal Adrenal Development.

K Minegishi MD, PhD¹, H Ishimoto MD, PhD², SH Kim MD¹, M Umezu MD¹, I Kadohira MD, PhD¹, K Miyakoshi MD, PhD¹, M Tanaka MD, PhD¹ and Y Yoshimura MD, PhD¹.

¹Keio Univ Tokyo, Japan and ²Tokai Univ Isehara, Japan.

Syndecan-3 (SDC3), a transmembrane heparan sulfate proteoglycan, acts as a co-receptor for fibroblast growth factor-2 (FGF2) in various cells, including those of the nervous system. Although the human adult adrenal gland is among tissues with the highest expression of SDC3, its functions in adrenocortical cells have been unknown. We have recently examined SDC3 expression in the human fetal adrenal (HFA) gland. The HFA primarily consists of two zones, the outer DZ and inner FZ. DZ cells have structural characteristics typical of cells in an undifferentiated proliferative state, whereas FZ cells have those typical of differentiated, steroidogenic cells. We have demonstrated that SDC3 protein is exclusively expressed in the DZ where FGF2 predominantly localizes. Of interest, DZ cells are known to be more responsive to the proliferative actions of FGF2 than FZ cells. In this study, to elucidate SDC3 functions, we transiently silenced endogenous expression of SDC3 by specific small interfering RNA (siRNA) in human adrenocortical NCI-H295A cells. MTS proliferation assays demonstrated that SDC3 siRNA pretreatment significantly attenuated NCI-H295A cell proliferation induced by FGF2 (10 ng/ml for 72 hrs). Quantitative TaqMan real-time RT-PCR revealed that SDC3 silencing simultaneously increased transcripts encoding the steroidogenic enzyme P450c17, the LDL receptor, and SPARC (osteonectin), the three of which are established markers for FZ cells. Additionally, the SDC3 knockdown increased cAMP-stimulated dehydroepiandrosterone sulfate (DHEAS) secretion by NCI-H295A cells (by 1.5-fold, with 8-Br-cAMP [1 mM] incubation for 48 hrs). The results indicate that SDC3 appears to be involved in FGF2-induced proliferation of human adrenocortical NCI-H295A cells, possibly by acting as a co-receptor for FGF2. Based on the results in NCI-H295A cells that share several phenotypical characteristics with HFA cortical cells, SDC3 might, at least in part, contribute to maintaining the DZ phenotype, because the SDC3 knockdown suppressed a DZ cell phenotype (i.e., FGF2 inducible cell proliferation) while displayed more of the characteristics of FZ cells (i.e., increased expression of the FZ cell markers, and DHEAS secretion). This possibility awaits further investigation.

Nothing to Disclose: KM, HI, SHK, MU, IK, KM, MT, YY

P1-626

The Pattern Dependent Effects of Glucocorticoid Exposure on Prefrontal Cortex Transcriptional Output.

CL George¹, MA McKenna¹, JR Pooley¹, JA Douthwaite¹, SL Lightman¹ and BL Conway-Campbell¹.

¹Univ of Bristol Bristol, UK.

The adverse effects of abnormal endogenous glucocorticoid (GC) secretion (resulting from stress or disease) or treatment with synthetic GC analogs are plentiful and well documented, however the function and molecular dynamics of the evolutionary conserved ultradian GC secretion pattern in the brain remains unclear. We propose that the pulsatile secretion of adrenal GCs (which occurs at approximately hourly intervals in rats during the circadian peak [1]) creates a digital signal that crosses the blood-brain-barrier [2,3] and directs tissue-specific responses to GCs depending on the transcriptional kinetics that decode the GC signal.

Previously we demonstrated that pulsatile GC administration induces cyclical GC receptor (GR) activation, DNA binding and transcriptional activation of the Period 1 (*Per1*) gene in the liver of intact rats [4]. Here we report the effects of pulsatile GC exposure on the transcriptional output of the brain, specifically the prefrontal cortex (PFC), an area that is functionally sensitive to GC exposure levels [5].

Using a model of exogenous corticosterone administration that temporally mimics pulsatile GC secretion in adrenalectomised male Sprague Dawley rats [3] we demonstrate that the transcriptional outputs evoked by pulses of GCs in the PFC are gene-dependant and tissue-specific. Notably the detection of GC pulses by the PFC is surprisingly rapid with significant GR activation occurring within 5 minutes of the administration of the exogenous corticosterone pulse (10.95 ± 4.37 fold induction of GRE oligonucleotide binding). However, interestingly a single pulse of corticosterone fails to evoke a transcriptional induction of *Per1* mRNA despite being sufficient to induce a 7.48 ± 2.01 fold induction of *Per1* mRNA in the liver of the same animals. This study thus provides evidence that pulsatile glucocorticoid signalling results in tissue-specific responses determined by a mechanism unrelated to GR activation kinetics.

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Sources of Research Support: Wellcome Trust Programme Grant (074112/Z/04/Z).

Nothing to Disclose: CLG, MAM, JRP, JAD, SLL, BLC-C

P1-627

Placental Adaptation to Maternal Low Protein Diet - Role of 11 β -Hydroxysteroid Dehydrogenase Type 2 (11 β -HSD2).

EC Cottrell PhD¹, L Kappeler PhD² and JR Seckl MBBS, PhD¹.

¹Univ of Edinburgh Edinburgh, UK and ²Hosp St-Antoine Paris, France.

11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), which rapidly converts glucocorticoids to inactive metabolites, is highly expressed in the placenta where it protects the developing fetus from excess maternal glucocorticoids [1]. Genetic deficiency or downregulation of placental 11 β -HSD2 (by maternal stress or undernutrition) associates with fetal growth restriction, low birth weight and the subsequent development of hypertension and metabolic disease in adulthood. We show here in the mouse that feeding an isocaloric low protein diet throughout gestation (8% protein vs 18% control diet) significantly decreased placental weight (by approximately 10%; $p < 0.001$) and that by embryonic day (E) 17.5, fetal weight was significantly reduced by 17% compared with control-fed fetuses ($p < 0.001$). The reduction in fetal weight at E17.5 was preceded by a downregulation of 11 β -HSD2 enzyme activity from E16.5 (24% and 36% decrease in activity in low protein placentas at E16.5 and 17.5 respectively; $p < 0.05$ overall). Unexpectedly, at earlier gestational ages (E13.5-15.5), maternal low protein diet increased placental 11 β -HSD2 activity (by 21%, 47% and 65% at E13.5, 14.5 and 15.5, respectively; $p < 0.05$ overall) and increased placental 11 β -HSD2 mRNA at E13.5 (by 44%; $p < 0.05$). Thereafter, there was a dramatic decrease in 11 β -HSD2 mRNA across E14.5-17.5, but this was unaffected by maternal diet. Epigenetic processes have been implicated in developmental programming in the fetus/offspring. In the placenta, the 11 β -HSD2 gene promoter was largely unmethylated ($< 10\%$) throughout gestation and this was unaffected by maternal diet. However, there was a marked 6-fold reduction in histone H3 acetylation (a marker of gene activation) at the 11 β -HSD2 promoter at E17.5 compared with E13.5 ($p < 0.01$), consistent with the gene expression profile. There was no effect of maternal diet on this modification. These data are compatible with the notion that a nutritionally-challenging maternal environment initially boosts the placental 'barrier' to maternal glucocorticoids, presumably to protect the fetus from their growth-restricting effects and then accelerates the normal pre-term decline in placental 11 β -HSD2, perhaps to increase maturational effects, at the expense of growth restriction. Thus, placental 11 β -HSD2 dynamically responds to alterations in the maternal environment plausibly to tailor fetal growth and maturation in the face of physiological challenge [2].

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Nothing to Disclose: ECC, LK, JRS

P1-628

Effects of Hypothalamic Estrogen on HPA-Axis Activity in Female Rats.

J Liu^{1,2}, PH Bisschop², XR Qi¹, E Fliers², P Hu¹, L Eggels², E Foppen^{2,3}, FT Meng¹, A Kalsbeek^{2,3} and JN Zhou¹.

¹Sch of Life Sci, Univ of Sci and Technology Hefei, China ; ²Academic Med Ctr, Univ of Amsterdam Amsterdam, Netherlands and ³Netherlands Inst for Neuroscience Amsterdam, Netherlands.

The stress-induced activation of the hypothalamus-pituitary-adrenal (HPA) axis is sexually dimorphic. Recent data suggest that the paraventricular nucleus (PVN) of the hypothalamus is an important site of interaction between estrogen and the control of HPA-axis activity. Based on these observations, we hypothesized that basal and stress-induced changes in HPA-axis activity during the menstrual cycle are related to changes in local estrogen concentrations in the PVN.

In intact female rats we determined baseline HPA-axis activity and stress-induced activation of the HPA-axis during the different phases of the menstrual cycle and measured 17beta-estradiol (E2) concentrations in the circulation as well as locally in the PVN by microdialysis. In ovariectomized (OVX) rats we determined the effect of systemic and intrahypothalamic (PVN and ventromedial hypothalamus (VMH)) estrogen administration on HPA-axis activity.

In pro-estrous, unstressed plasma corticosterone (CORT) and E2 concentrations were higher than in estrous, but PVN E2 concentrations were not different. Acute stress elevated plasma CORT and E2 concentrations both in pro-estrous and estrous, but PVN E2 concentrations and aromatase mRNA expression only in pro-estrous. Administration of E2 or PPT (ER alpha agonist) in the PVN increased plasma CORT, while DPN (ER beta agonist) infusion had no effect. In the VMH, estrogen administration had no effect on plasma CORT.

In conclusion, higher circulating estrogen concentrations during pro-estrous and stress are associated with increased HPA-axis activity. Increased concentrations of estrogen locally in the PVN during pro-estrous and stress maybe involved in this increased activity of the HPA-axis, as hypothalamic administration of estrogen restricted to the PVN increases HPA-axis activity.

Nothing to Disclose: JL, PHB, XRQ, EF, PH, LE, EF, FTM, AK, JNZ

P1-629

Antioxidant Treatment Normalizes Adrenocortical Nitric Oxide Synthase Activity and Steroid Secretion in Streptozotocin-Diabetic Wistar Rats.

R Sanchez¹, EM Repetto Biochemist¹, JM Cipelli Biochemist¹, EF Giordanino¹, F Astort Licentiate¹, C Martinez Calejman Licentiate¹, ME Mercau¹, P Arias PhD/MD² and CB Cymeryng PhD¹.

¹Sch of Med-Univ of Buenos Aires-CEFYBO-CONICET Buenos Aires, Argentina and ²Sch of Med-Univ of Buenos Aires Buenos Aires, Argentina.

We previously demonstrated that streptozotocin-induced (STZ) diabetes enhances adrenocortical oxidative / nitrosative stress, modulating basal and ACTH dependent steroid production. Consequently, present studies were designed to evaluate the effect of antioxidant treatment on oxidative stress parameters, nitric oxide synthase (NOS) activity, and steroidogenic function in the adrenal cortex of STZ-diabetic rats.

Control (CON) and STZ-treated male Wistar rats (40 mg/kg ip for two consecutive days) received α -tocopherol (α -T, 200 mg/kg po), tiotic acid (TA, 90 mg/kg ip), or the corresponding vehicle every 48 hours, beginning simultaneously with the detection of hyperglycemia. After 4 weeks, animals were sacrificed and adrenocortical oxidative stress parameters and NOS activity were measured. In a second series of experiments, basal and ACTH-stimulated corticosterone secretion was determined.

Antioxidant treatment with α -T or TA had no effect on glycemic levels or on body or relative adrenal weight. On the other side, these treatments abolished the increase in TBARS and carbonyl content, and in the expression levels of catalase and heme oxygenase-1 detected in adrenal cortex homogenates of STZ-treated rats. Both antioxidants prevented the increase in NOS activity (CON 88.5 ± 0.5 , STZ 216.7 ± 9.2 , STZ+ α -T 108.6 ± 7.4 , STZ+TA 117.4 ± 16.8 pmol/min/mg protein; $p < 0.01$ vs STZ), and normalized basal (CON 7.3 ± 2.5 , STZ 71.7 ± 9.2 , STZ+ α -T 5.8 ± 3.4 , STZ+TA 19.1 ± 5.5 ng/ml; $p < 0.001$ vs STZ) and ACTH-stimulated corticosterone output in diabetic rats. Neither antioxidant treatment modified NOS activity or corticosterone secretion in CON animals.

Our results show that, in STZ-diabetic rats, enhanced oxidative stress is associated with an increase in the activity of the NO-generating system, a well-known local modulator of steroid production in the adrenal cortex, and with altered corticosterone secretion. Systemic antioxidant treatment not only normalized adrenocortical oxidative stress parameters and NOS activity in STZ-treated rats, but also corrected the dysregulation in steroid secretion present in these animals.

Sources of Research Support: ANPCyT PICT 2005 N° 38283, UBACYT M014 2008-2010; CONICET PIP5525.

Nothing to Disclose: RS, EMR, JMC, EFG, FA, CMC, MEM, PA, CBC

P1-630

Ultradian Corticosterone Secretion Is Maintained in the Absence of Circadian Cues.

EJ Waite PhD¹, M McKenna¹, YM Kershaw¹ and SL Lightman¹.

¹Univ of Bristol Bristol, UK.

Corticosterone secretion exhibits both a circadian and an ultradian rhythm of secretion. Hourly pulses are secreted from the adrenal gland in response to ACTH with the peak of secretory activity observed at the onset of the dark phase in rats. The suprachiasmatic nucleus (SCN) is known to influence the diurnal variation of corticosterone observed in rats (1, 2). Lesioning the SCN leads to a loss of the light-phase nadir, elevating levels to those seen during the dark-phase peak. The role of the SCN in ultradian rhythmicity, however, is unclear.

Two experimental approaches have been used to address this. In the first study, rats were exposed to a constant light environment (LL) for 5 weeks. At the end of the experiment, when body temperature and locomotor activity were arrhythmic, blood was sampled for 24 hours at 10 minute intervals using an automated sampling system. Control animals were maintained on a standard light-dark cycle (LD). As expected, rats kept in constant light no longer exhibited a diurnal pattern of corticosterone secretion. Interestingly, pulsatile secretion was maintained across the 24 hours in treated rats. The number of pulses increased significantly (16.22 ± 1.36 vs. 12.29 ± 0.99 ; LL vs. LD; $p < 0.05$) but pulse amplitude, pulse area and interpeak interval remained unchanged.

In the second study, the SCN of treated rats was lesioned electrolytically. Sham-lesioned animals were used as a control. When body temperature, activity and drinking behaviour were considered arrhythmic, blood was sampled for 24 hours at 10 minute intervals. Immunohistochemistry was used to determine the degree of ablation caused by the lesion. Preliminary data suggests that in SCN-lesioned rats the diurnal pattern of corticosterone secretion is lost but, as with the LL rats, the ultradian rhythm remains.

Our data suggest that the ultradian rhythm of corticosterone secretion is differentially regulated to the circadian rhythm. As previously demonstrated circadian corticosterone secretion is influenced by the SCN and the external cues to which this central pacemaker is entrained. In contrast, the pulsatile pattern of corticosterone secretion is not organised through the SCN or light cues suggesting the existence of a separate mechanism for pulse generation. For example, it may be possible that it is a combination of intrinsic feedforward and feedback loops existing within the HPA axis that act to generate a pulse of secretion (3).

(1) Abe K. et al., *Neuroendocrinology* 1979; 29:119

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Nothing to Disclose: EJW, MM, YMK, SLL

P1-631

Morphological and Functional Changes Detected in Rat Adrenal Cortex during the Induction of Insulin Resistance by Means of a Sucrose-Rich Diet.

C Martinez Calejman Licentiate¹, EM Repetto Biochemist¹, F Astort Licentiate¹, JM Di Gruccio¹, R Sanchez¹, M Mercau¹, P Arias PhD/MD² and CB Cymeryng PhD¹.

¹Sch of Med Univ of Buenos Aires-CEFYBO-CONICET Buenos Aires, Argentina and ²Sch of Med Univ of Buenos Aires Buenos Aires, Argentina.

Hyperactivation of the hypothalamic-pituitary-adrenal axis (HPAA) has been demonstrated both in humans and animals with insulin resistance (IR), but evidence on a direct adrenocortical impact of IR-associated abnormalities (e.g. elevated serum insulin, triacylglycerides and/or FFA levels, oxidative stress, inflammatory state) is lacking. We evaluated, in adult male Wistar rats fed a sucrose-enriched diet (SED, sucrose 30% w/v in drinking water), the presence of changes in adrenocortical structure and function (corticosterone secretion, insulin signaling, and expression of nitric oxide synthase (NOS) and cyclooxygenase 2 (COX-2), two well known local modulators of steroidogenesis).

As compared to control animals, SED-treated rats showed, after 7 weeks, higher fasting serum glucose (130.6 vs. 74.5 mg/dl; $p < 0.001$), triacylglyceride (604.60 vs. 104.52 mg/dl; $p < 0.001$) and insulin concentrations (2.0 vs. 1.0 ng/ml; $p < 0.005$), as well as elevated basal serum corticosterone levels (6.6 vs. 9.6 ng/ml; $p < 0.001$). At this time point, impaired insulin signaling (i.e., lower phosphorylated Akt levels) and increased NOS activity and expression levels of eNOS, iNOS, COX-2, StAR and of the macrophage marker F4/80 were detected in adrenocortical homogenates obtained from the SED group. Interestingly, adrenal glands of SED-treated animals showed a significant lipidic infiltration, as demonstrated by histochemistry. In a second series of experiments rosiglitazone (RSG), a PPAR agonist, administered during SED treatment, reverted the observed changes in NOS activity and corticosterone levels and diminished adrenocortical lipidic infiltration.

In summary, SED-associated IR might affect adrenocortical cells by inducing lipidic infiltration. Without disregarding possible effects exerted at higher levels of the HPAA and/or on steroid metabolism, the observed changes in steroid production could be attributed to effects of locally produced adipokines or lipid metabolites. Also augmented inflammatory activity could result in COX-2 activation, thus increasing steroid production. In addition, augmented NO generation could trigger post-transcriptional modifications of proteins involved in steroid biosynthesis and/or its modulation. These changes were reverted by RSG, by means of previously described effects (diminished insulin resistance and lipidic infiltration, reduced systemic inflammation, suppression of COX-2 activity), and/or perhaps through other still unknown mechanisms.

Sources of Research Support: ANPCyT PICT 2005 N° 38283, UBACYT M014 2008-2010.

Nothing to Disclose: CMC, EMR, FA, JMDG, RS, MM, PA, CBC

P1-632

Dysregulation of Acetylcholine and Monoamine Neurotransmission in the Brain Regions of Adolescent Rats by Experience of Neonatal Maternal Separation.

Jeong Won Jahng PhD¹, Sang Bae Yoo PhD¹, Jin Young Kim¹ and Jong-Ho Lee DDS, PhD¹.

¹Seoul Natl Univ Sch of Dentistry Seoul, Korea.

Repeated maternal separation (MS) during neonatal period leads to a long-term dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis in rats. We have previously reported that rats experienced MS during the first two weeks of birth exhibits anxiety- and depression-like behaviors including anhedonia with altered responses of the HPA axis to stress challenges later in life. In this study, we examined the brain monoamine and acetylcholine levels in young MS rats with/without restricted access to highly palatable food (HPF) during adolescent period. Sprague-Dawley pups were separated from dam for 3 h daily during PND 2-14 (MS) or left undisturbed (NH). Half of MS or NH pups received free access to chocolate cookies for 1 h every other day from PND 28 thru 40 in addition to *ad libitum* access to standard chow (NH/HPF or MS/HPF), and the rest half of pups in each group remained with chow only (NH/C or MS/C). Rats were sacrificed on PND 40 immediately after the cookie session, and brain tissues were collected on ice. The tissue levels of acetylcholine, dopamine and serotonin in the hippocampus, pre-frontal cortex, striatum and midbrain were analyzed by HPLC. The hippocampal contents of acetylcholine, dopamine and serotonin appeared to be decreased by MS experience. Limited cookie access decreased acetylcholine, but increased serotonin, in the prefrontal cortex and striatum as well as in the midbrain of MS pups, and these changes were not found in NH pups. The midbrain dopamine and serotonin contents were decreased in NH pups, but not in MS pups, by cookie access. Decreases in the midbrain dopamine and serotonin contents by MS experience supported the neural basis of depression-like behaviors of MS pups. Together with our previous reports, it is suggested that the HPA dysfunction and the psycho-emotional disorders by MS experience may be mediated by dysregulation of acetylcholine, serotonin and dopamin neurotransmission in the brain regions observed.

Sources of Research Support: Grant from the Brain Research Center of the 21st Century Frontier Research Program (2009K001269) funded by the Korea Government (Ministry of Education, Science and Technology).

Nothing to Disclose: JWJ, SBY, JYK, J-HL

P1-633

Results of the Treatment of Congenital Adrenal Hyperplasia in a Man by the Use of a Subcutaneous Continuous Infusion of Hydrocortisone during 2 Years.

E Sonnet MD¹, N Roudaut MD¹ and V Kerlan MD¹.

¹CHU Brest, France.

Introduction: We report the case of a young man with Congenital Adrenal Hyperplasia (CAH) who has been treated during 2 years by a subcutaneous continuous infusion of hydrocortisone (SCIH). The principal aim was to reduce the volume of his bilateral testicular adrenal rest tumors (TARTs).

Hydrocortisone was delivered with an insulin infusion device. Circadian dosage of Cortisol and 17hydroxyprogesterone were measured, before, 1 day, 8 days, 2 months, 1 year and 2 years after the beginning of the use of the device. We studied the levels of testosterone, delta4androstene-dione, the evolution of TARTs by echography, sperm count, and the evolution of the quality of life by the SF36 questionnaire.

4 rates were determined, ranging from 0.1-1.6 mg/h, with a total of 47 mg a day (for 111 kgs). Biochemical parameters were normalized at 2 months. The SF36 questionnaire showed an increase of the well being. Weight decreased (-5 kgs; at the end: 106 kgs for 1.71 m; BMI: 36.25 kg/m²). Unfortunately, there was no decrease of the volume of the TARTs. Two episodes of dermo-hypodermatitis, with abscess at the infusion site, were described.

This case demonstrates the feasibility of the long-term use of the SCIH in patients with CAH. We did not show a decrease of the TARTs. It suggests that this mode of replacement could be used in presence of TARTs, but at early stages. This method could have positive effects in quality of life and in patients with weight problems. But further studies are needed.

Nothing to Disclose: ES, NR, VK

P1-634

The Use of Supprelin LA for Central Precocious Puberty Secondary to 11 β -Hydroxylase Deficiency Congenital Adrenal Hyperplasia.

TA Lee MD¹, S Sheffer-Babila MD¹, M Gangat MD¹ and LS Lam MD¹.

¹Children's Hosp at Montefiore, Albert Einstein Coll of Med Bronx, NY.

11 β -hydroxylase deficiency congenital adrenal hyperplasia (11 β -OH CAH) is the second most common cause of CAH accounting for 5-8% of all cases. It typically presents with virilization and later hypertension. Various factors, including the triggering of central precocious puberty and early epiphyseal maturation can result in short adult stature.

A 3 year 11 month old previously healthy male was brought for evaluation of pubic hair and penile enlargement. He developed acne when he was 3 years 5 months old and then pubic hair and penile enlargement over the subsequent six months. He recently had a growth spurt in height. On physical exam he had hypertension, height and weight above the 97th percentile, 12 year old molars, facial acne, Tanner 2 pubic hair, penile length of 8 cm, and testicular volumes of 4 cc. Initial laboratory results were significant for elevated testosterone and 17-hydroxyprogesterone levels, FSH 1.4 mIU/ml (nl <3) and LH 0.4 mIU/ml (nl <0.1). Bone age was 13 years. ACTH stimulation test results were consistent with 11 β -OH CAH. Since diagnosis he has required supra-physiologic doses of hydrocortisone (15-20 mg/m²/day) to suppress the hyperandrogenism .

A leuprolide stimulation test confirmed central precocious puberty (CPP). He was started on Supprelin LA, a once yearly subcutaneous GnRH agonist implant, to suppress CPP. Though his height at diagnosis was above the 97th percentile, his height prediction was only 136.5 cm. He was also started on GH therapy to maximize final adult height. After approximately two years of treatment he has been normotensive, his pubertal status has remained stable, and his gonadotropins have been appropriately suppressed. Additionally, his bone age has remained at 13 years, giving him an improved height prediction currently of 155 cm.

We introduce a case of 11 β -OH CAH complicated by CPP and very poor height prediction treated with Supprelin LA. The use of a once yearly GnRH agonist implant rather than monthly injections has benefits. With steady drug delivery over a year, there is less potential for reactivation of CPP. Additionally, it requires less clinic visits for injections and subsequent avoidance of pain and stress associated with monthly injections.

Nothing to Disclose: TAL, SS-B, MG, LSL

P1-635

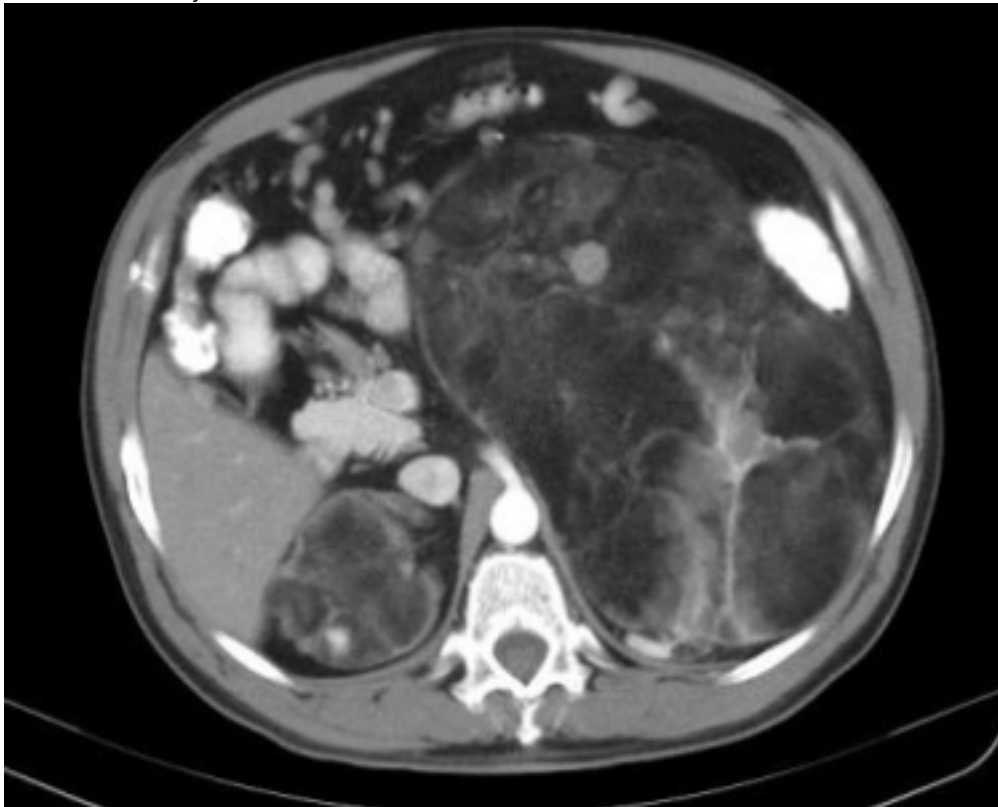
Giant Adrenal Myelolipomas in a Patient with 21-Hydroxylase Deficiency Congenital Adrenal Hyperplasia.

E German-Mena MD¹ and SN Levine MD¹.

¹Louisiana State Univ Hlth Scis Ctr Shreveport, LA.

Introduction: Adrenal myelolipomas are rare, hormonally inactive, benign neoplasms composed of adipose and hematopoietic tissues, most commonly diagnosed by their characteristic appearance on CT scans or MRI. They are usually asymptomatic, although occasional patients present with abdominal pain due to mechanical compression from tumor bulk, hemorrhage or necrosis. The etiology of most adrenal myelolipomas is unknown but they may arise in patients with chronic increases in ACTH, such as those with congenital adrenal hyperplasia or Cushing's disease.

Case Report: A 45 year old white male with untreated 21-hydroxylase deficiency congenital adrenal hyperplasia presented with a 2-3 year history of increasing abdominal girth and an episode of severe abdominal pain, nausea and vomiting 4 months prior to admission. CT of the abdomen showed 22 x 11 cm left and 6.0 x 5.5 cm right adrenal masses that were of very low density consistent with adrenal myelolipomas. As a child he was told he was "born without salt glands". He was treated with corticosteroids until age 10 when they were discontinued by his parents. He was a construction worker and denied any episodes of dizziness, syncope, anorexia, or weakness. He was 131 lbs, 61 inches tall, BP 117/85, pulse 86 with moderate hyperpigmentation of the skin. His abdomen was distended by a 15 cm mass occupying the epigastric region and LUQ. The remainder of the exam was unremarkable. Pertinent lab tests: Na 138 mmol/L, K 3.4 mmol/L, HCO₃ 26 mmol/L, chloride 103 mmol/L, morning cortisol 1.7 mcg/dL (3.7-19.4 mcg/dL), ACTH 1,050 pg/mL (6-48 pg/mL), 17-OH progesterone 28,179 ng/dL (5-160 ng/dL), testosterone 1,137 ng/dL (241-827 ng/dL), androstenedione 2,120 ng/dL (44-186 ng/dL), renin 20.60 ng/mL/hr (1.31-3.95 ng/mL/hr), aldosterone 13.7 ng/dL (1.0-16.0 ng/dL). He was covered with stress doses of hydrocortisone and had an uncomplicated resection of a 24.4 x 19.0 x 9.5 cm left adrenal myelolipoma weighing 2557 grams. The mass was composed largely of adipose tissue with areas of hematopoietic tissue. Following surgery he has done well on treatment with 5 mg of prednisone and 0.1 mg of fludrocortisone daily.



Nothing to Disclose: EG-M, SNL

P1-636

Prenatal Diagnosis and Treatment of Congenital Adrenal Hyperplasia Owing to 11-beta Hydroxylase Deficiency.

AA Parsa MD¹, O Lakarev DO¹ and MI New MD¹.

¹Mt Sinai Med Ctr New York, NY.

Congenital Adrenal Hyperplasia (CAH) owing to 11-beta hydroxylase deficiency (CYP11B1) is the second most common form of CAH affecting 1:100,000 births worldwide with a incidence in North Africa. Females with this disorder are born with ambiguous genitalia (clitoromegaly, labial fusion, formation of urogenital sinus). This can be prevented by prenatal treatment with dexamethasone according to established protocols. Here we describe such a case:

An Islamic family from Yemen had 2 daughters born with CYP11B1. Both had ambiguous genitalia (Prader IV). The mother announced that she was pregnant again and wished to have prenatal diagnosis and treatment. The parents and 2 daughters were genotyped. Fetal tissue from chorionic villous sampling (CVS) at 10.6 weeks gestation supplied by the obstetrician was sent to Mt. Sinai lab for prenatal diagnosis by DNA analysis. The DNA analysis revealed a homozygous mutation at codon 318 in the CYP11B1. This was the same genotype as her 2 older affected sisters.

Treatment: Dexamethasone 1.25mg daily (20 microgram/kg/day at pre-pregnancy weight) divided in 3 daily doses was administered orally to the mother from 6.6 weeks gestation till term. At birth the child had normal female genitalia (Prader I). She has since been reared as a girl and is happy and doing well both in school and with her family. Conclusion: This child with a homozygous CYP11B1 mutation was at risk for ambiguous genitalia as seen in her mutation identical sisters. By administering a low dose of dexamethasone to the mother she was born with normal genitalia. In our experience there have been no detrimental long term effects from this treatment but we suggest close monitoring for any side effects of steroid treatment such as diabetes, cushings and hypertension.

Nothing to Disclose: AAP, OL, MIN

P1-637

46, XY Female with Congenital Lipoid Adrenal Hyperplasia: Atypical Presentation and Novel StAR Protein Gene Mutation.

O Lekarev DO¹, Y Morel MD, PhD² and MI New MD¹.

¹Mount Sinai Sch of Med New York, NY and ²Hospices Civils de Lyon Lyon, France.

Introduction: Congenital lipoid adrenal hyperplasia is a severe disorder of adrenal and gonadal steroidogenesis. It is an autosomal recessive disorder caused by mutations in the steroidogenic acute regulatory protein (StAR) gene, leading to a defect in the conversion of cholesterol to pregnenolone. Patients usually present with adrenal crisis in early infancy. Those with 46, XY karyotype have complete sex reversal. However, the phenotype can be variable with adrenal insufficiency occurring later in life, and patients can have partially virilized or even normal male genitalia. We report an atypical female patient with a 46, XY karyotype, who had mild genital virilization and presented with a late-onset adrenal crisis at age 6 months. She carries a previously unreported StAR protein gene mutation.

Patient Report: Our patient presented to us at age 7 months. At birth she had mild clitoromegaly, mild posterior labial fusion, a single urogenital sinus, and bilateral inguinal hernias with gonads present. A pelvic sonogram revealed no uterus or ovaries. No mutations in the androgen receptor were identified. At age 3 months she underwent a bilateral orchiectomy, inguinal hernia repair and vaginoplasty. Testicular pathology was consistent with prepubertal seminiferous tubules, spermatic cord with vas deferens, epididymis and hernia sac. At age 6 months she presented with vomiting, dehydration and adrenal crisis. Na⁺ was 124 meq/mL, K⁺ was 7.7 meq/mL. She had low levels of all adrenal hormones, including testosterone, with no response to ACTH stimulation. Baseline cortisol was 10 mcg/dL, stimulating to only 12 mcg/dL 60 min after ACTH was administered. Plasma renin activity was 14,345 ng/dL/hr. Baseline ACTH level was 4,781 pg/mL. Genetic analysis revealed c.178+1G>C intron 2 homozygous splicing mutation of the StAR gene. The patient's father is Sephardic Jewish from Syria, Iraq, and Turkey and the mother is Sephardic from Syria and Ashkenazi (countries of origin unknown). There is no reported consanguinity.

Conclusion: Our patient's atypical late presentation of adrenal insufficiency, her tolerating the stress of surgery without developing adrenal crisis, having a detectable cortisol level and having genital virilization all suggest that the StAR gene was partially active. The above newly identified mutation appears to be associated with this atypical phenotype.

Nothing to Disclose: OL, YM, MIN

P1-638

Long-Term Clinical Follow Up and Molecular Genetic Finding in 8 Patients with Triple A Syndrome.

Miroslav Dumic MD, PhD¹, Nina Barisic MD, PhD¹, Vesna Kusec MD, PhD¹, Biserka Stanjker¹, Katrin Koehler MD, PhD² and Angela Huebner MD, PhD².

¹Univ Hosp Zagreb Zagreb, Croatia and ²Universitätsklinikum Carl Gustav Carus Dresden, Germany.

Introduction. The triple-A syndrome (Allgrove syndrome) is caused by autosomal recessively inherited mutation in the AAAS gene on chromosome 12q13, encoding the nuclear pore protein ALADIN. This multisystemic disease is characterized by achalasia, alacrimia, adrenal insufficiency and neurological impairment.

Aims. To analyse long-term clinical follow-up and results of sequencing AAAS gene of 8 Croatian patients with triple A syndrome.

Patients. Five males and 3 females patients with triple A syndrome from 5 nonconsanguineous families aged from 2 years to 35 years.

Results. At time of diagnosis all patients (aged 2 years to 8 years) presented with alacrimia, latent or manifest adrenal insufficiency, peripheral and/or autonomic nervous dysfunction, dermatological abnormalities and 5 of them with achalasia.

Sequencing of the AAAS gene identified S263P mutation in 5 of 8 patients. One of the patients is homozygous for S263P mutation, two compound heterozygous for S263P and Q15K mutations, two compound heterozygous for S263P and novel mutation S296T, two compound heterozygous for Q15K and Q387X mutations and one homozygous for Q387X mutation.

In the course of the follow-up during 2 to 23 years, progression of existing and appearance of new symptoms, particularly neurological, was noted. All of the six patients older than 18 years presented with pronounced peripheral polyneuropathy, postural hypotension, osteoporosis, male patients with erectile dysfunction, achalasia required repeated dilatation and/or surgical procedure and decreased saliva production resulted with loos of all or most of the teeth.

Genotype /phenotype analysis revealed a highly variable occurrence, age of onset and severity of all clinical symptoms between patient with same AAAS mutation, as well as intrafamilial phenotypic heterogeneity.

Conclusion. Triple A syndrome is multisystemic progressive debilitating disorder which may present great handicap for patients, especially in case of advanced neurological impairment. Although very helpful in establishing final diagnosis of triple A syndrome, DNA analysis is not useful for the prediction of clinical expression and outcome of the disorder.

Molecular results support the hypothesis that p.Ser263Pro mutation is founder mutation in the European population. The evident lack of genotype/phenotype relationship is suggestive of modifying gene/factors which need to be determined.

Nothing to Disclose: MD, NB, VK, BS, KK, AH

P1-639

Legs Progressive Spasticity in an Adult Male Diagnosed with Autoimmune Addison's Disease: A Reason for X-Linked Adrenoleukodystrophy Screening.

B Lecumberri MD, PhD¹, FJ Pardo MD², M Giros PhD³, M Casado MD, PhD¹, A Marco MD², M Garcia MD¹, C Alvarez MD, PhD¹ and LF Pallardo MD, PhD¹.

¹La Paz Univ Hosp Madrid, Spain ; ²Hosp Quirón Madrid Madrid, Spain ; ³Hosp Clins and CIBERER U737 Barcelona, Spain ; ⁴La Paz Univ Hosp Madrid, Spain and ⁵Hosp Quirón Madrid Madrid, Spain.

Introduction: X-linked adrenoleukodystrophy (ALD) is an inherited neurodegenerative disorder diagnosed in 1/20000 males, and caused by defects of the *ABCD1* gene on Xq28, resulting in an impairment of peroxisomal beta-oxidation and the accumulation of saturated very-long-chain fatty acids (VLCFAs) that affects mainly adrenal glands, myelin and testes. Recent studies suggest that the prevalence of the adult-onset form called adrenomyeloneuropathy (AMN), in which 70% of patients have Addison's disease (AD), is higher than previously thought. **Clinical Case:** A 28-year-old male patient diagnosed with autoimmune AD at the age of 14 years consulted for a sense of trembling on his lower limbs without paresthesia that started 8 years ago and had slowly and progressively worsened until interfering with his walking. He also referred sexual dysfunction with no sphincter insufficiency. Although he had negative anti-adrenal antibodies (AA), the autoimmune origin was considered based on the AD diagnosis of his paternal grandmother and the reported absence of circulating AA in 30-40% of autoimmune ADs. Physical examination showed a moderate diffuse hair loss involving the entire scalp, eyelashes, eyebrows, and axillary area, distal amyotrophy, hyperreflexia and proximal lower limbs paresis 4/5. The electromyography suggested a demyelinating distal process of the lower limbs and the magnetic spectroscopy of cervical and thoracic cervical cord confirmed a dorsocervical medullary atrophy, as well as decreased noradrenaline levels and slightly elevated coline levels. Serum VLCFAs levels were high in the proband, his mother and oldest sister. Mutation analysis from cutaneous biopsy showed a heterozygotic mutation p.Y181X (c.929C>G) in *ABCD1* gene that confirmed the ALD diagnosis. The same mutation was also carried by his mother, that exhibits mild spastic paraparesis resembling the AMN phenotype, and oldest sister. **Conclusions:** This case highlights the importance of ALD screening in adult males with AD, regardless time from diagnosis, especially in those without AA that complain about neurological symptoms of unknown origin like slowly progressive legs spasticity and disturbances of hair growth. A correct and early diagnosis is essential for appropriate genetic counselling and might help in the effectiveness of therapeutic interventions. Future research is needed to understand the mechanisms that explain the phenotype-genotype relationship in AMN patients and female carriers.

Nothing to Disclose: BL, FJP, MG, MC, AM, MG, CA, LFP

P1-640

Addison's Disease Due to Isolated Tuberculous (TB) Adrenitis - Case Report and Review of Literature.

DS Lim MBBS, MRCP¹ and PC Kek MBBS, M Med, MRCP¹.

¹Singapore Gen Hosp Singapore, Singapore.

Background: Isolated TB adrenitis is rare but needs to be considered as a cause of Addison's disease in the presence of bilateral enlarged adrenal glands.

Clinical Case: A 63-year old Chinese lady with diabetes, hypertension and dyslipidemia presented with 3 days of upper respiratory tract symptoms and lethargy. She was drowsy, hypotensive and capillary glucose was 3.2mmol/L. Her oral mucosa, tongue, palmar creases and nails were hyperpigmented. Sodium was 117 mmol/L (NR: 135-145). Baseline ACTH was 1430 ng/ml (NR: 10-60) and peak response to a short synacthen test was inadequate at 180nmol/L. 8am-aldosterone was 55.2pmol/L (NR: 97.3-834), renin 16.52mcg/L/hr (NR: 0.66-3.08) and DHEAS 0.1mcmol/L (NR: 1.1-11.8), confirming initial suspicion of Addison's disease. Blood pressure and sodium normalized with hydrocortisone and fludrocortisone replacement. Abdominal CT showed bilateral enlarged, hypodense adrenals with rim and septae enhancement. CT-guided adrenal biopsy showed fibrous tissue and no viable adrenal tissue. TB polymerase chain reaction and TB monospot test were positive. She had no history of TB and no evidence of other organ involvement. Upon starting quadruple anti-TB therapy, hyponatremia and symptoms of hypocortisolism recurred, resolving with hydrocortisone dose increment. Decrease in adrenal size was seen on CT 4 months later and pigmentation had decreased markedly.

Clinical lessons: The incidence of tuberculous Addison's disease has decreased from 70% in 1930 to 30-40% in the 1990s due to an overall decline in TB. Isolated adrenal TB without hematogenous spread from active infection at distant sites occurs in 6-7% of cases. CT features correlate with disease duration - bilaterally enlarged adrenals and rim enhancement occurring early in the disease and fibrosis, atrophy and calcification later, usually after several years. Although calcification strongly suggests adrenal TB, its absence does not exclude it, as in this case which appears to have presented in its early stages. Except for a few reported cases, recovery of adrenal function after effective anti-tuberculosis therapy is uncommon especially if minimal viable adrenal tissue remains, making it likely that this patient will require long-term steroids. Rifampicin-accelerated steroid metabolism due to hepatic enzyme induction has been reported to occur, precipitating adrenal crises. Increasing hydrocortisone replacement may be required to prevent this.

Nothing to Disclose: DSL, PCK

P1-641

Complete Adrenal Failure Following Treatment of Cushing's Disease with Mitotane.

C L Feeney MBBS MRCP MA¹, K Meeran FRCP MD¹, E Hatfield MBBCh MRCP¹ and N Martin MBChB MRCP PhD¹.

¹Imperial Coll Healthcare NHS Trust London, UK.

A 63 year old man presented in France in 2006 with features of Cushing's syndrome: centripetal obesity, facial plethora, 'moon' face, bruising on minimal trauma and osteoporosis. ACTH dependent Cushing's syndrome was diagnosed on the basis of elevated urinary free cortisol, unsuppressed ACTH and failure to adequately suppress serum cortisol following a low dose dexamethasone suppression test (LDDST). No adenoma was visible on a pituitary MRI. However, Cushing's disease was suspected as cortisol decreased by more than 50% compared to baseline following a high dose dexamethasone suppression test. Following these results, mitotane was given for a five month period as a treatment for Cushing's disease.

Over the following months he complained of increasing lethargy, weakness and weight loss. He presented to our centre for a second opinion. On examination, he had palmar pigmentation and significant postural hypotension. A low 9am cortisol (73 nmol/L) and elevated ACTH (163 ng/L), indicated primary adrenal failure. This was confirmed following a failed long synACTHen test (peak cortisol = 109nmol/L). Hydrocortisone and fludrocortisone was commenced and the patient's symptoms improved. A repeat MRI pituitary still demonstrated no adenoma. Periodic short synACTHen tests have confirmed persistent adrenal failure (cortisol <30nmol/L) and ACTH remains static over the last two years (pre-hydrocortisone 107-141ng/L, post-hydrocortisone 49-83ng/L).

Our concerns for this gentleman's long term management have been that he is at risk of Nelson's syndrome, since he probably has a pituitary source of ACTH and has received a medical adrenalectomy with mitotane. We are conscious that an ectopic source of ACTH has not been excluded, but felt that in view of his primary adrenal failure, ACTH levels during an IPSS (Inferior Petrosal Sinus Sampling) would be impossible to interpret.

Nothing to Disclose: CLF, KM, EH, NM

P1-642

Acute Adrenal Insufficiency Due to 'Apparent Unilateral' Adrenal Hemorrhage.

M Assal MD¹, A Gupta MD¹ and H Narula MD¹.

¹Stony Brook Univ Hosp Stony Brook, NY.

Objective: To present a case of acute adrenal insufficiency secondary to apparent 'unilateral' adrenal hemorrhage due to heparin induced thrombocytopenia (HIT) syndrome.

Case Presentation: A 58 year old man presented with bilateral lower extremity weakness and unsteadiness. He was diagnosed with Guillain-Barré syndrome. The patient was started on subcutaneous heparin for deep vein thrombosis (DVT) prophylaxis. His platelet count dropped from 500,000/ml on admission to 50,000/ml. The patient became unresponsive, severely hypotensive and bradycardic and had to be resuscitated. Adrenal insufficiency was suspected and confirmed by cosyntropin stimulation test (baseline serum cortisol was 3.1 mcg/dl and post cosyntropin, serum cortisol was 3.6 & 3.0 mcg/dl at 30 & 60 minutes)

The patient developed lower extremity DVT and cyanosis of toes. Heparin Induced thrombocytopenia was suspected and HIT antibody panel was positive. CT scan of the abdomen showed 4.5 x 3.1 cm hyperdense mass in the right adrenal gland consistent with hemorrhage, and the left adrenal gland was initially reported to be 'normal'. As adrenal insufficiency is extremely unusual after just unilateral adrenal hemorrhage, the CT scan was reviewed again with two other radiologists, who felt that the patient had a 'small' hemorrhage in the left adrenal as well. Patient was started on IV hydrocortisone and became hemodynamically stable. The patient was subsequently switched to oral hydrocortisone & fludrocortisone. He was discharged to rehab.

Conclusion: Adrenal insufficiency can be rarely seen due to bilateral adrenal hemorrhage. Clinicians should be careful and should not ignore apparent 'unilateral adrenal hemorrhage'. Adrenal imaging should be reviewed with an expert radiologist, and/or repeated if the clinical situation warrants and there is clinical concern for adrenal insufficiency.

Nothing to Disclose: MA, AG, HN

P1-643

A Case of Plasmodium Vivax Malaria with Adrenal Insufficiency.

Seungeun Lee¹, Jihyun Ahn¹ and Jaetaek Kim¹.

¹Chung-Ang Univ Coll of Med Seoul, Korea.

Background: Fatal complications have been often reported in Plasmodium falciparum malaria, however, complicated P. vivax malaria is rare. We experienced a case of P. vivax malaria who presented with clinical pictures of toxic shock. Clinical case: A 56-year-old male, resident in Seoul, presented with a 3-day history of fever, rigors, and epigastric pain. He had recently travelled to Taebaek and Paju. He showed petechiae and ecchymosis on abdomen. He showed hypoglycemia (glucose 52mg/dl), complication of DIC including marked thrombocytopenia, and septic shock condition. Examination of initial blood smears showed a P. vivax parasitemia of 65,442/ μ L. Abdominal CT showed mild splenomegaly and a 1.3 cm sized left adrenal mass, which was nonfunctioning on hormonal study. In spite of antimalarial agents, fluid replacement, and inotropes, shock was not recovered. Because rapid ACTH stimulation test revealed adrenal insufficiency, he was treated with intravenous hydrocortisone. On 6th day, malarial parasites were not found on peripheral blood smear.

Conclusion: This case emphasize the importance of considering the possibility of adrenal insufficiency in patients with P. vivax malarial infection combined with shock.

Nothing to Disclose: SL, JA, JK

P1-644

All That Infertility Is Not Always PCOS! A Salutory Tale....

L R Simmons Bsc MBBS (Hons)^{1,2}, K Williams MBBS¹, N Perera FRACP MBBS¹ and GP Ross FRACP MBBS¹.

¹Royal Prince Alfred Hosp Sydney, Australia and ²Univ of Sydney Sydney, Australia.

Cushing's syndrome (CS) during pregnancy is a rare entity. As CS results in increased maternal and fetal morbidity and mortality, early diagnosis and treatment are critical. The diagnosis can be challenging as features such as fatigue, weight gain, striae, hypertension, and hyperglycemia can be seen in both conditions. In addition, the diagnosis is made more difficult, in pregnancy, by increased hypothalamic-pituitary-adrenal axis activity and elevated cortisol binding globulin (CBG) leading to elevated total and free plasma cortisol levels as well as the blunted response to exogenous steroids.

We report the case of a 32 year old female admitted to hospital at 22 weeks gestation with severe hypertension. She had undergone successful in vitro fertilization after being told that polycystic ovarian syndrome (PCOS) was the cause of her infertility.

On admission, examination revealed a typical cushingoid appearance including moon facies, facial hirsutism, acne, buffalo hump, truncal obesity and purple abdominal striae. Glycosuria was observed at this time, with gestational diabetes subsequently confirmed on glucose tolerance testing. Elevated 24-hour urinary free cortisol levels and suppressed ACTH were also noted. An MRI at 24 weeks gestation revealed a right adrenal adenoma.

The patient underwent a laparoscopic right adrenalectomy at 26 weeks and postoperatively received ongoing steroid replacement. Her hypertension and diabetes control improved following adrenalectomy, although she still required antihypertensive and insulin therapy throughout the remainder of her pregnancy. A healthy infant was delivered at 36 weeks via caesarean section. The patient's ACTH-cortisol axis remained suppressed postpartum and she continues to exhibit undetectable basal cortisol levels requiring ongoing steroid replacement therapy.

Undiagnosed CS complicated the patient's pregnancy and may have caused her infertility. She had little evidence to support the diagnosis of PCOS, yet this was assumed to be the cause of her inability to fall pregnant. This case illustrates the importance of comprehensive screening to exclude ALL possible causes of infertility prior to assisted conception. The case further emphasizes the benefits of timely intervention in CS occurring in pregnancy.

Nothing to Disclose: LRS, KW, NP, GPR

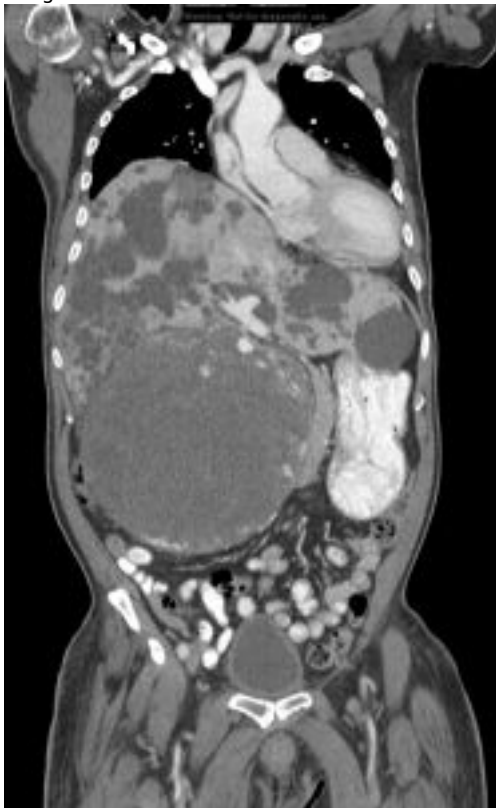
P1-645

Pulmonary Hypertension, Anemia, and Thrombocytopenia. Would You Think about an Adrenal Adenoma in the Differential Diagnosis?.

A Wallia MD¹, M Agulnik MD¹, J Wayne MD¹, J Horowitz MD¹, N Berg MD¹, J Kravarusic MD PhD¹ and P Kopp MD¹.

¹Northwestern Univ Feinberg Sch of Med Chicago, IL.

Background: Adrenal adenomas are typically small (≤ 3 cm) round masses with well-defined margins. Unless they are associated with excessive secretion of adrenal hormones, they are rarely symptomatic. **Case:** A 74 year old male presented with an ankle fracture after trauma. He had thrombocytopenia (platelets 85 K/ μ l; 140-390) and anemia (hemoglobin 6.1 g/dl; 13 -17.5). There were no signs or laboratory findings suggestive for an endocrine disorder. A CT of the abdomen revealed hepatomegaly, multiple liver and kidney cysts, and a large heterogeneous abdominal mass measuring 16.7 x 18.5 x 19 cm (**Figure 1**). The heart was displaced, the pulmonary artery was enlarged at 3.5 cm from compression of the lung base by the mass, resulting in pulmonary hypertension. The left adrenal was of normal size, the right was not identified. FNA and core biopsy of the mass were not diagnostic; spindle cell neoplasm was considered. The patient underwent an exploratory laparotomy and the specimen was a mass of 1804.5 g measuring 24.9 x 19.8 x 9.5 cm. The center was necrotic and liquefied. Histopathologic analysis showed adrenal cortical cells devoid of any cytologic atypia. On immunohistochemistry, tumor cells were positive for inhibin, melan-A, chromogranin and synaptophysin. No mitotic activity was identified by mitotic index and a Ki-67 stain showed no proliferative activity. There was no vascular or capsular invasion. The mass was characterized as an adrenal cortical adenoma with extensive hyaline fibrosis and infarction. **Conclusion:** An adrenal adenoma was not considered on initial evaluation and a definitive diagnosis of an adrenal tumor could only be established after surgery. This unusual adenoma led to displacement of inner organs, pulmonary hypertension, as well as anemia and thrombocytopenia secondary to extensive hemorrhage of the neoplasm. Large adrenal tumors have an increased risk for malignancy and about 76% of adrenal cortical carcinomas are larger than 6 cm. Extensive analyses of the neoplasm did not reveal histological features suggestive for malignancy, and the absence of vascular or capsular invasion and metastases support that the lesion is benign.



Nothing to Disclose: AW, MA, JW, JH, NB, JK, PK

P1-646

A Case of a Patient with Idiopathic Unilateral Adrenal Hematoma.

Seungeun Lee¹, Jihyun Ahn¹ and Jaetaek Kim¹.

¹Chung-Ang Univ Coll of Med Seoul, Korea.

Background: Adrenal hematoma can be spontaneous or be an incidentaloma. Adrenal hematomas result from trauma, sepsis, hypotension or anticoagulation therapy. Unilateral adrenal hematoma is usually caused by blunt abdominal trauma and involves the right adrenal gland more often than the left. We describe a case of left adrenal hematoma which was discovered as an incidentaloma.

Clinical case: A 71-year-old woman was admitted because of exertional dyspnea and headache. Initial vital signs were stable. Physical examination and laboratory findings showed no significant abnormalities. Abdominal CT showed 6×5.4 cm sized round mass in the left suprarenal region. Although a functioning adrenal mass was ruled out, laparoscopic adrenalectomy was done because of its size. The mass was soft tissue aggregating 11×8 cm in diameters. Histology confirmed hemorrhage and thick fibrous capsule with calcification with no neoplastic cells. Pathologic diagnosis was consistent with organizing adrenal hematoma.

Conclusion: Spontaneous unilateral adrenal hematoma is rare and difficult to distinguish from a hemorrhagic tumor preoperatively. Surgery will be needed to make a definitive diagnosis.

Nothing to Disclose: SL, JA, JK

P1-647

A Novel Polymorphism in 11 β -Hydroxysteroid Dehydrogenase Type 2 Gene (HSD11B2) Is Associated with High Urinary Excretion of Tetrahydro-11-Deoxycortisol (THS) in Essential Hypertensive Patients.

C Campino BS¹, H Quinteros BS¹, C Carvajal MSc¹, M Morales BS¹, O Olivieri MD², G Guidi MD², G Faccini PhD², F Passini PhD², J Sateler MD¹, R Baudrand MD¹, G Owen PhD¹, O Padilla¹, A Kalergis PhD¹ and C Fardella MD¹.

¹Pontificia Univ Catolica Santiago, Chile and ²Univ of Verona Verona, Italy.

The 11 β -HSD2 enzyme is expressed in adrenal gland and in tissues where the mineralocorticoid receptor is present, converting cortisol (F) to inactive cortisone (E). Impairment in its enzymatic activity, results in an inefficient conversion of F to E which triggers hypertension (through the activation of mineralocorticoid receptor). In a previous study, we had found that 15.7% of essential hypertensive patients had a high urinary F/E ratio suggesting a deficit in 11 β -HSD2 activity, which may be due to mutations or polymorphisms (1). **Aim:** To investigate in essential hypertensive patients, the presence of a new HSD11B2 polymorphism recently identified by our group and to assess the correlation of this polymorphism with the glucocorticoid profile. **Patients and Methods:** Chilean essential hypertensive outpatients (n=199) were recruited. The new polymorphism was identified analyzing the genomic DNA isolated from hypertensive patients who had the highest urinary F/E ratio in the aforementioned study. The 5 exons of HSD11B2 gene were amplified by PCR. We found a G/A substitution in exon 3 (position 7136 respect Gene Bank, GI:9989705). This change (GAG to GAA) did not alter the resulting glutamate but eliminated an *A*/*I* restriction site which was subsequently utilized as a test to screen PCR amplified DNA isolated from peripheral leucocytes. Plasma renin activity and serum aldosterone, F, E were measured by RIA and the F/E ratio was calculated. The tetrahydrometabolites of 11-deoxycortisol (THS); F (THF, allo-THF) and E (THE) were measured by GC/MS from the urine of each patient. Data were expressed as median [Q₁-Q₃]. **Results:** Nineteen of the 199 hypertensive patients possessed the G/A polymorphism. When the hypertensive patients were divided into 2 groups, based on presence or absence of the polymorphism, the THS excretion was significantly higher in patients possessing the polymorphism: 0.07 [0.04-0.12] mg/24h vs 0.04 [0.03-0.07] mg/24h (p<0.05, Mann-Whitney test). We found no significant differences in serum F/E ratio (3.58 [2.73-4.33] vs 3.61[2.90-4.59] respectively) nor in others variables measured between both groups. **Conclusions:** We describe a novel HSD11B2 gene polymorphism which is presented in 9.5% of this hypertensive cohort. This polymorphism is associated with high urinary THS excretion suggesting a regulatory role of 11 β hydroxylase to maintain cortisol homeostasis.

1.-Campino C, Carvajal C, Cornejo J, San Martin B, Olivieri O, Guidi G et al., Endocrine 2009 Oct 31. [Epub ahead of print] PubMed PMID:19882252

Sources of Research Support: Fondecyt n°1070876 and n°1100356, FONDEF D08I1087 and Millenium on Immunology and Immunotherapy Grant P04/030F.

Nothing to Disclose: CC, HQ, CC, MM, OO, GG, GF, FP, JS, RB, GO, OP, AK, CF

P1-648

In Vivo Systematic Screening Reveals High Prevalence of Aberrant Hormone Receptors in Patients with Bilateral Macronodular Adrenal Hyperplasia or Unilateral Adrenal Adenomas with Sub-Clinical or Overt Cortisol Secretion.

S Grunenwald MD¹, TL Mazzuco PhD¹, I Bourdeau MD¹ and A Lacroix MD¹.

¹Ctr Hosp de l'Univ de Montréal Montréal, Canada.

Background: In ACTH-independent Cushing's syndrome (CS) and sub-clinical Cushing's syndrome (SCS), cortisol secretion can be regulated by the aberrant expression of G-protein coupled receptors in unilateral tumors and bilateral macronodular adrenal hyperplasia (AIMAH) [1-3]. The presence of aberrant receptors has been reported in several cases and small series of patients but its exact prevalence is unknown [4-8].

Objective: To systematically investigate patients with cortisol secreting unilateral adenoma or AIMAH for the presence of aberrant regulation of cortisol secretion in vivo.

Patients and Methods: 59 patients (43 F, 16 M) with ACTH-independent abnormal cortisol secretion (11 adenomas and 48 AIMAH) were studied. A systematic in vivo screening protocol, performed under dexamethasone suppression when ACTH was not fully suppressed, included measurements of plasma cortisol, ACTH, aldosterone concentration (PAC) and renin activity during upright posture, mixed meal, or stimulation with ACTH, GnRH, TRH, vasopressin and metoclopramide or tegaserod. Additional tests were performed in specific cases. Cortisol or PAC increase > 50% over baseline was defined as a positive response.

Results: 95% of the patients (12/13 of CS and 44/46 of SCS) had at least one response of cortisol other than ACTH. Renin-independent aldosterone secretion was associated in 11 patients (4 adenomas, 7 AIMAH), including 2 CS. Aberrant responses were more prevalent in AIMAH (98%) than in adenomas (82%). The most frequent cortisol response (71%) was found after 10 IU vasopressin administration (40/56 of patients), followed by metoclopramide or tegaserod in 35% (17/48) and upright posture in 25% of the patients (13/51). Seven of 10 patients had a positive response of cortisol to isoproterenol infusion. After infusion of GnRH, cortisol increased in 12% (6/50) of the subjects (4 of them responded to LH). Following mixed meal, 12% (6/48) of patients had cortisol increase (2 of them responded to oral glucose and GIP infusion). Aberrant responses to TRH, glucagon and desmopressin were observed in less than 10% of patients (2/46, 2/28 and 2/29 respectively).

Conclusions: This study confirms that aberrant adrenal hormone receptors are frequent in CS and SCS. Aberrant responses were highly prevalent in AIMAH and slightly less in adenomas. Concomitant renin-independent hyperaldosteronism can occur in some cases with aberrant regulation of cortisol and aldosterone secretion.

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Sources of Research Support: Grant MT-13189 from the Canadian Institutes of Health Research.

Nothing to Disclose: SG, TLM, IB, AL

P1-649

Somatostatin and Dopamine Receptor (Co-)Expression in Neuroendocrine Tumors with Ectopic ACTH Production: Potential Targets for Medical Treatment.

RA Feelders MD, PhD¹, C de Bruin MD, PhD¹, AM Pereira MD, PhD², JA Romijn MD, PhD², AM Waaijers¹, RR de Krijger MD, PhD¹, DJ Kwekkeboom MD, PhD¹, SWJ Lamberts MD, PhD¹, WW de Herder MD, PhD¹ and LJ Hofland MD, PhD¹.

¹Erasmus Med Ctr Rotterdam, Netherlands and ²Leiden Univ Med Ctr Leiden, Netherlands.

Introduction. Neuroendocrine tumors (NET) can be associated with ectopic ACTH production leading to hypercortisolism. Somatostatin analogs (SA) and dopamine agonists (DA) that target somatostatin receptor subtype 2 (sst₂) and dopamine receptor subtype 2 (D₂), respectively, are occasionally used to decrease ACTH production. Sst₂ expression may be down-regulated by high cortisol levels if glucocorticoid receptor (GR) signalling is intact. However, in NET, GR signalling is frequently impaired, reflected by absent ACTH and cortisol suppression after (high-dose) dexamethasone (dex) administration. We examined sst₂ and D₂ (co-)expression in NET with ectopic ACTH production and the relation between *in vitro* sst₂ expression and *in vivo* performed octreoscan and dex testing, respectively.

Methods. Tumor tissue was examined from 18 patients with ectopic ACTH-producing tumors: bronchial carcinoid (n=5), thymic carcinoid (n=3), small cell lung carcinoma (SCLC) (n=3), pancreatic NET (n=2), medullary thyroid carcinoma (n=2), gastrinoma (n=1), non-SCLC (n=1) and esthesioneuroblastoma (n=1). Sst_{2a} and D₂ expression was examined immunohistochemically using a rabbit polyclonal and a mouse monoclonal antibody, respectively. Octreoscan and dex suppression test results were available in 13 and 12 patients, respectively.

Results. Positive sst₂ and D₂ staining was present in 10/18 and 13/18 tumors respectively. Co-expression of both receptors was found in 9/18 tumors, but negative staining for both sst₂ and D₂ was found in 4/18 tumors. In tumors with a positive sst₂ staining, octreoscan was positive in 7/8 patients, whereas in tumors with a negative sst₂ staining octreoscan was positive in 3/5 patients. Positive sst₂ staining was associated with an absent cortisol suppression after dex administration in 7/7 patients, whereas negative sst₂ staining was accompanied by adequate cortisol suppression after dex administration in 3/5 patients.

Conclusion. In ACTH-producing tumors of various origin, sst₂ and D₂ are co-expressed in approximately 50 % of tumors. The inverse relation between sst₂ expression and dex-mediated cortisol suppression suggests that if GR signalling is intact, as shown by adequate dex-induced cortisol suppression, endogenous glucocorticoids suppress sst₂ expression by these tumors and vice versa. Sst₂ and D₂ are potential targets for (combined) treatment with sst₂ preferential SA and DA to control hypercortisolism in the ectopic ACTH syndrome.

Nothing to Disclose: RAF, CdB, AMP, JAR, AMW, RRdK, DJK, SWJL, WWdH, LJH

P1-650

The ER22/23EK Polymorphism of the Glucocorticoid Receptor Gene Is Associated to Lower Blood Pressure in Elder Spanish Population.

J Blanco¹, L Sanchez¹, M Serra-Prat², E Palomera², M^a J Perales¹, X Buquet², J Oriola¹ and M Puig-Domingo¹.

¹Hosp Clin Barcelona, Spain and ²Hosp de Mataro Mataro, Spain.

Introduction: Genetic variations in the glucocorticoid receptor (GC-R) gene have been associated to longevity, mental status and some protection against metabolic diseases. The prevalence of ER22/23EK polymorphism of the GC-R gene in Caucasian europids has been reported of about 5- 8% (1, 2)

Objectives: Our study investigated the prevalence of the ER22/23EK polymorphism of the GC-R gene and its relationship with metabolic syndrome components, muscle strength and functional capacity in old Spanish population.

Patients and methods: 313 subjects (153 men and 160 women, mean age 77.0 ± 5.4) participating in the Mataro aging study, a population-based study, were included. Anthropometric variables, lipid profile, glucose and blood pressure were measured, as well as the presence of the ER22/23EK polymorphism of the GC-R gene. Genotypes were determined by polymerase chain reaction using the forward primer labelled with FAM. **Results:** Metabolic syndrome according to IDF criteria was found in 57.9% (54.9 % in men and 61 % in women). The ER22/23EK polymorphism of the GC-R gene was found in 8/313 (2.5%) of the total population. No differences in anthropometric variables, including waist circumference, and lipid profile were found in relation to this polymorphism; abnormal glycaemia was found in 51.9% of non-carriers vs 42.9% of carriers; a blood pressure >130/85 was found in 88% of non-carriers vs 62.5% of carriers (p=0.05); metabolic syndrome (IDF criteria) was found in 58% of non-carriers and in 28.6% of carriers. Barthel score and muscle strength were not different among carriers and non-carriers; in the heterozygous carriers of ER22/23EK, cortisol was lower (15.4 ± 4.9 vs 18.4 ± 6.8 µg/dl) as well as DHEAs (28.1 ± 15 vs 48.4 ± 23 µg/dl), although without reaching statistical significance (p=0.2 and 0.17, respectively). **Conclusion:** The prevalence of the ER22/23EK polymorphism of the GC-R gene in old Spanish population is low. Carriers of this genetic variant show less hypertension and a tendency to present less metabolic disturbances in comparison to non-carriers.

(1) Van Rosum E et al., J Clin Endocrinol Metab 2004 89:4004.

(2) Van Rosum E et al., Am J Med 2004; 117:158.

Sources of Research Support: FIS PI0070601.

Nothing to Disclose: JB, LS, MS-P, EP, MJP, XB, JO, MP-D

P1-651

Association of *CYP3A7*, *POR*, *SRD5A2* and *HSD17B5* Gene Polymorphisms with the Prader Score in Females with Classical Form of CAH.

L C Kaupert Fellow¹, R P P Moreira Fellow¹, M P de Mello MD, PhD², S H V Lemos-Marini MD, PhD³, G Guerra-Junior MD, PhD³, V N Brito MD, PhD¹, L G Gomes MD, PhD¹, M de Castro Professor⁴, B B Mendonca Professor¹ and T A S S Bachega MD, PhD¹.

¹Fac de Med, Univ de São Paulo São Paulo, Brazil ; ²Univ Estadual de Campinas Campinas, Brazil ; ³Fac de Ciências Med, Univ Estadual de Campinas Campinas, Brazil and ⁴Fac de Med de Ribeirão Preto, Univ de São Paulo Ribeirão Preto, Brazil.

Classical form of 21-hydroxylase deficiency (CAH) is the most frequent cause of 46,XX disorder of sex development and it is caused by *CYP21A2* mutations. Despite the strong correlation between genotype and clinical forms, there is no correlation with the degree of external genitalia virilization. It has been suggested that individual variability in the androgen sensitivity could explain the different Prader scores in patients harboring the same genotype. We previously described an influence of CAG repeats of the androgen receptor gene on genital virilization of some CAH females. However, variability in the fetal androgen metabolism can also modulate the genital phenotype. **Objective:** to analyze if allelic variants of genes involved in the fetal androgen metabolism and peripheral androgen activity could influence the severity of external genitalia virilization in a large cohort of CAH females. **Patients:** 150 females diagnosed with 17OHP levels >50 ng/mL in a multicentric study. The degree of external genitalia virilization was graded according to Prader score (I and II in 21 patients, III in 76, IV in 42, V in 11 patients). **Methods:** genomic DNA was PCR amplified and submitted to direct sequencing in order to identify *CYP3A7**1B, *1C, *1D, *1E, *2, A503V *POR* and -71G *HSD17B5* polymorphisms. The V89L and A49T variants of the *SRD5A2* gene were screened by RFLP. The *CYP21A2* genotype was divided into 2 groups, with complete (A) and severe (B) impaired enzymatic activity. **Statistical analysis:** Logistic regression was performed to evaluate the association between polymorphisms and Prader scores in each CAH genotype group. **Results:** *CYP3A7**2, A503V *POR*, -71G *HSD17B5*, and V89L *SRD5A2* were found in 23%, 24%, 33% and 31% of alleles, respectively. The variants *CYP3A7**1B, *1C, *1D, *1E and A49T *SRD5A2* were not evaluated since their frequencies were lower than 3%. The distribution of polymorphisms was similar among Prader scores of females carrying CAH genotype groups A and B. **Conclusions:** We observed that the allelic variants of *CYP3A7*, *POR*, *HSD17B5*, *SRD5A2* genes, involved in the fetal androgen metabolism, did not explain the variability of external genitalia virilization in CAH females.

Sources of Research Support: FAPESP # 08/55546-0, 08/57616-5.

Nothing to Disclose: LCK, RPPM, MPdM, SHVL-M, GG-J, VNB, LGG, MdC, BBM, TASSB

P1-652

Tissue-Microarray of Beta-Catenin in a Cohort of Pediatric and Adult Adrenocortical Tumors.

LO Lima MD¹, IC Soares MD¹, MQ Almeida MD¹, MCBV Fragoso MD¹, AM Lerario MD¹, TC Ribeiro¹, TC Mariani¹, GD Hammer PhD², BB Mendonca PhD¹ and AC Latronico PhD¹.

¹Hosp das Clins da Fac de Med da Univ de São Paulo São Paulo, Brazil and ²The Univ of Michigan Ann Arbor, MI.

Introduction: Wnt signaling pathway has an important role in embryogenesis and tissue homeostasis. Abnormal activation of Wnt signaling pathway has been frequently identified in cancers. Somatic activating mutations of *CTNNB1*, the gene encoding beta-catenin, have been identified in adrenocortical tumors (ACT) diagnosed in adults. In addition, cytoplasmic and/or nuclear beta-catenin staining, a feature that suggests Wnt activation was found in a subset of these tumors, suggesting that Wnt pathway activation can be involved in adult adrenocortical tumorigenesis. However, Wnt pathway involvement in childhood adrenocortical tumorigenesis has not been assessed so far.

Objectives: Our aim was to investigate the frequency of Wnt/ beta-catenin pathway dysregulation and its effects on the outcome of a Brazilian cohort of pediatric and adult ACTs.

Patients and methods: A cohort of 111 patients with ACTs (68 adults and 43 children) was studied. Thirty one adult tumors were classified as carcinomas (Weiss score ≥ 3) as well as 11 pediatric ACTs due to the presence of metastasis. In all tumors, beta-catenin immunoeexpression was analyzed by tissue microarray (TMA). Somatic mutations of exon 3 of the *CTNNB1* were screened in 33 tumor samples (19 adenomas and 14 carcinomas) by automatic sequencing.

Results: Abnormal cytoplasmic and/or nuclear accumulation of beta-catenin was detected in 15% (6 out of 40) and 23.4% (15 out of 64) of pediatric and adult ACTs, respectively. The pattern of beta-catenin staining was similar between adenomas and carcinomas. However, Cox-proportional hazards regression survival analysis identified anomalous nuclear beta-catenin staining as an adverse prognostic factor in carcinomas ($p < 0.05$). Automatic sequencing revealed 6 somatic mutations (5 missense mutations affecting codons 37 or 45, and a frameshift mutation) in *CTNNB1* of ACTs (3 children and 3 adults). The presence of *CTNNB1* mutations was associated with nuclear beta-catenin staining, as expected.

Conclusion: Wnt/beta-catenin signaling pathway is involved in adrenocortical tumorigenesis in both children and adults. In addition, anomalous cytoplasmic and/or nuclear beta-catenin accumulation was associated with a dismal follow up of children and adults with adrenocortical carcinomas.

Nothing to Disclose: LOL, ICS, MQA, MCBVF, AML, TCR, TCM, GDH, BBM, ACL

P1-653

Long Term Follow-Up of Eight Patients with Sub-Clinical Cushing's Syndrome and Aberrant Adrenal Hormone Receptors.

S Grunenwald MD¹, TL Mazzuco PhD¹, I Bourdeau MD¹ and A Lacroix MD¹.

¹Ctr Hosp de l'Univ de Montréal Montréal, Canada.

Background: As described previously in ACTH-independent overt Cushing's syndrome [1], cortisol secretion in sub-clinical Cushing's syndrome (SCS) can be regulated by the aberrant expression of a diversity of G-protein coupled receptors (GPCR) [2]. The natural history of SCS regulated by aberrant GPCR is unknown.

Objective: To describe the evolution of patients with SCS with aberrant regulation of cortisol secretion followed-up for at least 5 years.

Patients and Methods: Patients with incidentally found bilateral adrenal lesions were studied by systematic in vivo screening protocol for aberrant adrenal hormone receptors. The protocol was performed under dexamethasone suppression. Cortisol increase > 50% over baseline was defined as a positive response. Follow-up consisted of annual clinical examination, along with dexamethasone suppression test (DST), adrenal CT and/or cortisol stimulation tests in selected cases.

Results: 8 women with AIMAH and SCS (normal urinary free cortisol (UFC), but plasma cortisol > 50nmol/L after 1mg-DST) had at least one aberrant cortisol response at time of diagnosis; 4 patients had multiple aberrant responses. At a mean follow-up of 7 years, 3 patients developed diabetes (DM), 3 dyslipidemia and 1 hypertension (HT). There were a slight progression in adrenal CT abnormalities for 3 patients. Seven patients had no progression of cortisol level after multiple DST over the follow-up with stability of aberrant cortisol responses.

In three cases, a specific treatment was required

| Treatment | Clinical context | Follow-up data |
|--------------------------------|---|---|
| Leuprolide monthly | SCS + hyperandrogenism. HT, obesity and hirsutism. Aberrant responses to LH, AVP and cisapride. | 9 years: stable SCS and normal androgen levels. Development of DM. |
| Unilateral adrenalectomy (ADX) | SCS + primary aldosteronism. Aberrant hormone responses to posture and AVP. | 4 years: improved SCS and stable contralateral adrenal CT. |
| Unilateral ADX | Adrenal size and UFC increasing, development of CS clinical signs after 5 years. | After ADX: persistence of CS requiring small doses of ketoconazole. |

Conclusions: Aberrant regulation of cortisol can remain stable over many years but a clinical follow-up is justified by the development of metabolic complications or progression to overt hypercortisolism. Long-term studies using specific antagonists for aberrant hormone receptors will be required to examine whether it is possible to block the progression of AIMAH.

(1) Lacroix A et al., Clin Endocrinol (Oxf) 2009 Aug 29. [Epub ahead of print]

(2) Bourdeau I et al., J Clin Endocrinol Metab 2001;86(11):5534-40

Sources of Research Support: Grant MT-13189 from the Canadian Institutes of Health Research.

Nothing to Disclose: SG, TLM, IB, AL

P1-654

A Novel Dax-1 Mutation in an Argentinian Infant with X-Linked Adrenal Hypoplasia Congenita.

G Cecchi MD¹, RM Gomez MD¹, L Francipane MD¹, V Kunstas MD¹, M Saitta MD¹, R Marino MD², N Perez Garrido MD² and A Belgorosky MD².

¹Hosp de Clins Buenos Aires, Argentina and ²Hosp de Pediatria JP Garrahan Buenos Aires, Argentina.

Introduction: Adrenal hypoplasia congenita (AHC) is a hereditary disorder that leads to adrenal insufficiency and hypogonadotropic hypogonadism (HHG) in childhood. The gene responsible for the X-linked form of AHC, NR0B1(nuclear receptor subfamily 0, group B, member 1) / DAX1 (dosage-sensitive sex-reversal, AHC, on the X-chromosome, gene 1), encodes for a nuclear factor which lacks the characteristic zinc finger DNA-binding domain that is highly conserved in nuclear receptors. Deletions and point mutations in the DAX1 gene have been described in more than 70 AHC families. **Clinical case:** We present the clinical and genetic data of a six-week-old boy, previously assisted in another institution due to dehydration, hyponatremia and hypotonia, who was referred to our hospital because of clinical signs of acute adrenal insufficiency. He was also hypotonic and lethargic. ACH was suspected because blood tests showed high levels of plasma ACTH and renin, and low values of serum androstenedione, cortisol and 17-hydroxyprogesterone(17OHP).

Table 1

| | Normal Range | Admission Values |
|-----------------|-----------------|------------------|
| Cortisol | 5-25 μ g/dL | < 2.0 |
| ACTH | 7-64 pg/mL | > 60.0 |
| 17OHP | 1.22-2.80 ng/mL | 0.9 |
| Renin | 6-50 mIU/mL | > 2000 |
| Androstenedione | 0.6- 2.8 ng/mL | < 0.6 |

Under treatment with glucocorticoids and mineralocorticoids he experienced a marked improvement in the first two days and therefore, after a month, he was discharged with replacement doses of hydrocortisone and fludrocortisone. Preliminary studies allowed us to exclude HSC, adrenoleukodystrophy and hypopituitarism as causes of the adrenal deficiency, so congenital adrenal hypoplasia was considered. The NR0B1/DAX1 gene was amplified in three PCR fragments from the patient's and his mother's gDNA obtained from peripheral lymphocytes. In the infant, sequencing revealed a novel single nucleotide deletion in codon 55 from exon 1 that resulted in a frameshift and a stop codon at position 84 (c.165 del G). The mother was heterozygous for this mutation.

In conclusion, we report clinical data in a boy with neonatal adrenal insufficiency and a novel DAX-1 mutation that predicts a severely truncated protein in the NH2 terminal domain (DBD) that lacks entire LBD-like region, and is thus without biological activity

Nothing to Disclose: GC, RMG, LF, VK, MS, RM, NPG, AB

P1-655

Genetic Assessment of a Subgroup of Kuwaiti Females with Non-Classical Congenital Adrenal Hyperplasia (NCCAH).

K.A.S. Al-Shoumer FRCP, PhD, FACE¹, V.S. Nair PhD¹ and A.H. Ali BSc¹.

¹Fac of Med, Kuwait Univ Jabriyah, Kuwait.

INTRODUCTION:

Congenital Adrenal Hyperplasia (CAH) due to the deficiency of 21-hydroxylase enzyme (21-OH) is mainly divided into Classical (CAH) and non-Classical (NCCAH) forms. It arises mostly from mutations in the steroid 21-hydroxylase (CYP21) gene. No previous attempts were made to assess the genotypic CAH variants in Kuwait. This study aimed to assess genotypic variation in CAH Kuwaiti subjects compared with controls.

SUBJECTS & METHODS:

We studied 10 NCCAH patients and 10 normal controls. Patients and controls were Kuwaiti females who were matched for age and BMI. Genetic analyses were performed to all subjects.

For 21-OH genotyping, genomic DNA was isolated from fresh whole blood of subjects using standard salting out procedure. To distinguish between the active CYP21 and the non-functional CYP21P genes, two pairs of oligonucleotide primers were designed. On Primary PCR, the CYP21 and CYP21P genes respectively were amplified. The products generated from CYP21P and CYP21 were distinguished by digestion with *EcoRI*. Using primary PCR product as template, the secondary ACRS PCR was done. 5 mL of the secondary PCR product was incubated for three hours with 5 to 10 units of specific restriction enzymes and then was analyzed by electrophoresis in a 2.0 % agarose. In secondary ACRS PCR, region specific primers for 11 known mutation loci associated with CAH were used. ACRS PCR products were then digested with appropriate restriction enzymes.

RESULTS:

At least one mutation was found in each patient studied. Out of 11 mutations studied, 7 mutations were found in the patients. These 7 mutations were (Exon 1 Codon 30 - detected in 3 patients; Exon 4 Codon 172 - detected in one patient; Exon 6 Codon 237 - detected in one patient; Exon 7 Codon 281 - detected in all 10 patients; Exon 7 Codon 306 - detected in one patient; Exon 8 Codon 318 - detected in 3 patients; Exon 8 Codon 356 - detected in one patient). These data clearly indicate that majority of the cases showed mutations in Exon 7 codon 281, followed by Exon 1 codon 30 and exon exon 8 codon 318. Three of the controls appear to have hitherto un-identified mutations. All of these mutations in the patients are considered part of the genetic basis of their phenotypic presentation with features of NCCAH.

CONCLUSION:

To our knowledge, this is the first demonstration of these mutations in Kuwaiti females with NCCAH that would trigger assessment of a larger population of such patients with clinical correlations.

Nothing to Disclose: KASA-S, VSN, AHA

P1-656

The Value of Salivary Cortisol in Clinical Endocrinology.

RJ Santos MD¹, S Prazeres PhD¹, V Leite MD, PhD¹ and LG Sobrinho MD, PhD¹.

¹Inst Português de Oncologia de Lisboa Lisboa, Portugal.

Introduction: The measurement of salivary cortisol is a valuable tool in the assessment of the function of the adrenal cortex. In the present report we review the basic principles involved and describe our experience with a radioimmunoassay method to measure salivary cortisol.

Objectives: To define the reference values in the normal population and to find out the diagnostic validity of salivary cortisol in selected groups of patients.

Material and methods: 247 morning and late evening saliva samples were collected from healthy volunteers. Salivary and urinary cortisol measurements were obtained in 49 patients with adrenal disorders and in 60 with miscellaneous, non-adrenal, conditions.

Results: The reference intervals for salivary cortisol were: Morning = 2.6 - 60.7 nmol/L; Late evening = 1.0 -14.9 nmol/L. There were no significant differences between men and women or between age groups.

In adrenal hyperfunction evening salivary cortisol had a sensitivity of 83% and a specificity of 87%. There was a significant positive correlation (R = 0.6) between evening salivary cortisol and urinary cortisol. In 26 adrenal "incidentalomas" the evening salivary cortisol was elevated in two while the urinary cortisol was elevated in eight.

Discussion / Conclusions: The reference intervals for salivary cortisol were higher than those referred by other authors. It is, therefore, essential that each laboratory defines its values. Our series confirms that the evening salivary cortisol is an excellent method in the screening for adrenal hyperfunction. The possible reasons for the dissociation between salivary and urinary cortisol in incidentalomas are discussed.

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Nothing to Disclose: RJS, SP, VL, LGS

P1-657

Decreased Salivary Cortisol Concentrations and Disturbed Circadian Cortisol Profiles in Children with Hyperactivity.

P Pervanidou Dr, MD¹, L Thomaidis Dr, MD², E Laios PhD¹ and G P Chrousos Dr, MD¹.

¹Athens Univ Med Sch, Aghia Sophia Children's Hosp Athens, Greece and ²Athens Univ Med Sch, A & P Kyriakou Children's Hosp Athens, Greece.

Introduction: Hyperactivity is a core clustering of symptoms in a variety of developmental and behavioral disorders of childhood and represents failure of behavioral inhibition. The Hypothalamic-pituitary-adrenal (HPA) axis is involved in the physical and behavioral adaptation to stress and as a constituent component of the stress system participates in behavioral inhibition. Basal and stress-related circulating cortisol concentrations and circadian rhythm may be related to pathophysiology or be markers of poor inhibition. A limited number of studies have shown under-reactivity of the HPA axis to stress in individuals with Attention Deficit Hyperactivity Disorder (ADHD).

Methods: Nineteen children (fifteen males and 4 females, mean age 8.4 ± 0.4 years) with hyperactivity, recruited from the Developmental and Behavioral Pediatrics Unit of the First Department of Pediatrics, were examined. Nineteen age-matched, typically developed children served as controls. Morning (8.00) serum cortisol and serial salivary cortisol concentrations obtained 5 times a day (8.00, 12.00, 15.00, 18.00 and 21.00 hours) were measured.

Results: Morning serum cortisol concentrations did not differ between the two groups. However, salivary cortisol concentrations were significantly lower in the hyperactivity group at all five time points ($p=0.04$, $p<0.001$, $p<0.001$, $p=0.02$, $p<0.01$ respectively). A repeated measures analysis revealed a significant difference between the two groups, within the 5 time points ($p=0.03$).

Conclusions: Cortisol concentrations were decreased and circadian cortisol profile was disturbed in children with hyperactivity compared to those of typically developed children. Further studies are needed to examine whether low cortisol is a neurobiological characteristic of hyperactivity and whether it represents a pathophysiologic component or a marker of the condition.

Nothing to Disclose: PP, LT, EL, GPC

P1-658

Plasma NOx Is Not a Suitable Tissue Marker To Guide Glucocorticoid Therapy in Severe Sepsis.

JT Ho MBBS, PhD¹, S O'Connor¹, MJ Chapman MBBS, PhD¹, J Edwards¹, S Lam MBBS¹, G Ludbrook MBBS¹, JG Lewis PhD² and DJ Torpy MBBS, PhD, FRACP¹.

¹Royal Adelaide Hosp Adelaide, Australia and ²Canterbury Hlth Labs Christchurch, New Zealand.

Objectives

Nitric oxide concentrations are elevated in sepsis and their vasodilatory action may contribute to the development of hyperdynamic circulatory failure. Hydrocortisone infusion has been reported to reduce nitric oxide metabolite (NOx) concentrations and facilitate vasopressor withdrawal in septic shock. Our aim was to determine if NOx concentrations relate to (1) protocol-driven vasopressor initiation and withdrawal, and (2) plasma cortisol concentrations, from endogenous and exogenous sources. Demonstration of a relation between NOx, cortisol and vasopressor requirement may provide an impetus towards the study of hydrocortisone mediated NOx suppression as a tool in sepsis management.

Design

A prospective study of sixty-two patients with severe sepsis admitted to the intensive care unit. Measurements Plasma NOx, total and free cortisol, and corticosteroid-binding globulin (CBG) concentrations were measured and related to protocol-driven vasopressor use for 7 days following admission.

Results

Patients who developed septic shock (n=35) had higher plasma NOx, total and free cortisol, and lower CBG concentrations than the non-septic shock group (n=27). Cortisol, CBG and NOx concentrations correlated with illness severity. Free cortisol, and to a lesser extent total cortisol, but not NOx concentrations, predicted septic shock. NOx concentrations were higher in non-survivors and the concentrations were characteristically stable within individuals but marked inter-individual differences were only partly accounted for by illness severity or renal dysfunction. NOx concentrations did not correlate with cortisol, did not relate to vasopressor requirement and were not suppressed readily by hydrocortisone treatment.

Conclusions

Nitric oxide production was increased in sepsis and related to survival, but not related to vasopressor requirement. Perhaps due to unexplained high interindividual variability NOx was not suppressed reproducibly by hydrocortisone. The results suggest that NOx may not be a suitable target for individualized hydrocortisone therapy.

Nothing to Disclose: JTH, SO, MJC, JE, SL, GL, JGL, DJT

P1-659

The Founder Effect of E180splice Mutation in the Growth Hormone Receptor (*GHR*) Gene Causing Laron Syndrome in Israel, Ecuador, Brazil and Chile.

AAL Jorge¹, EM Pinto¹, D Meyer², Z Laron³, J Guevara-Aguirre⁴, FG Cassorla⁵, D Damiani⁶, TS Lins⁷, O Shevah³, V Hwa⁸, AL Rosenbloom⁹, RG Rosenfeld¹⁰ and IJP Arnhold¹.

¹Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil ; ²Univ de Sao Paulo Sao Paulo, Brazil ; ³Schneider Childrens' Med Ctr Petah-Tikva, Israel ; ⁴Inst for Endocrinology Metabolism and Reproduction Quito, Ecuador ; ⁵Univ of Chile Santiago, Chile ; ⁶Hosp das Clins da FMUSP São Paulo, Brazil ; ⁷Inst Materno Infantil de Pernambuco Recife, Brazil ; ⁸Oregon Hlth and Scis Univ Portland, OR ; ⁹Univ of Florida Coll of Med Gainesville, FL and ¹⁰Lucile Packard Foundation for Children's Hlth Palo Alto, CA.

Introduction: Laron syndrome (LS) is a genetic disorder caused by defects in the *GHR* leading to severe GH insensitivity. The E180splice mutation, the most prevalent *GHR* mutation observed in LS patients, was described in a large inbred population of Spanish descent from the Andes of Southern Ecuador, in one Jewish patient from Israel of Moroccan origin, in one Chilean family and in 7 Brazilian families.

Objective: To determine if the E180splice mutation arose from a common origin.

Patients and Methods: Genomic DNA was available from 16 LS subjects (8 Brazilian, 6 Ecuadorian, 1 Israeli and 1 Chilean) homozygous for the E180splice mutation and their 8 first degree relatives. DNA from 42 normal individuals (30 Brazilian and 12 Israeli) and 5 LS subjects (3 from Israel and 2 from Brazil) caused by other *GHR* mutations than E180splice was also analyzed. We genotyped 3 polymorphic markers in the short arm of chromosome 5 (D5S2082, D5S665 and D5S2087) localized surrounding (<2cM) the *GHR*, several intragenics SNPs (rs6179, rs6180, IVS9 haplotype) and the presence or absence of *GHR* exon 3.

Results: An identical haplotype was identified in all but one of the apparently unrelated patients carrying the E180splice mutation: D5S2082: 192/192; D5S665: 150/150; D5S2087: 246/246; rs6179 G/G rs6180 C/C, IVS9 haplotype-I and GHRfl. One Ecuadorian patient differed from other patients only by D5S2082 marker (168/192). This haplotype segregated perfectly in available parents and was not found in LS patients harboring other mutations or in the control group. The microsatellite frequencies of controls indicated that this haplotype is relatively rare (~3%). Using this result, we rejected the hypothesis that the E180splice-associated haplotype is present in all families due to independent origins ($p < 10^{-8}$). The alternative hypothesis is that individuals harboring the mutation share a common ancestor, from which they also inherited this haplotype. Jews were expelled from Spain in 1492, but many had converted to the Christian faith. To flee continued persecution by the Inquisition, many of these "New Christians" immigrated from the Iberian Peninsula to the New World. The founder effect of E180Splice mutation may be explained by this historic event.

Conclusion: We demonstrated strong evidence of co-segregation between several polymorphisms and the germline E180splice mutation in LS patients from different countries, indicating that this mutation originated from a single common ancestor.

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(4) Espinosa C et al., J Pediatric Endocrinol Metab 2008; 21:1119

Nothing to Disclose: AALJ, EMP, DM, ZL, JG-A, FGC, DD, TSL, OS, VH, ALR, RGR, IJPA

P1-660

A Novel Heterozygous STAT5b Variant, N160S, Associated with Modest Short Stature.

V Hwa PhD¹, NE Friedman MD², B Ozhan MD³, P Fang PhD¹, MA Derr MS¹, B Ersoy MD³ and RG Rosenfeld MD¹.

¹Oregon Hlth and Sci Univ Portland, OR ; ²Duke Univ Med Ctr Raleigh, NC and ³Celal Bayar Univ Manisa, Turkey.

Growth hormone (GH) promotes normal human post-natal growth primarily by regulating the production of circulating insulin-like growth factor (IGF)-1. The STAT5b signaling pathway, activated by the interaction of GH with its cognate receptor, the GH receptor (GHR), is critical for this process, as homozygous mutations in the human *STAT5b* gene have been associated severe growth retardation, GH insensitivity (GHI) and IGF-I deficiency (IGFD).

Objective: Two patients who presented with poor growth, GHI and IGFD, were evaluated.

Case Reports: (1) Proband 1, a young male, was normal sized at birth with a birth length of 48.2 cm and birth weight of 3400g. By age 2.6y, his height was 79 cm (-3 SDS) and, at age 6.75 y, was 103.1 cm (-3 SDS), despite 14 months of IGF-I therapy, with a weight of 16.6 kg (-1.9 SDS). His parents were of normal stature (mother, 163 cm, 0 SDS; father, 173 cm, -0.67 SDS). Stimulated serum GH (arginine and clonidine) was 13.2 ng/ml (base of 0.9ng/ml). Serum GH binding protein (GHBP) was normal (280 pmol/L; normal, 267-1638); IGF-I, pre-IGF-I treatment, was low (18 ng/ml); IGF binding protein (IGFBP)-3 (0.9 mg/L; normal, 2.1-4.2) and acid labile subunit (ALS; 1.8 mg/L; normal, 2.3-11) were below normal. (2) Proband 2, a young male of Turkish ethnicity, normal sized at birth (weight, 3400 g; length, 47 cm), was more growth retarded than Proband 1, with a height of 67 cm (-5.84 SDS) by age 2 y. His basal serum GH was modestly elevated at 17.9 ng/ml. Serum IGF-I was undetectable and remained undetectable upon GH-stimulation.

Results: The clinical profiles of Probands 1 and 2 suggested a possible defect in the GH-IGF-I axis. Sequencing of the *GHR* gene revealed that Proband 2 carried a heterozygous, previously reported, splicing mutation in intron 2, *70+IG>A*. Proband 1 was normal for the *GHR* gene. The *IGF1* gene in both probands were normal. Interestingly, both were heterozygous for a novel missense mutation in exon 5 of the *STAT5b* gene that resulted in an asparagine (N) to serine (S) substitution at residue 160 of the protein.

Summary: A novel, heterozygous, *STAT5b* missense mutation was identified in two subjects who presented with short stature, GHI and IGFD. Investigations are currently underway to determine if this heterozygous mutation could be the etiology for the modest short stature of Proband 1, and, in combination with the heterozygous *GHR* mutation, for the more profound growth retardation observed in Proband 2.

Sources of Research Support: Tercica, Inc (RGR).

Nothing to Disclose: VH, NEF, BO, PF, MAD, BE, RGR

P1-661

Heterozygous *STAT5b* Gene Mutations: Impact on Clinical Phenotype.

OV Fofanova-Gambetti MD, PhD, DrMedSci¹, V Hwa PhD¹, AAL Jorge MD, PhD², CA Tonelli MD³, JM Wit MD, PhD⁴, MJE Walenkamp MD, PhD⁵, H Domene MS⁶, HG Jasper MD⁶, A Martinez MD⁶, M Berberoglu MD⁷, G Ocal MD⁷, A Belgorosky MD, PhD⁸, R Marino PhD⁸, M Miras MD⁸ and RG Rosenfeld MD¹.

¹Oregon Hlth & Sci Univ Portland, OR ; ²Univ of Sao Paulo, Sch of Med Sao Paulo, Brazil ; ³Extremo Sul of Santa Catarina Univ Criciuma, Brazil ; ⁴Leiden Univ Med Ctr Leiden, Netherlands ; ⁵VU Univ Med Ctr Amsterdam, Netherlands ; ⁶Ricardo Gutierrez Children's Hosp, Endocrinology Res Ctr Buenos Aires, Argentina ; ⁷Ankara Univ Ankara, Turkey and ⁸Hosp de Pediatria Garrahan Buenos Aires, Argentina.

Background/Aims. In humans, the impact of homozygous and/or compound heterozygous defects in the *STAT5b*, *GHR*, *IGFI*, *IGF1R*, and *ALS* genes on short stature is well described. The possibility that heterozygous defects in these same genes may also modulate growth has become of increasing interest. Recently, a clinically significant impact of approximately 1.0 SD height loss was shown for heterozygous *IGFALS* and *IGF1* mutations. The present study was, therefore, undertaken to evaluate the impact of heterozygous expression of *STAT5b* mutations on height.

Patients/Methods. To date, a total of 7 novel mutations of the *STAT5b* gene in 10 patients from 8 non-related families have been identified. Patients who carry homozygous *STAT5b* mutations (n=10) and family members, who were heterozygous carriers for *STAT5b* mutations (n=16) were analyzed. Three wild type siblings were not analyzed due to unavailability of height data. Height at diagnosis was expressed as SDS (HSDS) for US references. Within each family in whom height data of two or more heterozygous carriers was available, the effect on stature of two mutant alleles versus one was calculated. The differences in HSDS were then pooled for all the families, and evaluated in the entire cohort.

Results. The cohort of patients with *STAT5b* mutations represented high divergency in ethnicity; 50% families came from Argentina. Out of 8 families, 3 (37.5%) were consanguineous, and 3 (37.5%) were non-consanguineous; in 2 families the probands were adopted. All reported *STAT5b* mutations were homozygous. According to the type of mutation, 3 (38%) were missense, 2 (25%) insertion, 2 (25%) deletion, and 1 (12%) a nonsense mutation. In the whole cohort, mean \pm SD height SDS in patients was -5.68 ± 1.60 (below -5 SDS in 70%); in heterozygous carriers -0.98 ± 0.99 (below 0 SDS in 81%, below -2 SDS in 2 parents (14%) from the same family). The difference in mean HSDS between patients and heterozygous carriers was -4.47 ± 1.48 (n=8, 6 families). **Conclusions.** Homozygosity for *STAT5b* mutations results in approximately 4.5 SD height loss in comparison with heterozygosity. To avoid ascertainment bias issues in further "genotype: phenotype" studies, the family analysis approach should include, as much as possible, wild type and heterozygous carriers of first degree relatives in families of patients harboring *STAT5b* mutations.

Nothing to Disclose: OVF-G, VH, AALJ, CAT, JMW, MJEW, HD, HGJ, AM, MB, GO, AB, RM, MM, RGR

P1-662

Mutated IGF-I Receptor (D1105E) Extinguish Autophosphorylation: A Family of Short Stature Born Intrauterine Growth Retardation Bearing a Novel Missense Mutation of the IGF-I Receptor.

Y Kawashima MD,PhD¹, S Okada MD,PhD¹, T Botsubo MD², N Miyahara MD¹, R Nishimura MD¹, J Nagaishi MD.PhD¹, K Hanaki MD.PhD¹ and S Kanzaki MD.PhD¹.

¹Fac of Med Tottori Univ Yonago, Japan and ²Sapporo Med Ctr NTT EC Sapporo, Japan.

The insulin-like growth factor (IGF) plays key roles in intrauterine fetal growth as well as postnatal growth via the IGF-I receptor (IGF-IR). Heterozygous *IGF-IR* mutations presenting with the small for gestational age (SGA) and short stature were observed in over 5 families, including our patient (1). Recently, we determined another family with SGA short stature bearing a heterozygous missense mutation at the tyrosine kinase domain of IGF-IR (D1105E) and the mechanism of this mutation.

We identified heterozygous mutation (D1105E) of the *IGF-IR* gene in an 7-yr-old Japanese girl and her mother with SGA short stature. The girl was born at 37 weeks of gestation, with a birth weight of 2175 g (-1.5 SD) and birth height of 42.5 cm (-2.5 SD). She was healthy except for SGA at birth. She had not been diagnosed with mental retardation. Her height is 93 cm (-2.7 SD) at 5 yrs of age. The serum levels of IGF-I at age 5 were high [353 ng/ml (4.3 SD)]. Her mother was born at 40 weeks of gestation and her birth weight was 2300 g (-2.2 SD); she also had severe short adult stature (height: 137 cm (-4.0 SD)).

R⁻ cells, which are 3T3-like mouse embryo cells with targeted disruption of the *IGF-IR* genes, were transfected with the mutated *IGF-IR* gene. These cells showed absence of autophosphorylation in response to IGF-1. DNA synthesis induced by IGF-1 is currently being tested.

This mutation site is at tyrosine kinase domain, and reported cooperation with activation-loop tyrosine site, which is most important site for autophosphorylation of IGF-IR. Therefore, R⁻ cells bearing D1105E mutation did not show any IGF-IR autophosphorylation. The results of this study provide important new information on the IGF-IR mutation in SGA short stature and on the role of tyrosine kinase domain of IGF-IR. In addition, her high serum IGF-I levels also suggests IGF-IR dysfunction. We identified 3 families with a biologically significant *IGF-IR* gene mutation from among the over 30 patients with SGA short stature until now. Several new mutated IGF-IR may be identified in patients with SGA short stature who show higher serum GH and IGF-I levels or are resistance to GH treatment.

(1)Kawashima Y et al., J Clin Endocrinol Metab 2005;90:4679

Nothing to Disclose: YK, SO, TB, NM, RN, JN, KH, SK

P1-663

Novel Mutations (p.R511W and P.G6R) in *IGF1R* Gene in Children Born Small for Gestational Age (SGA) without Catch-Up Growth.

A C Leal MD¹, LR Montenegro MD¹, DC Coutinho MD¹, B B Mendonca MD, PhD¹, I J P Arnhold MD, PhD¹ and A A L Jorge MD, PhD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

IGF-1 insensitivity caused by *IGF1R* mutations was identified as one of the causes of growth impairment in children born SGA who did not present catch-up growth.

Objectives: to study *IGF1R* of SGA children suspected to have IGF-1 insensitivity.

Patients and methods: 25 SGA children without catch-up growth were selected. IGF-1 insensitivity was suspected by the presence of IGF-1 SDS >0. The *IGF1R* cDNA was sequenced and allelic variants were confirmed by direct sequencing of genomic DNA. Functional analysis of mutated *IGF1R* in fibroblasts were evaluated by AKT phosphorylation and IGF-1-stimulated DNA synthesis.

Results: We identified 2 children (8%) carrying a nucleotide substitution in heterozygous state in *IGF1R*. The first variant (c.16G>A) was located in exon 1, leading to a substitution of glycine by arginine in the pro-IGF-1R signaling peptide (p.G6R). The second variant was located in exon 7 (c.1531 C>T), leading to a substitution of arginine by tryptophan in codon 511 of the IGF1-R (p.R511W). These variations were not observed in 306 alleles from control individuals. Clinical data and the response to rhGH therapy are shown below.

Laboratory and clinical data of affected patients

| | Patient 1 | Patient2 |
|------------------------------------|-----------|----------|
| Birth weight SDS | -2.2 | -2.0 |
| Birth length SDS | n.a. | -1.9 |
| Fathers height SDS | +0.5 | -3.7 |
| Mothers height SDS | -3.0* | -2.3* |
| <i>IGF1R</i> mutation | p.R511W | p.G6R |
| At the start of rhGH therapy | | |
| Age (y) | 5.8 | 7.3 |
| Head circumference SDS | -2 | +0 |
| Height SDS | -2.3 | -3.3 |
| BMI SDS | -1.5 | -0.8 |
| GH peak at stimulation test (µg/L) | 12.1 | 5.8 |
| IGF-1 SDS | +1.3 | +0.5 |
| IGFBP-3 SDS | +1.0 | +1.3 |
| After rhGH therapy | | |
| Duration of rhGH therapy (y) | 2.9♦ | 4.1 |
| rhGH dose (µg/kg/day) | 50 | 50 |
| Height SDS | -1.5 | -1.7 |
| IGF-1 SDS | +0.8 | +0.8 |
| IGFBP-3 SDS | +1.0 | +0.7 |

* - affected parent. ♦ - still in treatment; n.a.- not available.

Fibroblast cultures were developed from affected patients and age-matched controls. Cell proliferation induced by IGF-1 was 50% lower in fibroblasts from affected patients in comparison to controls. Leucocytes and fibroblasts from the patient harboring p.G6R mutation presented 50% decrease in *IGF1R* expression, also confirmed at protein level by Western blot. AKT phosphorylation was decreased in both mutated fibroblast lineages.

Conclusion: Two novel mutations in *IGF1R* gene associated with pre and postnatal growth impairment are described. Mutations in *IGF1R* gene emerge as a relevant cause of intrauterine growth retardation.

Sources of Research Support: Grants from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP) (08/57915-2 to A.C.L.) and from Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) 301339/2008-9 to B.B.M.; 300982/2009-7 to I.J.P.A. and 301477/2009-4 to A.A.L.J.).

Nothing to Disclose: ACL, LRM, DCC, BBM, IJPA, AALJ

P1-664

Severe Short Stature Caused by Novel Compound Heterozygous Mutations of the Insulin-Like Growth Factor 1 Receptor.

P Fang PhD¹, YH Cho MD², MA Derr MS¹, V Hwa PhD¹, CT Cowell MD² and RG Rosenfeld MD¹.

¹Oregon Hlth and Sci Univ Portland, OR and ²The Children's Hosp at Westmead Sydney, Australia.

Background: Insulin-like growth factor 1 (IGF-I), essential for normal human growth, mediates its effects through the IGF-I receptor (IGF1R), a cell-surface tyrosine kinase receptor that is widely expressed. In humans, about 40 heterozygous *IGF1R* mutations have been reported to date. Patients with these mutations have presented with varying degrees of intrauterine and postnatal growth retardation. Here we report the second case of compound heterozygous *IGF1R* mutations in a patient presenting with profound growth failure.

Case Report: The male proband, born at 36-week gestation to consanguineous Lebanese parents, had a birth weight of 1550 g and a length of 39 cm. The parents had heights within the normal range (father, -1.44 SDS; mother, -1.16 SDS). At age 3.4 y, the proband presented with a height of 72.2 cm (-7.33 SDS), weight of 6.9 kg (-9.31 SDS), and head circumference of 42.5 cm (-5.1 SDS); growth velocity was 3.5 cm/y; bone age was 1.8 y. A one-year trial of GH therapy (0.25 mg/kg/wk), initiated at age 3.4 y, did not lead to catch-up growth. At age 18.9 y, a final height of 132.6 cm (-5.96 SDS) was attained, with a weight of 47.4 kg (-2.86 SDS) and head circumference of 50.8 cm. Stimulated serum GH levels were normal; serum levels of IGF-I were consistently elevated, with IGFBP-3 concentrations within the normal range. A diagnosis of antibody-negative insulin-requiring diabetes was made at age 14 y.

Results: Molecular analyses revealed novel compound heterozygous mutations, E121K in exon 2 and E234K in exon 3 of the *IGF1R* gene. *In vitro* reconstitution studies indicated that the prepeptide forms of the IGF1R variants carrying either E234K or E121K were expressed at levels comparable to wild-type IGF1R, but levels of the mature forms of these variants were consistently lower than that of mature wild-type IGF1R. As a consequence, the mutant IGF1R variants exhibited significantly reduced IGF-I-induced signal transduction compared to wild-type IGF1R. When either one of the two IGF1R variants was co-expressed with wild-type IGF1R, the IGF-I-induced activation of the IGF1R pathway was not significantly affected. Neither mutation, therefore, appears to function in a dominant-negative manner, suggesting that the presence of both mutations is required for the significant effect on growth. It remains unclear whether the mutations are also responsible for the insulin-dependent-diabetes observed in the proband.

Nothing to Disclose: PF, YHC, MAD, VH, CTC, RGR

P1-665

SHOX Gene Abnormalities in Children with Idiopathic Short Stature and Leri-Weill Dyschondrosteosis in the French Population.

M Rosilio MD¹, C Huber PhD², H Sapin MS¹, M Vincent MD¹, JC Carel MD, PhD³ and V Cormier-Daire MD, PhD².

¹Lilly France Suresnes, France ; ²Hosp Necker Enfants Malades Paris, France and ³Hosp Robert Debré Paris, France.

The prevalence of SHOX gene abnormalities in children evaluated for short stature is highly variable (2 to 15%) in various series (1, 2).

We describe a series of 537 children with short stature and SHOX gene analysis performed in a central laboratory, as part of the SHOX module of the GeNeSIS observational study. Patients were evaluated at pediatric endocrinology centers throughout France and SHOX gene analysis was performed after exclusion of other causes of short stature as part of the routine workup of these patients. The molecular study included segregation of polymorphic microsatellite markers on the SHOX gene and the PAR 1 region, and direct sequencing of the gene if no deletion was found.

Statistical analyses were mostly descriptive.

537 children (313 females, 58%) were screened at 38 centers from January 2003 to October 2007. Mean (SD) age was 11.2 (4.2) years. 178 patients (33%) were clinically defined with Leri-Weill Dyschondrosteosis (LWD) and 290 (54%) were classified by the physician as having idiopathic short stature (ISS), ie short stature with no apparent Madelung deformity, prior to genetic diagnosis.

For 69 pts (13%), no information was available allowing for a clinical classification ("other" group). 380 pts (71%) had no detectable SHOX region defect, 149 (28%) had a SHOX region defect and for 8 patients the genetic analysis was not conclusive. Among the 149 patients with a SHOX region defect, 61 (41%) had a partial or complete gene deletion, 62 (42%) had a deletion downstream of the gene and 26 (17%) had a point mutation. The frequency of SHOX region defect was 49% in the LWD group, 17% in the ISS group and 19% in the "other" group.

69 (46%) patients with a SHOX region abnormality had Madelung deformity reported: 39% for those with a deletion downstream the gene, 51% for partial or complete deletions of the SHOX gene and 54% for point mutations. In 67 patients (45%) the mutation was inherited from the mother, in 64 (43%) from the father, in 14 (9%) it was a neo mutation or deletion, while the family history was unknown in 4 patients.

We conclude that a high frequency (28%) of SHOX gene abnormalities were detected in a large sample of patients with LWD or ISS, evaluated in an observational setting in France. Given the fact of clinical variability of dyschondrosteosis, it is highly probable that some ISS patients have been inadequately classified when they had mild forearm deformity.

1 Rappold et al. J Clin Endocrinol Metab 2002

2 Huber et al. J Med Genet 2006

Disclosures: MR: Employee, Lilly USA, LLC. HS: Employee, Lilly USA, LLC. MV: Employee, Lilly USA, LLC. JCC: Investigator, Lilly USA, LLC, Ipsen; Advisory Group Member, Pfizer, Inc.

Nothing to Disclose: CH, VC-D

P1-666

Pre- and Postnatal Growth Pattern in Noonan Syndrome (NS) Patients According to *PTPN11*, *SOS1* and *RAF1* Genotypes.

AC Malaquias¹, AS Brasil², LT Wanderley², AC Pereira³, DR Bertola² and AAL Jorge¹.

¹Hosp das Clins da Univ de Sao Paulo Sao Paulo, Brazil ; ²Inst da Criança da Univ de Sao Paulo Sao Paulo, Brazil and ³Inst do Coração da Univ of Sao Paulo Sao Paulo, Brazil.

The Noonan syndrome (NS) is an autosomal dominant disorder characterized by dysmorphic face, congenital heart disease and short stature. Heterozygous mutations in the *PTPN11*, *SOS1*, *RAF1*, and *KRAS* genes, leading to activation of MAPK pathway, cause 60-70% of NS cases. There were no clear correlations between genotype and growth phenotype in NS and even patients with the same mutation in *PTPN11* gene had distinct phenotype.

Objectives: To study the correlation between *PTPN11*, *SOS1* and *RAF1* mutations in NS patients and pre- and postnatal growth.

Patients and Methods: 38 NS patients with mutations identified in the *PTPN11*, *SOS1* or *RAF1* genes were selected. Auxological measurements were obtained at birth and during childhood. The analyzed variables were congenital heart defect, gestational age, birth weight and length SDS, chronological age, height SDS and BMI SDS. The differences between genotypes were analyzed by student t-test and Fisher's exact test.

Results: Among patients carrying *PTPN11* and *SOS1* mutations a wide phenotype variation was observed. Whereas, *RAF1* mutated patients have a more homogenous phenotype. The frequency of hypertrophic cardiomyopathy was higher in patients harboring *RAF1* mutation ($p < 0.05$). No other difference among genotype groups was found. Clinical characteristics are shown in Table 1.

Clinical characteristics of patients with *PTPN11*, *SOS1* and *RAF1* mutation

| | PTPN11 | SOS1 | RAF1 |
|-----------------------------|------------|---------------|------------|
| Sex (M:F) | 19:10 | 2:2 | 2:3 |
| Gestacional Age SDS | 38.4 ± 1.2 | 37.6 ± 2.8 | 36.9 ± 2.9 |
| Birth weight SDS | 0 ± 1.3 | -0.3 ± 1.1 | 0.3 ± 0.8 |
| Birth length SDS | -1.0 ± 1.0 | -1.8 ± 0.8 | -1.8 ± 1.3 |
| Birth length SDS < -2 (%) | 10 | 25 | 20 |
| Congenital cardiopathy (%) | 72 | 100 | 100 |
| Hypertrophic Cardiomyopathy | 5 | 0 | 80 |
| Pulmonary Stenosis | 67 | 100 | 20 |
| Others | 29 | 0 | 0 |
| Age (years) | 8.4 ± 3.8 | 10.1 ± 8.0 | 6.1 ± 5.9 |
| Short Stature (%) | 38 | 50 | 80 |
| Height SDS | -2.0 ± 1.0 | -2.0 ± 0.8 | -2.8 ± 0.6 |
| BMI SDS | -0.4 ± 1.1 | Not available | 0.1 ± 0.9 |

Conclusions: Hypertrophic cardiomyopathy was more frequently observed in patients with *RAF1* mutation compared to other genotype patients. Prenatal growth, assessed by birth length SDS, was slight compromised in NS patients regardless the genotypes. This growth impairment becomes more evident during childhood. The severity of short stature was not associated with any specific gene mutation or with the presence of cardiopathy.

Sources of Research Support: FAPESP 07/59555-0.

Nothing to Disclose: ACM, ASB, LTW, ACP, DRB, AALJ

P1-667

Linear Growth and IGF-I in Infants and Children with Congenital Cyanotic Heart Disease before vs. after Surgical Intervention.

A T Soliman MD PhD FRCP¹ and A Khella MD¹.

¹Hamad Med Ctr Doha, Qatar.

This prospective controlled study recorded the anthropometric data and measured the circulating insulin-like growth factor-I (IGF-I) in 16 children with congenital cyanotic heart disease before and a year after surgical intervention. At presentation patients were significantly shorter [length SD scores (LSDS) = -2.44 +/- 1.31], vs. controls (LSDS = -0.25 +/- 0.18). After surgical treatment the LSDS and growth velocity SD scores (GVSDS) increased significantly to (-) 0.25 +/- 0.95 and 3.7 +/- 2.1, respectively. IGF-I increased from 45.7 +/- 26.3 ng ml(-1) to 67.7 +/- 16.4 ng ml(-1). The GVSDS after treatment was correlated with the body mass index (BMI) ($r = 0.339$, $p < 0.05$) and negatively with the LSDS before surgery ($r = -0.461$, $p < 0.05$). The percentage increase of IGF-I after operation was correlated significantly with the BMI after surgical intervention ($r = 0.82$, $p < 0.001$). It appears that the postoperative growth spurt in infants with cyanotic congenital heart disease (CHD) is mediated through activation of the GH/IGF-I system and improved nutrition.

Nothing to Disclose: ATS, AK

P1-668

Effect of Body Mass Index on Peak Growth Hormone Response to Clonidine in 187 Children with Short Stature.

Sabrina Pilia¹, Chiara Guzzetti¹, Maria Rosaria Casini¹, Valeria Marras¹, Manuela Porcu¹, Luigi Minerba² and Sandro Loche¹.

¹Ospedale Microcitemico Cagliari, Italy and ²Univ di Cagliari Cagliari, Italy.

Background. Obesity is characterized by reduced spontaneous as well as stimulated GH secretion. An inverse relationship has been shown between BMI and the peak GH response to stimulation in adults and in children with short stature. Interestingly, this relation is observed within a normal range of BMI.

Objective. The aim of the study was to investigate the effect of BMI on the GH response to clonidine in a large number of children with short stature.

Subjects and Methods. This was a retrospective study in 187 short children (age 2.5-17.7; 124 M and 63 F; 123 prep and 62 pub; mean height (H)-SDS -2.46 ± 0.71) who underwent clonidine testing in the last 3 years. Clonidine was administered orally (150 mg/m^2) between 8.00 and 9.00 am, and blood samples were collected at times 0, 30, 60, 90 and 120 min for GH determination. IGF-I was also determined in all children at baseline. GH and IGF-I were measured by chemiluminescence assay. All continuous variables were normally distributed with the exception of IGF-I which was log transformed (lnIGF-I).

Results. 126 patients had a GH peak $>10 \text{ } \mu\text{g/L}$. 27 of the 61 patients with a GH peak $<10 \text{ } \mu\text{g/L}$ underwent a second GH stimulation test (arginine or ITT) and GHD was confirmed in 19. Mean (\pm SD) BMI-SDS in the entire cohort was -0.56 ± 1.2 (range -3.4 - 2.75). In univariate regression analysis BMI-SDS was negatively associated with the peak GH response to clonidine ($r = -0.37$, $P < 0.0001$). After correction for lnIGF-I, H-SDS, height velocity (HV)-SDS, target height (TH)-SDS and age the correlation increased ($r = -0.43$, $P = 0.0001$). In the multivariate stepwise regression analysis including age, sex, pubertal status, H-SDS, HV-SDS, TH-SDS, BMI-SDS and lnIGF-I concentrations as independent variables, and peak GH as the dependent variable, BMI-SDS and HV-SDS were the only significant predictors of peak GH. The model explained 23.5% of the variance in peak GH. In the Generalized Linear Model (GLM), BMI-SDS was the only significant predictor, explaining 24.7% of the variability. When only subjects with BMI-SDS between -2.0 and $+2.0$ were analyzed ($n = 158$), BMI-SDS remained the only significant predictor of the peak GH explaining 17.8 and 18.5 % of the variability in the multivariate stepwise regression analysis and the GLM, respectively.

Conclusions. BMI contributes substantially to the variability of the GH response to clonidine in children with short stature, and it should be considered when interpreting the results to the stimulation test.

Stanley TL et al., J Clin Endocrinol Metab 2009; 94:4875

Nothing to Disclose: SP, CG, MRC, VM, MP, LM, SL

P1-669

Growth While Treated (Rx) with Recombinant Human Growth Hormone (rhGH) after Bone Age (BA) 14 for Boys and 13 for Girls: Data from the Genentech National Cooperative Growth Study (NCGS).

AH Perelman MD¹, J Frane PhD² and BM Lippe MD³.

¹Southwest Pediatric Endocrinology, PLC Scottsdale, AZ ; ²Biostatistical Consultant Santa Monica, CA and ³Genentech Inc South San Francisco, CA.

Background: Although rhGH Rx therapy is commonly continued after the BA has reached a relatively late stage, there are scant data on the extent of growth that can be achieved and whether the Bayley-Pinneau (B-P) tables, which estimate, for a given BA, the % of growth likely achieved at mature height, are applicable when GH Rx is used.

Methods: Data were extracted from the NCGS for rhGH Rx patients with GH deficiency(D), Organic (O) GHD, and Idiopathic Short Stature (ISS) who continued Rx for: 1) ≥ 3 years if BA 14 for boys and ≥ 2.5 years if BA 13 for girls, 2) for ≥ 2 years if BA is 15 for boys and ≥ 1.5 years if BA is 14 for girls, and 3) ≥ 1 year if BA is 16 for boys and 15 for girls. BAs were reported individually by sites, using Greulich and Pyle method in most cases. Girls with Turner syndrome were excluded.

Results: There were no significant differences for growth achieved among the 3 etiologies so data for combined groups are in Table.

Growth in GHD, OGH and ISS Children Treated with rhGH and Relevant B-P predictions

| BOYS | BA 14 Rx ≥ 3 y N=96 | BA15 Rx ≥ 2 y N=68 | BA 16 Rx ≥ 1 y N=84 |
|------------------------------|----------------------------|---------------------------|--------------------------|
| CA at BA yr | 14.9 \pm 1.4 | 15.1 \pm 1.1 | 15.9 \pm 1.0 |
| Yrs Rx after BA yr | 3.4 \pm 0.3 | 2.6 \pm 0.5 | 1.5 \pm 0.6 |
| cm after BA yr | 13.4 \pm 4.0 | 8.8 \pm 4.1 | 3.3 \pm 2.4 |
| B-P expected cm to mature ht | 12.7 \pm 1.3 | 5.6 \pm 0.6 | 3.1 \pm 0.1 |
| GIRLS | BA 13 Rx ≥ 2.5 y N=31 | BA14 Rx ≥ 1.5 y N=29 | BA 15 Rx ≥ 1 y N=27 |
| CA at BA yr | 13.6 \pm 1.3 | 14.5 \pm 1.4 | 14.8 \pm 1.2 |
| Yrs Rx after BA yr | 2.9 \pm 0.4 | 2.3 \pm 0.6 | 1.3 \pm 0.2 |
| cm after BA yr | 9.6 \pm 3.3 | 5.9 \pm 3.8 | 2.4 \pm 1.6 |
| B-P expected cm to mature ht | 6.3 \pm 0.9 | 3.0 \pm 0.5 | 1.7 \pm 0.3 |

Data are Mean \pm 1 SD CA= chronologic age

Conclusion: The mean incremental attained growth met or slightly exceeded the mean B-P predictions in boys who continued rhGH for ≥ 3 yrs after BA 14 and in girls ≥ 2.5 yrs after BA 13, or fewer years at more advanced BA, as shown in the Table. B-P predicts what % of mature height has been attained at a given BA and thus predicts what amount is expected to be achieved to reach mature height. The rhGH data were only the heights recorded in the database, which may not be mature height. Therefore, the NCGS data show what are at best conservative estimates of late growth potential in rhGH treated GHD, OGH and ISS children and confirm that B-P can be used to determine conservative expectations in the late treatment phase.

Disclosures: AHP: Study Investigator, Genentech, Inc., Lilly USA, LLC, Pfizer, Inc., Serono, Novo Nordisk. JF: Consultant, Genentech, Inc., Tercica. BML: Employee, Genentech, Inc.

P1-670

Age and Severity of Growth Retardation at Treatment Start, but Not Degree of Prematurity or IUGR Are Related to One and Two Year GH Treatment Response in Short Children Born SGA: Data from the Nordinet® IOS and ANSWER® Program.

Peter A Lee MD¹, Oliver Blankenstein MD², Catherine Pienkowski MD³, Isabelle Oliver MD³, Marta Snajderova MD⁴, Viatcheslav Rakov MD⁵, Birgitte T Pedersen⁶, John Germak MD⁷ and Lars Savendahl MD⁸.

¹Indiana Univ Sch of Med Indianapolis, IN ; ²Charité-Univ Med Berlin, Germany ; ³Hosp des Enfants Toulouse, France ; ⁴ Univ Hosp - Motol Prague, Czech Republic ; ⁵Novo Nordisk Hlth Care AG Zurich, Switzerland ; ⁶Novo Nordisk AS Soeborg, Denmark ; ⁷Novo Nordisk Inc Princeton, NJ and ⁸Karolinska Hosp Stockholm, Sweden.

Objectives: The aim was to analyse how prematurity and degree of intrauterine growth retardation (IUGR) measured by weight SDS at birth relate to short term growth hormone (GH) treatment response in short children born SGA.

Methods: Short children born SGA and enrolled in the observational studies on Norditropin® were included in this analysis. SGA was defined based upon birth weight or birth length or both parameters <-2 SDS (Usher, Mclean). Children born ≥38 weeks of pregnancy were defined as born at term, <38 week - prematurely born, and ≤32 - very prematurely born. Children with birth weight <-3 SDS were defined as very small. Multivariate analyses were used. The model included change in HtSDS after one and two years of treatment in relation to gestational age (normal, premature and very premature), birth weight (<-3, ≥-3), age and HtSDS at GH treatment start, gender and GH average relative dose. **Results:** 561 short children born SGA were identified. Birth and baseline characteristics are shown in the table.

Birth and baseline characteristics in short children born SGA

| Database (n) | Gest. age, w. | B. weight, g | B. weight, SDS | Basel. age, y. | Basel. HtSDS | GH d. ug/kg/d |
|--------------|---------------|--------------|----------------|----------------|--------------|---------------|
| | mean (SD) | mean (SD) | mean (SD) | mean (SD) | mean (SD) | mean (SD) |
| IOS (461) | 36.8 (3.9) | 1971 (701) | -2.4 (0.9) | 7.9 (3.1) | -3.4 (0.7) | 37.6 (10.0) |
| ANSWER (100) | 33.2 (5.4) | 1487 (673) | -2.9 (0.8) | 7.6 (3.5) | -3.0 (0.8) | 51.4 (12.9) |
| Total (561) | 36.2 (4.5) | 1885 (720) | -2.5 (0.9) | 7.9 (3.2) | -3.3 (0.8) | 40.1 (11.8) |

There were more SGA children born very prematurely (≤32 weeks) in the ANSWER® database (46.9%) compared to the NordiNet® IOS (18.2%). Also more children with severe IUGR (weight <-3 SDS) were found in the ANSWER® population than in the European database (38.7% vs. 18.5% respectively). Neither prematurity, nor severity of growth retardation at birth significantly influenced GH treatment response. However, HtSDS and age at treatment start (p<0.0001), dose of GH (p=0.0022) and in the second year also gender (p=0.0004), boys grew better than girls) were significantly related to HtSDS change. **Conclusions:** Age and severity of growth retardation at treatment start, but not degree of prematurity or IUGR were related to short term GH treatment response in this SGA cohort. Also the dose of GH, gender and target height have to be considered as factors which may significantly influence GH treatment results in this patient population.

Disclosures: VR: Employee, Novo Nordisk. BTP: Employee, Novo Nordisk. JG: Employee, Novo Nordisk.

Nothing to Disclose: PAL, OB, CP, IO, MS, LS

P1-671

Vocal Perception, Acoustic Analysis and Anthropometric Parameters in Short Stature with and without Growth Hormone Deficiency.

EHO Valenca MSc¹, AHO Souza MSc¹, R Salvatori MD², LA Oliveira-Neto MSc¹, MIR Goncalves PhD³, RA Meneguz-Moreno MD¹, CRP Oliveira MD¹, VP Araujo MD¹, RMC Pereira MSc¹ and MH Aguiar-Oliveira MD, PhD¹.

¹Fed Univ of Sergipe Aracaju, Brazil ; ²The Johns Hopkins Univ Sch of Med Baltimore, MD and ³Fed Univ of São Paulo São Paulo, Brazil.

Body dimensions are important determinant of vocal quality. We have reported that individuals with lifetime congenital untreated isolated GH deficiency (IGHD) have short stature (SS) with a doll-like facies, reduced muscle mass (1), high pitched voice (2), but normal quality of life (3). We asked what was the individual impact of the two features (SS and IGHD) on voice quality of these individuals. The aims of this study were: 1) analyze the vocal perception by the total score (TS), physical domain (PD), social-emotional domain (SED) and the vocal self-perception (VSP); 2) measure the fundamental frequency of their voice (f₀, Hz); 3) measure the height and cephalic perimeter and 4) study the influence of age on f₀. A cross-sectional study was carried out in 73 adults: 33 with SS caused by IGHD (16 F, 47.1±16.7 yr and 17 M, 45.5±19.0 yr), 10 with SS without IGHD (SS) (6 F, 43.0±16.5 yr all of them with 3-M syndrome, and 4 M, 33.7±11.1 yr, 1 with 3-M syndrome and 3 with acondroplasia), and 30 with normal stature (control group, CO, 15 F, 47.1±16.7 yr and 15 M, 47.6±19.5 yr). Height (cm) of the SS (124.2±10.3) and IGHD (123.3±8.0) groups was similarly reduced in comparison to the control group (161.0±9.4). TS, PD, SED and VSP were all lower in IGHD than in SS and CO. No difference was observed between SS and CO. f₀ was similar in SS women with (239.5±33.6) and without IGHD (239.1±30.4), and higher than CO (201.9±16.5, p<0.05). f₀ of SS men with IGHD (205.7±29.9) was higher than the SS without IGHD (138.6±18.4, p=0.01) and CO (138.3±22.4 p= 0.001). In CO f₀ was higher in males older than 50 years in comparison to younger subjects (156.0±0.6 vs. 126.7±20.7, p=0.007) while it was reduced in females (212.3±9.7 vs. 186.3±11.1, p<0.0001). IGHD subjects did not show any effect of aging on f₀ either in males (201.5±24.0 vs. 211.7±38.1) or females (244.2±27.3 vs. 234.8±40.1). The cephalic perimeter of the SS without IGHD was similar to CO and increased in comparison to IGHD. In conclusion, lower vocal perception and high f₀ are present in adults with untreated, congenital lifetime IGHD in comparison to SS without IGHD suggesting that specifically GH-related factors besides height, like cephalic perimeter, craniofacial dimensions, or laryngeal muscle mass can influence the quality of voice in IGHD. Conversely, stature seems to be a relevant factor influencing f₀ in females but not in males. Finally, IGHD seems to abolish the effect of aging on the quality of voice.

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(2)Barreto VM et al. Otolaryngol Head Neck Surg 2009; 140:37

(3)Barbosa JA et al. Psychoneuroendocrinology 2009;34:894

Disclosures: RS: Advisory Group Member, Novo Nordisk; Researcher, Novo Nordisk.

Nothing to Disclose: EHOV, AHOS, LAO-N, MIRG, RAM-M, CRPO, VPA, RMCP, MHA-O

P1-672

Pituitary Volumes and Functions in Children with Growth Hormone Deficiency: Volumetric Magnetic Resonance Findings.

M Takasu MD¹, C Tani MD¹, M Ishikawa MD¹, K Tanitame MD¹, H Fukuda MD¹, J Horiguchi MD¹, K Awai MD¹, Y Nishi MD² and S Okada MD¹.

¹Hiroshima Univ Hosp Hiroshimashi, Japan and ²Hiroshima Red Cross Hosp Hiroshimashi, Japan.

Purpose: To compare pituitary volumes in patients with idiopathic GHD calculated from MR imaging with the severities of clinical and biochemical features of this disease.

Materials and methods: MR images of the head were obtained for 50 affected males and 23 affected females (5-21 years of age). Maximal whole pituitary dimensions were determined from sagittal and coronal images, and pituitary volumes were estimated from cubic and ellipsoid formulas. Pituitary volumes were compared with measurements of pituitary function, including GH, IGF-1, IGFBP-3, urinary GH, ACTH, cortisol, testosterone, PRL, FSH, LH, free T4, free T3, TSH, and peak GH values on insulin, arginine, glucagon, clonidine, and levodopa tests.

Results: Endocrine evaluation revealed that IGF-1 level was significantly correlated with pituitary volume in both genders (multiple regression analysis, multiple correlation coefficient (R) = 0.67, P = .0001, n =48 for males; R = 0.82, P = .006, n =25 for females).

Pituitary hormone levels with idiopathic GHD patients

| | | Reference values | Patient values |
|---------|--------|------------------|----------------------------------|
| IGF-1 | Male | 64-509 ng/ml | 184.65±90.1 [$<.001+$, .013+]* |
| | Female | 74-542 ng/ml | 184.85±74.23 [.006+, .05+]* |
| IGFBP-3 | Male | 1.5-4.59 µg/ml | 2.24±0.53 [.94, .002+]* |
| | Female | 1.66-5.2 µg/ml | 2.30±0.38 [.05, .62]* |
| FSH | Male | n. a. | 2.76±1.51 [.84, .03+]* |
| | Female | n. a. | 2.81±1.62 [.03+, .29]* |
| LH | Male | n. a. | 0.86±0.92 [.03+, .01+]* |
| | Female | n. a. | 0.81±1.16 [ND] |

Note. - Numbers in brackets are P values. Patient data are mean values ± standard deviation. na = not available. ND = insufficient data to perform trend analysis. * multiple regression analysis [pituitary volume, age]. + Significant correlation ($P < .05$).

In female patients, IGFBP-3 exhibited a weak correlation with pituitary volume (R = 0.49, P = .05, n =18). LH levels of male patients and FSH levels of female patients were significantly correlated with pituitary volume (R = 0.56, P = .03, n =43 for LH; R = 0.75, P = .03, n =25 for FSH).

Conclusion: Pituitary volumes of GHD patients were significantly correlated with IGF-1 and FSH. It might be speculated that impairment of the most abundant somatotrophs cells in the anterior pituitary gland reduces pituitary size. Our results also demonstrate the possibility that the relationship between somatotrophic axis-related hormones and reproductive neuroendocrine function can be determined based on routine clinical MR imaging.

(1) Hamilton J et al.,AJNR Am J Neuroradiol 1998;19:1609-1615

Nothing to Disclose: MT, CT, MI, KT, HF, JH, KA, YN, SO

P1-673

The Primordial Growth Disorder 3-M Syndrome Is Associated with Reduced Cell Proliferation and Transcriptional Loss of IGFBP-2, IGFBP-5 and IGFBP-7.

PG Murray MBChB MRCPCH¹, D Hanson BSc¹, A Whatmore PhD¹, GCM Black DPhil FRCOphth¹ and PE Clayton MD FRCPCH¹.

¹Univ of Manchester/Manchester Academic Hlth Scis Ctr Manchester, UK.

Background: 3-M syndrome is an autosomal recessive disorder characterised by pre- and post-natal growth restriction, a characteristic facial appearance, variable radiological features and normal intelligence. It is caused by nonsense and missense mutations in the genes encoding Cullin 7 (*CUL7*) (1) and Obscurin-like 1 (*OBSL1*) (2). *CUL7* forms one component of an E3 ubiquitin ligase enzyme and interacts with the tumour suppressor p53 while *OBSL1* is postulated to have a role as a cytoskeletal adaptor protein.

Aims: To characterise cell proliferation, apoptosis and gene expression changes in primary fibroblast cell lines derived from a patient with a nonsense mutation in *CUL7* (c.4191delC p.H1379HfsX11) and a patient with a nonsense mutation in *OBSL1* (c.1273insA p.T425NfsX40).

Methods: Cell proliferation was assessed by the percentage of cells incorporating 5-ethynyl 2-deoxyuridine (EdU) and apoptosis by TUNEL. Changes in candidate gene expression were assessed with quantitative reverse transcriptase PCR with genome wide expression analysis assessed using Affymetrix 133 plus 2.0 arrays.

Results: EdU incorporation was reduced in both *CUL7* ($p < 0.001$) and *OBSL1* patient fibroblasts ($p = 0.002$) while there was no difference in the percentage of cells positive for TUNEL between 3-M and control cell lines.

OBSL1 mRNA expression was decreased in both patient cell lines (Relative fold expression (RF) 0.48 ± 0.12 , $p < 0.001$ for *CUL7* cells and 0.26 ± 0.12 , $p < 0.001$ for *OBSL1* cells) while *CUL7* expression was reduced only in *CUL7* cells (RF 1.37 ± 0.65 , $p = 0.15$ for *OBSL1* cells and 0.27 ± 0.04 , $p < 0.001$ for *CUL7* cells). *TP53* expression was unchanged in either patient cell line. *IGFBP-2* and *IGFBP-5* expression were reduced in both the *CUL7* cells (RF 0.64 ± 0.17 , $p < 0.001$ for *IGFBP-2* and 0.17 ± 0.09 , $p < 0.001$ for *IGFBP-5*) and the *OBSL1* cells (RF 0.11 ± 0.04 , $p < 0.001$ for *IGFBP-2* and 0.03 ± 0.01 , $p < 0.001$ for *IGFBP-5*).

Genome wide expression analysis identified 1926 genes differentially regulated between control and 3-M syndrome fibroblasts (defined as fold change > 2 and expression level > 50). 779 of these genes were upregulated and 1147 downregulated. The downregulated genes included *IGFBP7* as well as *IGFBP5*.

Discussion: 3-M syndrome is associated with reduced cell proliferation and a reduction in transcription of IGFBPs 2, 5 and 7. A reduction in IGF binding protein levels is likely to change the amount of IGFs available, which may influence their paracrine actions in promoting growth.

1. Huber C, et al. Nat Gen. 2005;37(10):1119-24.
2. Hanson D, et al. Am J Hum Gen. 2009;84(6):801-6

Nothing to Disclose: PGM, DH, AW, GCMB, PEC

P1-674

C-Type Natriuretic Peptide (CNP) Levels Are Altered in Boys with Klinefelter Syndrome.

RC Olney MD¹, TC Prickett PhD², EA Espiner MD² and JL Ross MD³.

¹Nemours Children's Clin Jacksonville, FL ; ²Christchurch Sch of Med and Hlth Scis Christchurch, New Zealand and ³ Thomas Jefferson Univ & Nemours/Alfred I duPont Hosp for Children Philadelphia, PA.

Klinefelter syndrome (47XXY) is associated with tall stature. It has been suggested that the extra copy of the short stature homeobox-containing gene (*SHOX*) is the genetic cause of the overgrowth but how overrepresentation of this gene results in accelerated growth remains unknown. A recent study reported that the *SHOX* protein regulates expression of B-type natriuretic peptide (BNP) and that BNP colocalized with *SHOX* protein in hypertrophic chondrocytes of the human growth plate¹. In rodents, it has been shown that it is not BNP but C-type natriuretic peptide (CNP) that is crucial to endochondral growth. We postulated that BNP, if augmented in Klinefelter syndrome, would increase the plasma concentration of CNP by inhibiting its local degradation. We studied the peripheral plasma concentrations of CNP and NTproCNP (a marker of CNP production²), together with NTproBNP in boys with Klinefelter syndrome and compared values with a cohort of normal boys of same age and height. Twenty-four boys with Klinefelter syndrome were studied. Plasma samples were assayed by commercial ELISA (NTproBNP) or by RIA (CNP and NTproCNP). NTproBNP levels were 5.6±4.6 pmol/L (mean±SD) and NTproBNP SD scores were -0.4±0.8 (*P*=0.1 vs. a healthy population). For CNP and NTproCNP comparisons, age- and height-matched controls were chosen from a database of 95 healthy boys.

| | <i>n</i> | age (y) | height SDS | CNP (pmol/L) | NTproCNP (pmol/L) | CNP:NTproCNP |
|--------------|----------|---------|------------|--------------|-------------------|---------------|
| Klinefelters | 24 | 8.2±3.0 | 0.3±1.0 | 1.2±0.5* | 42.6±10.6 | 0.027±0.008** |
| Controls | 24 | 8.2±3.1 | 0.3±1.0 | 1.5±0.5 | 38.3±8.5 | 0.040±0.009 |

The subject and control cohorts were very well matched. CNP levels were lower (*P*<0.05 by paired Student's *t*-test, *) and NTproCNP trended higher in the boys with Klinefelter syndrome (*P*=0.1). The CNP:NTproCNP ratio was substantially lower in the boys with Klinefelter syndrome (*P*<0.00001, **).

Our finding of reduced CNP:NTproCNP ratio in boys with Klinefelter syndrome suggests that CNP clearance is *increased*, and argues against the hypothesis that BNP (if augmented in Klinefelter syndrome) promotes linear growth by *decreasing* local CNP degradation. BNP production, as reflected by plasma levels of NTproBNP, is not increased in Klinefelter syndrome. The trend for increased plasma concentration of NTproCNP is likely to reflect augmented growth velocity² in these boys, but the basis of the lower concentration of CNP remains to be determined.

(1) Marchini A et al., Hum Mol Genet 2007; 16:3081

(2) Prickett TC et al., Pediatr Res 2005; 58:334

Sources of Research Support: Nemours Research Programs.

Disclosures: JLR: Consultant, Novo Nordisk.

Nothing to Disclose: RCO, TCP, EAE

P1-675

Growth of Kashmiri Schoolchildren in Relation to the WHO Child Growth Standards - It Is Time To Use a Universal Standard of Reference.

Abdul Hamid Zargar MD, DM¹, Shariq R. Masoodi MD, DM¹, Salim Sherwani MD¹, Mir Iftikar Bashir MD¹ and Arshad I. Wani MD¹.

¹Sher-i-Kashmir Inst of Med Scis Srinagar, India.

BACKGROUND: Many countries use their ethnicity specific growth standards for reference; in India, these reference standards were drawn several decades back from lower socio-economic groups.

OBJECTIVE: To compare the growth in the Kashmiri schoolchildren with the WHO child-growth standards and to study the secular trend by comparing with of Indian Council of Medical Research (ICMR, 1956-1964) reference data.

SUBJECTS: 9092 Kashmiri schoolchildren (4959 boys), aged 6-16 years.

METHODS: A multistage sampling procedure adopted; results expressed as SD scores relative to the WHO reference data.

RESULTS: Mean height was 0.13 for boys and 0.14 SD scores for girls below the mean for WHO growth standards. The mean values for weight were 0.9 and 0.5 SD scores less than that for boys and girls respectively. Comparison of current growth of Kashmiri schoolchildren with five-decade old ICMR reference data revealed that the mean height had increased by 1.2 SD in boys and 1.1 SD in girls. The prevalence of short stature (<3rd centile) was 7.3% by using WHO and only 0.3% by ICMR reference data; about 7% of Kashmiri schoolchildren will miss evaluation for short stature if ICMR criteria were used.

Growth standards of 9092 kashmiri children in comparison to WHO 2007

| Age (years) | Kashmiri boys | WHO boys | SD score | Kashmiri girls | WHO girls | SD score |
|-------------|---------------|-------------|----------|----------------|-------------|----------|
| 6+ | 116.7 (5.1)* | 116.0 (4.9) | 0.14 | 117.1 (4.5) | 115.1 (5.1) | 0.39 |
| 7+ | 123.0 (5.4) | 121.7 (5.3) | 0.24 | 123.1 (4.7) | 120.8 (5.5) | 0.42 |
| 8+ | 127.8(5.5) | 127.3(5.6) | 0.09 | 126.5(5.9) | 126.6(5.8) | -0.01 |
| 9+ | 133.5(6.4) | 132.6 (6.0) | 0.15 | 133.5(6.1) | 132.5(6.1) | 0.17 |
| 10+ | 137.7(6.7) | 137.8(6.4) | -0.02 | 136.8(6.6) | 138.6(6.4) | -0.28 |
| 11+ | 142.5(6.4) | 143.1(6.7) | -0.09 | 143.4(7.9) | 145.0(6.6) | -0.24 |
| 12+ | 146.9 7.4) | 149.1(7.1) | -0.30 | 148.6(7.8) | 151.2(6.8) | -0.39 |
| 13+ | 153.7(8.5) | 156.0(7.4) | -0.32 | 154.1(7.2) | 156.4(6.9) | -0.33 |
| 14+ | 160.6(9.5) | 163.2(7.7) | -0.34 | 157.6(6.8) | 159.8(6.9) | -0.32 |
| 15+ | 165.3 (9.0) | 169.0 (7.8) | -0.46 | 158.9 (6.0) | 161.7 (6.9) | -0.40 |
| 16+ | 168.9(7.6) | 172.9(7.8) | -0.52 | 159.1(5.9) | 162.5(6.8) | -0.50 |

*Mean height (cm) with SD (in parenthesis)

CONCLUSIONS: The growth of Kashmiri children in India is comparable to the 2007 WHO child-growth standards with a significant secular trend in the growth. Short stature in a Kashmiri-Indian child may not be due to ethnic origin.

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2. World Health Organization (2007). Growth reference data for 5-19 years. <http://www.who.int/growthref/en/> (last accessed: 22nd January 2010)
3. Gatrad AR, Birch N, Hughes M. Preschool weights and heights of Europeans and five subgroups of Asians in Britain. Arch Dis Child 1994;71:207-10.
4. Hauspie RC, Das SR, Preece MA, Tanner JM. A longitudinal study of the growth in height of boys and girls of West Bengal aged six months to 20 years. Ann Hum Biol 1980;7:429-41.
5. Kelly AM, Shaw NJ, Thomas AMC, Pynsent PB, Baker DJ. Growth of Pakistani children in relation to the 1990 growth standards. Arch. Dis. Child. 1997;77:401-405.

Nothing to Disclose: AHZ, SRM, SS, MIB, AIW

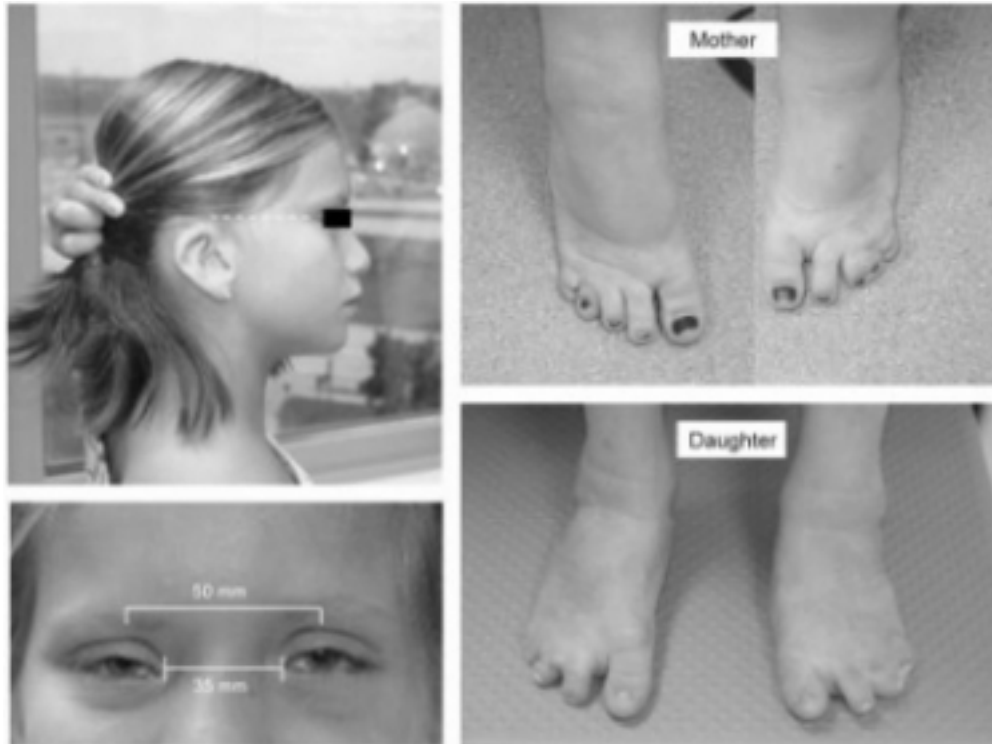
P1-676

Phenotypic Expression of a Pre-Pubertal Girl with STAR Syndrome Due to a Full Deletion of FAM58A Gene at Xq28 and Response to Growth Hormone Therapy.

J Kim MD¹ and ME Flores MD¹.

¹Univ of Illinois Coll of Med at Peoria Peoria, IL.

Background: STAR syndrome is a rare X-linked dominant disorder characterized by toe Syndactyly, Telecanthus, Anogenital and Renal malformations due to mutations in FAM58A gene at Xq28 (1). Short stature has been reported in virtually all cases (1,2). No previous studies have documented the use/efficacy of growth hormone (GH) therapy. Clinical Case: we report a prepubertal 12 year-old female born at 35 weeks with normal weight/length for gestational age. Multiples anomalies detected at birth included clitoromegaly, syndactyly of toes and small kidneys on ultrasound. Physical exam revealed short stature (-3.6 SD), telecanthus, low-set ears, 3-5 and 4-5 syndactyly of left and right toes, respectively. Mother had similar phenotype.



Informed consent was obtained for publication of photographs

Microarray analysis from mother/daughter revealed a 333.2 Kb deletion at Xq28 and later confirmed by FISH analysis. Blood work and imaging studies were performed prior to GH therapy.

Auxological Data

| | Pre-treatment | 1st year of therapy |
|-----------------------|---------------|---------------------|
| Chronological age | 12.3 | 13.3 |
| Bone age | 8.10 | 10.0 |
| Height cm | 126.2 | 134.0 |
| BMI | 14.9 | 16.2 |
| Linear growth cm/year | 4.5 | 7.8 |
| Tanner stage | I | I |

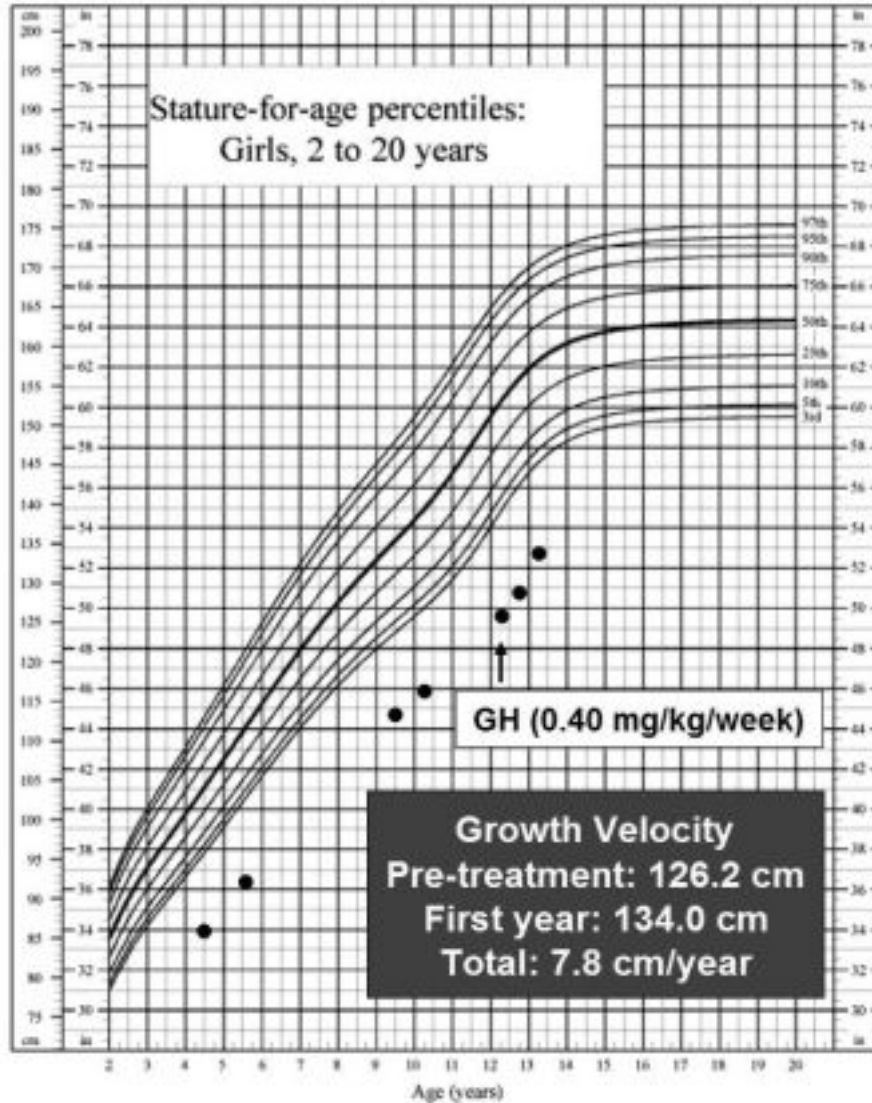
Laboratory/Imaging Results

| | Pre-treatment | 1st year of therapy |
|------------------------------|------------------------------|---------------------|
| *IGF-1 ng/dl | 127 (97-352) | 318 (49-461) |
| *IGFBP3 mg/L | 3.6 (1.6-6.5) | 5.2 (2.1-7.7) |
| Provocative GH testing ng/dl | Clonidine 12.6; Arginine 1.0 | |
| TSH mIU/ml (0.30-5.0) | 2.75 | 2.43 |

| | | |
|---|---|----|
| LH mIU/ml | <1 | <1 |
| Karyotype | 46,XX | |
| X-inactivation | Normal | |
| DNA microarray test (mother & daughter) | 333.2 Kb deletion at Xq28 | |
| Important genes deleted | FAM58A (STAR syndrome); ABCD1 (X-linked adrenoleukodystrophy) | |
| Very long chain fatty acids | Normal | |
| Brain MRI | Normal | |

* Bone age-matched

GH was started at 0.4 mg/kg/week (3). Our patient grew 7.8 cm during the 1st year of therapy.



SOURCE: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000)



Conclusion: This is the first case demonstrating optimal response to GH therapy in a short girl with STAR syndrome.

1. Green, A et al., J. Med. Genet. 33: 594-596, 1996.
2. Unger, S et al., Nature Genet. 40: 287-289, 2008.
3. Cohen, P et al., J Clin Endocrinol Metab. 93: 4210-4217, 2008

Nothing to Disclose: JK, MEF

P1-677

Relationship between 1 Month Biomarker Changes and 1 Year Growth Response in Prepubertal Children with Growth Hormone Deficiency (GHD) or Turner Syndrome (TS) Treated for 1 Year with GH: The PREDICT Multicentre, Observational Follow-Up Study.

P Chatelain¹, L Tato², V Peterkova³, A Belgorosky⁴, R Reynaud⁵, T Theocharis⁶, C Olivier⁶ and P Clayton⁷.

¹Hosp Debrousse Lyon, France ; ²Univ degli Studi di Verona Verona, Italy ; ³Inst of Pediatric Endocrinology Moscow, Russian Federation ; ⁴Hosp Garrahan Buenos Aires, Argentina ; ⁵CHRU de Marseille-La Timone Enfant Marseille, France ; ⁶Merck Serono SA - Geneva Geneva, Switzerland and ⁷St Mary's Hosp Manchester, UK.

Background: PREDICT follow-up investigates relationships between short-term biomarker changes, long-term auxological changes and genomic markers during GH therapy in children with GHD or TS. We previously reported significant 1 month changes in growth and metabolic markers in both GHD and TS, with IGF-I, insulin and IGFBP3 the most responsive.

Objective: To report auxological changes after 1 yr of GH therapy and their relationship with 1 month biomarker changes.

Methods: Auxological measurements were taken before and after 1 yr of r-hGH therapy at approved doses in GH-treatment-naïve prepubertal children with GHD or TS. Changes were analysed by Wilcoxon-signed rank test. Relationships between yr 1 auxological changes and month 1 changes in IGF-I, IGFBP3, total-, HDL- and LDL-cholesterol, triglycerides, insulin, TSH, free T4, fasting glucose and HOMA-IR were analysed by Spearman rank correlation coefficient.

Results: The ITT population was 121 for GHD and 83 for TS. Excluding protocol deviators, the PP population consisted of 115 GHD (69 males) and 67 TS. GH dose (mcg/kg/day) was 35 for GHD and 50 for TS. Growth response is similar to previously published data.

Table 1. Auxology

| | GHD, n=115 (median) | TS, n=67 (median) |
|-----------------------------|---------------------|-------------------|
| Age at baseline, yrs | 9.8 | 9.1 |
| Height SDS at baseline | -2.1 | -2.4 |
| Mid-parental height SDS* | -0.8 | -0.1 |
| Height change to 1 yr, cm | 8.5 | 7.2 |
| Height velocity SDS at 1 yr | 2.1 | 1.9 |
| Height SDS change to 1 yr | 0.6 | 0.5 |

*n=113 for GHD

Significant correlations were found between 1 month change in IGF-I SDS or IGFBP3 SDS and 1 yr growth parameters in GHD, but not in TS. Although all other biomarkers showed a significant change at 1 month, none correlated with growth response in either GHD or TS.

Table 2. Associations

| GHD, n=109 | 1 month IGF-I SDS change r_s (p value) | 1 month IGFBP3 SDS change r_s (p value) |
|--|--|---|
| Height velocity at 1 yr, cm/yr | 0.35 (<0.01) | 0.29 (<0.01) |
| Height velocity SDS at 1 yr | 0.20 (0.04) | 0.26 (<0.01) |
| Height SDS change to 1 yr | 0.28 (<0.01) | 0.27 (<0.01) |
| Distance to target height SDS change to 1 yr (n=107) | 0.26 (<0.01) | 0.30 (<0.01) |

Conclusions: These data show that 1 month change in IGF-I or IGFBP3 is associated with 1 yr growth in GHD only, leading to potential improvements over existing pharmacoprediction models. The relationship of these observations to candidate genetic markers is being investigated.

Sources of Research Support: Merck Serono S.A. - Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany).

Disclosures: PC: Investigator, Merck & Co.; Consultant, Merck & Co.; Speaker, Merck & Co. LT: Investigator, Merck & Co.; Speaker, Merck & Co. TT: Employee, Merck & Co. CO: Employee, Merck & Co. PC: Investigator, Merck & Co.; Speaker, Merck & Co.

Nothing to Disclose: VP, AB, RR

P1-678

Clinical Heterogeneity in Patients with Beckwith-Weidemann Syndrome(BWS) Due to Hypomethylation of the KvDMR1 Locus.

D Ismail¹, R Kapoor¹, C Gilbert¹, K Morgan¹ and K Hussain¹.

¹Great Ormond Street Hosp/Inst of Child Hlth London, UK.

Introduction: Beckwith-Weidemann Syndrome (BWS) is a congenital overgrowth disorder that is clinically heterogeneous. It is due to genetic and epigenetic mechanisms affecting the balance of imprinted genes on chromosome 11p15.5. This region has two imprinted control regions, ICR1 and ICR2. ICR1 contains the genes *H19* and *IGF2* genes with *H19* being maternally expressed and *IGF2* paternally expressed. ICR2 contains the *KCNQ1*, *KCNQ1OT1*, and *CDKN1C* genes. Hypomethylation of *KvDMR1* (an intronic CpG island within the *KCNQ1* gene) on the maternal allele is the most common (about 50%) genetic abnormality observed in patients with BWS. The *KvDMR1* locus regulates multiple genes on 11p15.5. **Methods:** We present a case series of 6 consecutive patients all with BWS due to hypomethylation of *KvDMR1* but with extremely marked clinical heterogeneity. **Results:** The clinical presentation of all 6 patients ranged from mild macroglossia, umbilical hernia and no hypoglycaemia to severe obstructive macroglossia, marked hemihypertrophy, severe medically unresponsive hypoglycaemia (requiring a near total pancreatectomy) and hepatoblastoma formation. **Conclusions:** BWS due to hypomethylation of the *KvDMR1* locus shows marked clinical heterogeneity. This suggests that other factors (genetic or environmental) might be influencing the mechanism by which hypomethylation of *KvDMR1* leads to the clinical features of BWS. Further studies are required to understand the mechanism of the clinical heterogeneity in patients with BWS due to hypomethylation of *KvDMR1*.

Nothing to Disclose: DI, RK, CG, KM, KH

P1-679

rhIGF-1 Therapy for Growth Failure and IGF-1 Deficiency in Congenital Disorder of Glycosylation Ia (PMM2 Deficiency).

BS Miller MD, PhD¹, MM Duffy¹ and K Sarafoglou MD¹.

¹Univ of Minnesota Amplatz Children's Hosp Minneapolis, MN.

Background: Congenital disorders of glycosylation (CDG) are a group of rare disorders in which glycosylation required for proper protein-protein interactions and protein stability is disrupted, manifesting clinically with multiple system involvement and growth failure.(1) The insulin-like growth factor (IGF) system plays an important role in childhood growth and has been shown to be dysfunctional with low IGF-1 levels in a previous study in children with CDG type Ia (PMM2 Deficiency).(2) rhIGF-1 therapy has not been reported in patients with CDG-Ia.

Case Report: A 3 year old Caucasian male with failure to thrive was diagnosed with CDG-Ia at 5 months of age. Initially, his height and weight were < -2 standard deviation score (SDS), IGF-1: <25 ng/mL (49-289), IGFBP-3: 1.0 mcg/mL (0.9-4.3), & ALS: 1.3 mg/L (0.7-7.9). Despite an aggressive feeding schedule, with overnight g-tube supplementation, he continued to show poor growth and weight gain. At 16 months, he underwent an IGF-1 generation test with growth hormone (0.1 mg/kg/day) for seven days. At baseline, the IGF-1 was 27 ng/mL (55-327) and stimulated only to 33 ng/mL. rhIGF-1 therapy was initiated at 21 months of age at 40 mcg/kg/dose twice daily and increased to 130 mcg/kg/dose. The child showed an excellent linear growth response with height increasing from -2.73 to -1.39 SDS over 22 months of rhIGF-1 therapy. IGF-1 and IGFBP-3 levels also increased.

Table 1: Growth Data

| Chronologic Age | Height (cm) | Ht SDS | Growth Velocity (cm/yr) | Weight (kg) | Weight SDS | IGF-1 (ng/mL) | IGFBP-3 (mcg/mL) |
|-----------------|-------------|--------|-------------------------|-------------|------------|---------------|------------------|
| 5 mo | 57.6 | -3.71 | | 5.0 | -3.37 | | |
| 9 mo | 63.2 | -3.32 | 20.0 | 6.1 | -3.75 | <25 | 1.1 |
| 11 mo | 67 | -3.05 | 15.8 | 6.7 | -3.86 | <25 | 1.0 |
| 1 yr 2 mo | 68.4 | -3.45 | 6.7 | 7.8 | -3.09 | 31 | |
| 1 yr 4 mo | 72.8 | -2.69 | 18.3 | 8.8 | -2.52 | 27 | 1.5 |
| 1 yr 9 mo | 75.8 | -2.73 | 8.6 | 8.8 | -3.11 | 64 | 1.0 |
| 2 yr 6 mo | 84 | -1.86 | 11.5 | 10.6 | -2.27 | 39 | 1.0 |
| 3 yr 1 mo | 89.2 | -1.72 | 8.5 | 11.0 | -2.67 | 53 | 1.2 |
| 3 yr 7 mo | 94 | -1.39 | 8.9 | 12.9 | -1.69 | 346 | 1.9 |

Interestingly, the child's protein losing enteropathy also improved while on rhIGF-1. Blood glucose levels have been monitored with only one episode of documented hypoglycemia (glucose ~60 mg/dL) during a febrile illness.

Conclusion: This is the first case report of rhIGF-1 therapy in a patient with CDG-Ia. The child had an excellent linear growth response. These results provide additional *in vivo* evidence for IGF dysfunction in CDG-Ia and suggest that rhIGF-1 may be a novel treatment for growth failure in CDG-Ia.

(1) Miller BS and HH Freeze, Rev Endo Metab Dis 2003; 4:103

(2) Miller BS et al., Clin Endocrinol 2009; 70:892

Disclosures: BSM: Consultant, Ipsen.

Nothing to Disclose: MMD, KS

P1-680

Pubertal Growth (TPG) in Adolescents with Idiopathic Growth Hormone Deficiency (GHD), Turner Syndrome (TS), Short Stature Born Small for Gestational Age (SGA) and Idiopathic Short Stature (ISS) Documents a Minor Role for GH Doses in Puberty.

MB Ranke Prof¹ and A Lindberg².

¹Children's Hosp, Univ of Tuebingen Tuebingen, Germany and ²Pfizer Endocrine Care Sollentuna, Sweden.

Context and Aims: There is still limited understanding of the factors governing TPG. The aims were to describe TPG in adolescents with GHD, TS, SGA and ISS treated with GH and to develop algorithms to predict TPG.

Patients and Methods: Patients from KIGS (Pfizer International Growth Database) as of Sept, 2009. TPG was defined as growth from the onset of puberty (PO) (Tanner stages: >B1 or testes > 3ml)-spontaneous (spPO), if pre-puberty was documented <6 months before - or induced with sex steroids (inPO) to adult height (AH). Diagnoses and analyses according to KIGS criteria (1): Idiopathic GHD (maxGH < 10 µg/L in 2 tests) (N=629; male (m) spPO=271, female (f) spPO=170, (m) inPO=81, (f) inPO=51); TS (karyotype) (N=463; spPO=91, inPO=324); SGA (weight or length at birth <-2.0 SDS) (N=59; (m) spPO=35, (f) spPO=24); ISS (based on exclusion)(N=130; (m) spPO=87, (f) spPO=43). Algorithms to predict TPG at puberty onset were developed (1). **Results:** Results [mean(SD)] of pub GH dose (mg/kg wk), age (yrs) at PO (CAPO); and TPG (cm) of patients (N see methods) and controls (CON; m=128, f =83) (2) are in Table 1.

| Diag | Pub | Male | | | Female | | |
|------|------|--------|------|-----------|--------|------|-----------|
| | | GHdose | CAPO | TPG | GHdose | CAPO | TPG |
| CON | spPO | - | 12.2 | 28.0(4.2) | - | 11.1 | 21.0(3.9) |
| GHD | spPO | 0.22 | 12.9 | 25.6(6.8) | 0.21 | 11.8 | 17.7(5.8) |
| | inPO | 0.20 | 14.8 | 19.0(8.0) | 0.21 | 14.0 | 12.1(6.2) |
| TS | spPO | | | | 0.30 | 12.6 | 11.3(6.2) |
| | inPO | | | | 0.30 | 13.5 | 9.3(4.7) |
| SGA | spPO | 0.26 | 12.9 | 23.0(6.2) | 0.25 | 11.8 | 17.3(6.3) |
| ISS | spPO | 0.24 | 12.8 | 25.0(6.2) | 0.23 | 11.7 | 18.9(5.0) |

TPG in GHD (m/f) and TS could be explained with the same 4 predictors at PO: 1) age (yrs) [neg], 2) age minus bone age [pos], 3) height minus MPH (SDS) [neg], and mean dose of GH during pub [pos]. The algorithms explained 66, 65 and 69% of the variability of TPG, - with error SDs of 4.5, 3.8 and 2.9 cm. Spontaneous and induced TPG could be explained equally. The algorithms for GHD were also applicable for SGA and ISS. Changes in GH dose induced only small changes in TPG. **Conclusions:** In GHD, TS, SGA and ISS treated with GH TPG is determined by the same anthropometrical factors at PO - age, delay of bone age over CA, distance of height to MPH. TPG depends on the conditions at the end of pre-pubertal growth. The impact of GH dose on TPG is generally small. At TPG can be predicted with a high degree of accuracy and precision. These algorithms can be utilised to optimise treatment strategies in short children treated with GH.

- (1) Growth Hormone Therapy in Pediatrics. 20 years of KIGS (2007) (Ranke MB, Price DA, Reiter EO, eds) Basel, Karger;
 (2) Buckler JM, Wild J. (1987) Longitudinal study of height and weight at adolescence. Arch Dis Child 62:1224-32

Nothing to Disclose: MBR, AL

P1-681

Influences of Growth Hormone Treatment to Psychological and Behavioral Aspects of Youths with Turner Syndrome and Their Parents in Korea.

EB Lee MD¹, MJ Park MD,PhD², B-Y Choi BA³, K-M Chung PhD³, H-S Kim MD¹ and DH Kim MD¹.

¹Yonsei Univ Coll of Med, Severance Children's Hosp Seoul, Korea ; ²Sanggye Paik Hosp, Coll of Med, Inje Univ Seoul, Korea and ³Yonsei Univ Seoul, Korea.

Context: Difficulties with psychological adjustment and learning problems have also been associated with Turner syndrome (TS), but few studies have investigated the effects of growth hormone(GH) treatment on the psychological aspects of youths with TS and their parents.

Objective: To explore the influences of the GH treatment on the psychological and behavioral aspects of youths with TS and their parents.

Design: This study examined psychological and behavioral aspects of 39 Korean youths with TS, 10 to 18 years of age(mean 13.5 years), and their parents. The informations about clinical characteristics and hormonal treatment were obtained from medical records. Mean duration of GH treatment was 63.9±30.7 months. Korean Child Behavior Checklist(KCBCL; reported by a parent), the Pediatric Quality of Life inventory(PedsQL; reported by both a child and a parent), Self-Concept Inventory(SCI), Child Depression Inventory(CDI) and Revised Children's Manifest Anxiety Scale(RCMAS) were used for patients , and the Parenting Stress Index-Short Form(PSI-SF), Satisfaction with Life Scale(SWLS) and Beck Depression Inventory(BDI) were used for their parents. Spearman's correlation and Mann-Whitney U test were conducted.

Results: Duration of GH treatment was negatively correlated with social immaturity subscales of KCBCL and positively correlated with self-concept on mathematics. The scores on attention problems, internalizing and externalizing problems and total CBCL problems were higher in non-GH treatment group than in GH treatment group. Duration of disease was positively correlated with BDI and negatively correlated with SWLS and PedsQL by parents. The self-concepts related to physical ability, appearance, emotion, general self and total self-concept were significantly lower in patients with other associated problems such as cardiac anomaly, hyper- or hypothyroidism.

Conclusion: GH treatment is helpful to reduce the behavioral problems and to improve self-concept on mathematics in youths with TS. Long-term and serial assessment of the effect of GH treatment on psychological and behavioral aspects is needed. Furthermore, assessment of the effects of GH on real mathematics performance such as numeration, geometry, addition, and measurement in youths with Turner syndrome might be encouraging for Turner syndrome patients with mathematics learning disabilities.

Nothing to Disclose: EBL, MJP, B-YC, K-MC, H-SK, DHK

P1-682

Factors Influencing Growth Response to Growth Hormone Therapy in Prepubertal Patients with Chronic Kidney Disease.

JH Kim M.D.¹, SH Lee M.D.¹, YA Lee M.D.¹, MJ Kang M.D.¹, HH Lim M.D.¹, IS Yoon M.D.¹, SW Yang M.D.¹ and CH Shin M.D.¹.

¹Coll of Med, Seoul Natl Univ Seoul, Republic of Korea.

Purpose: The aim of this study was to evaluate the factors affecting growth-promoting effect to rhGH therapy in growth-retarded prepubertal patients with CKD.

Subjects and Methods: This study included 36 patients, who were prepubertal during the rhGH therapy. Inclusion criteria were as follows: patients on dialysis or conservatively treated patients with glomerular filtration rate of <60 ml/min per 1.73 m²; height of <-2 standard deviation score (SDS) or height velocity (HV) of <4 cm/yr. All patients were treated with rhGH up to 3 years.

Results: Among patients, 23 were on conservative treatment, and 13 were on dialysis at initiation of rhGH treatment. All patients were treated with rhGH at least for a year, 21 for 2 years, and 12 for 3 years.

Table 1. Effects of 1 to 3 years of rhGH treatment on height SDS, height SDS difference and height velocity in prepubertal patients with CKD analyzed by repeated measures ANOVA

| | n | rhGH treatment | | | |
|-------------------------|----|----------------|-----------|-----------|------------|
| | | baseline | 1 yr | 2 yr | 3 yr |
| Height SDS | 36 | -2.7±0.9a | -2.1±1.0b | | |
| | 21 | -2.6±0.7a | -2.0±0.9b | -1.6±1.0c | |
| | 12 | -2.7±0.9a | -2.1±1.0b | -1.8±1.1c | -1.6±1.1d |
| Height SDS difference | 36 | -0.4±0.6a | 0.6±0.4b | | |
| | 21 | -0.4±0.6a | 0.7±0.4b | 0.3±0.4c | |
| | 12 | -0.1±0.5a | 0.7±0.4b | 0.3±0.4c | 0.2±0.3a,c |
| Height Velocity (cm/yr) | 36 | 4.5±1.9a | 8.0±1.9b | | |
| | 21 | 4.5±1.9a | 8.7±1.9b | 7.0±1.7c | |
| | 12 | 4.6±2.3a | 8.3±1.5b | 6.5±1.7c | 6.0±1.3a,c |

Values not sharing superscript a,b,c letters are significantly different from the values of other time points within each row.

rhGH response was positively correlated with the interval between CRF diagnosis and baseline in the first year of therapy and was better in conservative treatment group in the second year. In conservatively treated patients, predicted adult height (PAH) SDS was -1.6±0.7 before rhGH treatment and -0.9±0.7 after rhGH treatment (P<0.001); in patients on dialysis, PAH SDS before and after rhGH treatment was -1.8±1.6 and -1.1±1.5, respectively (P=0.073). However, PAH SDS of all subjects was increased significantly from -1.7±1.1 to -1.0±1.1 after rhGH treatment (P<0.001). **Conclusion:** In patients with CKD, rhGH treatment significantly increased height SDS, difference in height SDS, HV and PAH SDS in the short term. Long-term follow-up is to be warranted to elucidate the effect of rhGH to the final height.

Nothing to Disclose: JHK, SHL, YAL, MJK, HHL, ISY, SWY, CHS

P1-683

Long-Term Growth Hormone (GH) Therapy and Early Starting Age Are Associated with Better Height Outcome in Children with Turner Syndrome: Results from the ANSWER Program®.

Judith Ross MD¹, Robert Gut MD, PhD², John Germak MD² and Peter Lee MD, PhD³.

¹Thomas Jefferson Univ duPont Hosp for Children Philadelphia, PA ; ²Novo Nordisk Inc Princeton, NJ and ³Penn State Coll of Med Hershey, PA.

Since 2002, the American Norditropin Studies: Web-Enabled Research (ANSWER) Program® has collected clinical data on patients receiving Norditropin® (somatropin rDNA origin) at the discretion of the physicians. The purpose of this analysis was to compare the change in height standard deviation score (HSDS) in response to short-term vs. long-term GH therapy in patients with Turner Syndrome (TS) using data from the ANSWER Program®.

By November 2009, 382 GH treatment naïve girls with TS have been enrolled in the program (mean±SD pretreatment age=8.6±4.0 yrs, bone age=8.0±3.5 yrs, HSDS=-2.6±0.9). Mean treatment duration was 2.4±2.0 yrs. As shown in Figure 1, patients treated with GH for 3 yrs (n=114) reached a mean HSDS of -1.72 (HSDS > -2 in 60.0% of patients), whereas patients treated for 1 yr (n=246) reached a mean HSDS of -2.15 (HSDS > -2 in 39.9% of patients). When the patient population was stratified by baseline age into 4 quartiles, patients starting therapy at a younger age demonstrated a greater HSDS gain (DHSDS) than older patients during treatment for 1 to > 3 years (Table 1). For all quartiles, correlation analysis showed that DHSDS at 4 months and at one year predicted the DHSDS at 3 years (correlation coefficients range from 0.79 to 0.94, p<0.002).

In conclusion, girls with TS treated with GH for ≥3 years achieved a moderate increase in HSDS, resulting in the majority of girls reaching normalized height (HSDS>-2). Younger starting age is associated with better growth treatment response. Short-term treatment response is predictive of longer-term height outcome in girls with TS.

Figure 1: HSDS Change over Time (Cross-sectional Data)

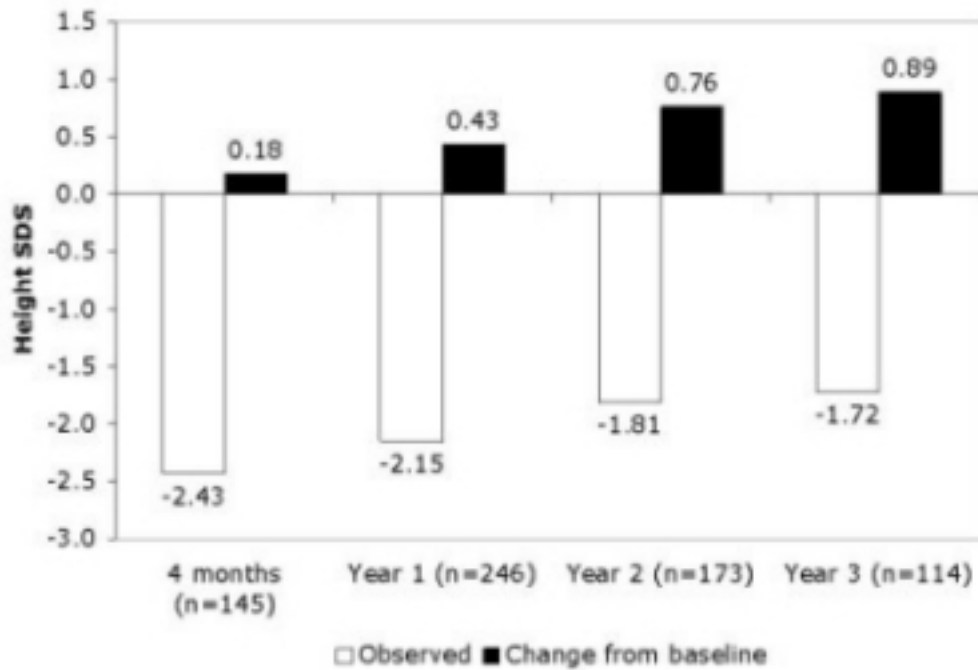


Table 1. Percentage of Patients Reaching HSDS > -2

| Baseline age | Mean HSDS, [HSDS>-2, N (%)] | | | |
|--------------|-----------------------------|--------------------|--------------------|--------------------|
| | Baseline | Year 1 | Year 3 | Year 3+ |
| <5.4 | -2.35, [31 (31.6)] | -1.65, [37 (63.8)] | -1.22, [21 (77.8)] | -1.48, [25 (69.4)] |
| 5.4 to <9.0 | -2.55, [24 (25.3)] | -2.01, [26 (41.9)] | -1.45, [20 (74.1)] | -1.58, [26 (70.3)] |
| 9.0 to <11.7 | -2.62, [20 (22.2)] | -2.39, [19 (31.2)] | -2.20, [13 (39.4)] | -1.96, [16 (43.2)] |
| ≥11.7 | -2.82, [16 (16.2)] | -2.49, [17 (25.4)] | -1.92, [15 (53.6)] | -1.68, [19 (63.3)] |

Disclosures: JR: Consultant, Novo Nordisk. RG: Employee, Novo Nordisk. JG: Employee, Novo Nordisk. PL: Consultant,

P1-684

Treatment Response of Growth Hormone (GH) in Children with Noonan Syndrome: Results from the ANSWER Program®.

Peter Lee MD, PhD¹, Judith Ross MD², John Germak MD³ and Robert Gut MD, PhD³.

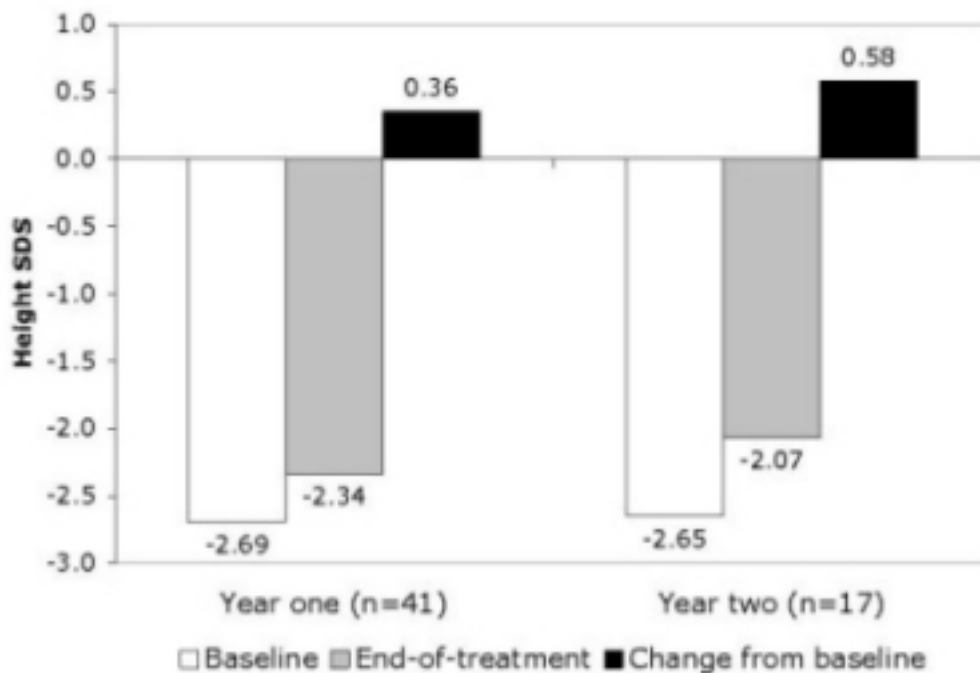
¹Penn State Coll of Med Hershey, PA ; ²Thomas Jefferson Univ duPont Hosp for Children Philadelphia, PA and ³Novo Nordisk Inc Princeton, NJ.

Noonan syndrome (NS) is a genetic disorder characterized by phenotypic features, including facial dysmorphology, cardiovascular anomalies, and short stature. It is estimated that NS affects 1: 1000 to 1: 2500 live births. In 2007, the US Food and Drug Administration approved the use of growth hormone for short stature in children with NS. Since 2002, the American Norditropin Studies: Web-Enabled Research (ANSWER) Program® has collected clinical data on patients receiving Norditropin® (somatotropin rDNA origin) at the discretion of the physicians. The purpose of this analysis was to evaluate the change in height standard deviation score (HSDS) in response to treatment with GH in patients with NS using data from the ANSWER Program®.

As of November 2009, 91 children with NS have been enrolled in the program: 79 (58 boys, 21 girls) being naïve to GH therapy. The 79 GH naïve patients had a mean±SD age=9.6±3.8 yrs, bone age=8.0±3.8 yrs, HSDS=-2.7±0.7, IGF-I SDS=-2.8±1.27, and peak GH=10.8±9.1 ng/mL. The mean GH dose, with a median treatment duration of 405 days, was 49.9 mcg/kg/day. Longitudinal data from 41 patients completing one year of treatment showed that, from baseline to Year 1, mean height velocity was 7.5±2.3 cm/year, mean HSDS increased from -2.69 to -2.34 (change = 0.36) and mean daily GH dose increased from 46 to 52 mcg/kg/day. During the first year, the change in HSDS was negatively correlated with age at treatment start (correlation coefficient R=-0.398, p=0.01). In seventeen patients who completed two years of treatment, mean HSDS at Year 2 was -2.07 (increase of 0.58 from baseline). The mean dose at end of Year 2 was 60 mcg/kg/day. A negative correlation between baseline age and HSDS change at two years was also observed (R=-0.608, p=0.0096).

In conclusion, patients with Noonan syndrome achieved a moderate increase in height SDS during the first two years of GH therapy. Younger starting age is correlated with better treatment response. Adjustment of GH dose, based on individual treatment response, may be necessary to achieve optimal growth.

Figure 1. HSDS Change over Time



Disclosures: PL: Consultant, Novo Nordisk; Researcher, Novo Nordisk. JR: Consultant, Novo Nordisk. JG: Employee, Novo Nordisk. RG: Employee, Novo Nordisk.

P1-685

GeNeSIS in Italy: Baseline Features of the Population and Preliminary Data on Outcomes.

L Tauchmanova¹, B Predieri², A Cicognani³, M Cappa⁴, F De Luca⁵, S Vanelli⁶, G Bona⁷, MG D'Avanzo⁸, S Loche⁹ and D Valle¹.

¹Eli Lilly Italia Sesto Fiorentino, Italy ; ²AO PoliClino Modena, Italy ; ³PoliClino S Orsola Malpighi Bologna, Italy ; ⁴Ospedale Pediatrico Bambino Gesù Rome, Italy ; ⁵Azienda PoliClino Univ Messina, Italy ; ⁶Ospedale Infantile Regina Margherita Torino, Italy ; ⁷Azienda Ospedaliera Maggiore di Novara Novara, Italy ; ⁸Ospedale SG Moscati Avellino, Italy and ⁹ Ospedale Regionale per le Microcitemie Cagliari, Italy.

Background GeNeSIS (B9R-IT-GDFC) is an observational, open-label, international multicenter study aiming at characterization of genetic defects associated with hypopituitarism, growth disorders or short stature, and their management.

Objective Here are presented the preliminary data of the Italian population.

Patients & methods Enrolment started in 2005; up to September 2008, 370 Italian children (151 F, 218 M, 1 unknown) were enrolled in 43 centers, most of them (n=305) presented with growth hormone deficiency (GHD, 232 idiopathic and 73 organic), 26 patients had Turner syndrome (TS), other or missing diagnosis were reported in 39 children. In GHD patients, the mean chronological age was 8.3 ± 4.1 yrs and mean height SDS was -2.55 ± 1.15 SD; the gap between chronological and bone age was -1.85 ± 1.3 yrs. Mean age of girls with TS was 8.2 ± 3.1 yrs. Most of the patients (n=248) were naïve to GH treatment. At baseline, a complete or partial endocrine workup was performed in about 1/3 of patients and mostly included thyroid hormones, prolactin, DHEAS, sex hormones and gonadotropins. IGF-1 levels were included in the database only for 181 children and IGFBP-3 for 56.

Results Patients received GH treatment at the dose of 0.23-0.32 mg/kg/weekly. In patients with GHD, the height velocity was 8.60 ± 1.95 cm/yr during the 1st year and 7.38 ± 1.67 cm/yr during the 2nd year. Height SDS were -2.21 ± 0.98 , -1.77 ± 1.00 and -1.54 ± 1.00 at baseline, after 1 and 2 years of treatment, respectively. IGF-I SDS were -2.44 ± 2.29 at baseline and -0.81 after 2 years. Among 10 patients with serious adverse events, only one was treatment-related (hypoglycemia); 48 patients discontinued treatment, reasons included final height achievement (n=5), patient/parent decision (n=3); lost to follow up (n=4), death (n=1, unrelated to treatment) and other reasons (n=35).

Among 41 patients eligible for the DNA sub-study, 33 had isolated GHD and 8 multiple pituitary hormone deficiency (MPHD). Mutations were revealed in 2 patients with MPHD, in the LHX3 and PROP1 genes.

Conclusion 370 patients with short stature enrolled during 5 years in GeNeSIS in Italy represent the largest national population ever evaluated for short stature and related treatments; GH treatment appeared efficacious and safe. Future detailed analyses of the Italian patient data in GeNeSIS should provide important data on the etiology of short stature, and safety and efficacy of GH treatment in common clinical practice in Italy.

Sources of Research Support: Eli Lilly Italia.

Disclosures: LT: Employer, Lilly USA, LLC. DV: Employee, Lilly USA, LLC.

Nothing to Disclose: BP, AC, MC, FDL, SV, GB, MGD, SL

P1-686

A Retrospective Review of Pituitary MRI Findings in Pediatric Patients on Growth Hormone Therapy.

SL Tsai MD, FRCPC¹, E Laffan MD² and S Lawrence MD, FRCPC¹.

¹Children's Hosp of Eastern Ontario Ottawa, Canada and ²The Children's Univ Hosp Dublin, Ireland.

Introduction[bold/]: Magnetic resonance imaging (MRI) is an important tool for delineating pituitary gland anatomy. Previous reports have shown that patients with multiple pituitary hormone deficiencies (MPHD) have more pituitary anatomy abnormalities compared to those with isolated growth hormone deficiency (IGHD). Correct identification of the classic triad (interrupted or thin pituitary stalk, absent or ectopic posterior pituitary and anterior pituitary hypoplasia) and its variants has important clinical implications.

Methods/Results[bold/]: The MRI findings in 55 pediatric patients, who were diagnosed with growth hormone deficiency from 1988 to January, 2010 were reviewed. Nine patients had MPHD, 44 had IGHD, and 2 patients were treated with growth hormone for other causes (β -thalassemia major and histiocytosis X). Fifteen patients (27%) had the classic triad. Of the 9 patients with MPHD, 7 had the classic triad. The two remaining patients with MPHD had a normal pituitary and two elements of the classic triad, respectively. Of the 44 patients with IGHD, 9 had the classic triad, 8 had varying degrees of pituitary anatomy abnormalities, and 27 had normal MRI findings. The patients with β -thalassemia major and histiocytosis X had one or two elements of the classic triad, respectively.

The MRI images were reviewed by an expert neuroradiologist, and the results were compared to the findings from previous reports. There were discrepancies found with the original reports in 12/55 cases. The discrepancies ranged from inappropriate identification of a pituitary microadenoma (n=4) to misidentification of one or more elements of the classic triad (n=8).

Conclusions[bold/]: The number of patients identified as having abnormal pituitary anatomy in this study is consistent with current literature. Imaging of the pituitary gland is an important clinical tool, as those with the classic triad are at higher risk of developing MPHD, and therefore, should be screened more closely. The level of discrepancy between the initial report by a non-neuroradiologist and that of an expert neuroradiologist is important to note, as this can have important clinical implications for patients and their families.

Nothing to Disclose: SLT, EL, SL

P1-687

1st and 2nd Year Response to Growth Hormone Treatment for Turner Syndrome in Australia (OZGROW).

IP Hughes¹, C Choong², A Cotterill³, M Harris³, W Cutfield⁴, P Hofman⁴, C Cowell⁵, G Ambler⁵, G Werther⁶ and PSW Davies¹.

¹The Univ of Queensland Herston, Australia ; ²Princess Margaret Hosp for Children Subiaco, Australia ; ³Mater Children's Hosp South Brisbane, Australia ; ⁴Univ of Auckland Auckland, New Zealand ; ⁵The Children's Hosp at Westmead Westmead, Australia and ⁶Royal Children's Hosp Parkville, Australia.

Background: Short stature is a common phenotype of Turner Syndrome (TS) and is due in part to SHOX gene haploinsufficiency. GH treatment improves growth and final height (Ht). Here we examine factors affecting 1sty and 2ndy growth response to GH treatment in Australian girls with TS.

Methods: All TS patients who completed 1y (n=198) or 2y (n=159) of treatment and were receiving GH as of 12.03.07 were included. Change in Ht-SDS (Δ SDS 1sty and Δ SDS 2ndy) was the outcome variable used in stepwise multiple regression analysis with predictor variables Age, Ht-SDS, and BMI at start of treatment, mean dose (mg/m²/wk), mean parental Ht-SDS, and Δ SDS 1sty (for Δ SDS 2ndy).

Results: Median age at start was 5.8y. Mean doses (mg/m²/wk) fell into 3 distinct ranges, Low (4.00-5.49), Middle (5.50-7.99), and High (8.00-9.99). Median mean dose 1sty: Low: 4.6 mg/m²/wk (0.19mg/kg/wk), Middle: 6.7(0.29), High: 8.9(0.34), Total: 5.4(0.22). 2ndy: Low: 4.7(0.19), Middle: 6.8(0.27), High: 8.7(0.32), Total: 6.5(0.26). Numbers of patients in each dose range changed from 1sty to 2ndy mainly following a dose increment. Low: 84 to 58, Middle: 34 to 63, and High: 41 to 38. Median Δ SDS 1sty = +0.440, Δ SDS 2ndy = +0.193. The regression model for Δ SDS 1sty = -0.156(Ht-SDS) -0.005(Age)+0.058(BMI)+0.047(Mean Dose), R²=0.195. For 2ndy response, no predictor variables significantly influenced Δ SDS 2ndy.

Summary: 1sty Δ SDS in Australia (0.44) is comparable to published studies (0.3-0.6) but notably applies a lower dose (0.22 cf. 0.23-0.36 mg/kg/wk) and an earlier age (5.8y cf. 6.5-11.9y) at initiation of therapy. Ht-SDS and Age were the most important factors influencing 1sty response with younger, shorter TS girls with a higher BMI on a higher dose most likely to have the best response. Response waned in the 2ndy and no variables predicted 2ndy response.

Conclusions: Growth response to GH in TS and sensitivity to dose level is greatest in the 1sty. These data support starting GH early, with the highest dose allowable from onset as optimal treatment of short stature in TS. Dose increments in the 2ndy may have limited effectiveness in improving growth response.

Nothing to Disclose: IPH, CC, AC, MH, WC, PH, CC, GA, GW, PSWD

P1-688

A Randomized Trial of Growth Hormone in Children with Optic Nerve Hypoplasia (ONH), Growth Hormone Deficiency (GHD), and Normal Height Velocity: One-Year Growth and Body Composition Outcomes.

AM Vedin M.D.¹, CA Fink M.P.H.¹, P Garcia-Filion M.P.H.^{1,2}, MS Borchert M.D.^{1,3} and ME Geffner M.D.^{1,3}.

¹USC Keck Sch of Med and Childrens Hosp Los Angeles Los Angeles, CA ; ²USC Keck Sch of Med Los Angeles, CA and ³ Childrens Hosp Los Angeles Los Angeles, CA.

Background: Some children with ONH and GHD have normal height (ht) velocity, a possible example of the “growth without GH” paradox.

Objective: To identify how GH affects growth/body composition in children with ONH and normal growth despite GHD.

Subjects/Methods: From a prospective study of 236 children with ONH, 12 <5 y of age with GHD (GH <10 ng/mL after glucagon) were enrolled. Subjects were randomized to either GH [0.3 mg/kg/wk (n=7)] or observation (n=5) and were seen every 4 mo for ht, weight (wt), and %body fat (BF) [by bioelectrical impedance analysis (BIA)]. Data were

non-normally distributed and are presented as median; 5th, 95th %iles. To compare baseline and 1-y data, non-parametric tests for between (2-sample) and within (paired) groups were performed.

Results: There was no difference in initial (i) ht SDS, wt/ht ratio, or %BF between GH and control subjects. Final ht SDS increased significantly in GH group, but not in controls; there was a significant difference in Δht SDS between GH and controls. There was no difference in Δwt/ht ratio in either group; however, at 1 y, a trend toward lower %BF was observed in GH vs control group.

Results

| Parameter | GH-treated Median | GH-treated 5th, 95th %ile | Control Median | Control 5th, 95 %ile | P-value |
|-------------------|-------------------|---------------------------|----------------|----------------------|---------|
| Age (mo) at start | 31 | 24, 61 | 23 | 18, 59 | 0.122 |
| Peak GH (ng/mL) | 2.3 | 0.8, 7.1 | 2.5 | 1.4, 9.3 | 0.570 |
| iht SDS | -1.94 | -3.0, 0.08 | -1.67 | -2.8, 1.2 | 0.465 |
| iwt SDS | -0.57 | -2.81, 1.49 | -1.36 | -2.97, 1.41 | 0.808 |
| iwt/ht ratio | 0.29 | -1.2, 2.56 | 0.82 | -1.69, 1.55 | 0.75 |
| i%BF | 13.1 | 3, 28.8 | 17.1 | 3.9, 20.7 | 0.222 |
| Ht SDS 1 y | -0.13 | -1.3, 1.5 | -2.14 | -3.3, 0.53 | 0.061 |
| ΔHt SDS 1 y | 1.6 | 0.99, 2.31 | -0.47 | -1.39, 0.33 | 0.005 |
| Wt SDS 1 y | 0.22 | -1.7, 2.7 | -0.85 | -3.28, 0.89 | 0.123 |
| ΔWt SDS 1 y | 1.07 | 0.42, 1.98 | -0.31 | -0.62, 0.6 | 0.019 |
| Wt/ht ratio 1 y | 0.41 | -1.44, 2.33 | 0.75 | -1.24, 1.28 | 0.935 |
| ΔWt/ht ratio 1 y | -0.04 | -0.62, 0.5 | 0.21 | -0.77, 0.46 | 0.935 |
| %BF 1 y | 8.7 | 3, 25.5 | 19.7 | 12, 20.7 | 0.08 |
| Δ%BF 1 y | -3.3 | -11.3, 6.3 | 3.4 | -8.7, 8.2 | 0.465 |

Conclusions: Children with ONH, GHD, and normal ht velocity grow faster when treated with GH for 1 y, but have a tendency toward decreased ht velocity when left untreated. Wt/ht ratio after 1 y was uninfluenced by GH, although there was a trend toward lower %BF with GH treatment.

Disclosures: MEG: Principal Investigator, Pfizer, Inc.; Poster Reviewer, Pfizer, Inc.; Scientific Board Member, Pfizer, Inc.; Principal Investigator, Pfizer, Inc.

Nothing to Disclose: AMV, CAF, PG-F, MSB

P1-689

A Novel Missense Variant in the *Necdin* Gene in a Male Patient with Kallmann Syndrome.

D. Beneduzzi Undergraduate¹, AP. Silveira-Neto Student¹, LFG. Silveira MD¹, E. Trarbach PhD¹, BB. Mendonca MD¹ and AC Latronico MD¹.

¹Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil.

Context: Congenital isolated hypogonadotropic hypogonadism (IHH) is one the major diagnostic criteria for Prader-Willi syndrome, a complex genetic disorder caused by loss of the expression of genes located at 15q11-13 chromosomal region. The human *Necdin* gene (*NDN*), mapped to chromosome 15q11.2, was recently considered a key regulator of GnRH gene expression in rodents. Loss of *Necdin* gene results in decreased number of GnRH neurons and impairs normal GnRH neuronal migration during mice development, suggesting that this gene might contribute to hypogonadotropic hypogonadism and infertility found in Prader-Willi syndrome. We hypothesized if *NDN* might be implicated in the pathogenesis of HH not associated with Prader-Willi syndrome in humans. **Aim:** To investigate the presence of mutations in the *NDN* gene in patients with IHH. **Patients and Methods:** One hundred and six Brazilian patients with sporadic or familial IHH (80 males) were studied. Fifty seven patients had abnormal olfactory sense (Kallmann syndrome). Genomic DNA was extracted from peripheral leucocytes and the single *NDN* exon was amplified and automatically sequenced. **Results:** A new heterozygous variant, p.Val318Ala, was detected in the carboxyterminal region of the *necdin* in a 23 yr-old male with Kallmann. At 23 ys of age he had basal prepubertal gonadotropin levels and low testosterone levels (50 ng/dL). Notably, the valine at position 318 of *necdin* is a highly conserved residue among the species. Moreover, this missense variant was not found in any of the 100 Brazilian control subjects. Additionally, two previously known polymorphisms, c.11C>T and c.971C>T, were also identified in 80 and 35 patients with isolated hypogonadotropic hypogonadism, respectively. **Conclusion:** A novel missense variant in the *NDN* gene was identified in a male patient with Kallmann syndrome, suggesting a potential role for *necdin* in the genetic etiology of idiopathic hypogonadotropic hypogonadism.

Sources of Research Support: FAPESP Grants 09 / 52256-3.

Nothing to Disclose: DB, APS-N, LFGS, ET, BBM, ACL

P1-690

Familial Isolated Hypogonadotropic Hypogonadism Caused by a Novel Homozygous Mutation in a Splice Site of the GPR54 Gene.

DH Zangen M.D.¹, M Abdulhadi-Atwan M.D.^{1,2}, P Renbaum PhD³, S Zeligson M.A.³, H Fridman B.A.³ and E Levy-Lahad M.D.³.

¹Hadassah Hebrew Univ Med Ctr Jerusalem, Israel ; ²Al Yamama Hosp Bet Lehem, Israel and ³Shaare Zedek Med Ctr Jerusalem, Israel.

Background: Isolated hypogonadotropic hypogonadism (IHH) is characterized by abnormal pubertal development due to low gonadotropin levels. Kisspeptin/GPR54 gene mutations have recently become a significant etiology of familial IHH. **Methods:** Clinical, endocrine, imaging, and molecular genetic characterization was performed in three IHH patients.

Results: A 16y old Palestinian girl and her 20y old brother born to consanguineous parents presented with no pubertal development, infantile uterus and prepubertal testes, respectively. Both were normosmic and stimulated gonadotrophins were prepubertal. The female's basal estradiol levels were low(50pmol/l) and failed to rise in response to hCG. Her brain CT scan was normal. The brother's low testosterone (1.87 nmol/l) did rise in response to combined hCG and HMG (LH and FSH) therapy but the testes remained small (1-2ml). Secondary sexual characteristics were attained by exogenous sex steroids replacement. DNA was extracted from 3 affected siblings (another affected sister) and other family members. Initial homozygosity studies using microsatellite markers located in proximity to candidate genes: GnRHR, GPR54, GnRH, and Kiss1 did not yield a molecular etiology. SNP array studies thereafter revealed a small area of homozygosity at the telomeric end of chromosome 19. Sequencing the GPR54 gene revealed a novel homozygous G>A mutation at the nt -1 canonical acceptor splice site of intron 1 in all 3 affected siblings. The mother (menarche at 14y) was heterozygous, while a healthy sister and 5 normal Jerusalem Palestinians had the normal sequence. The mutation results in skipping of exon 2, a frameshift which results in an altered protein from residue 82, and a premature stop codon at residue 151(p. A82GfsX151). To assure the aberrant transcript formation we extracted RNA from transformed lymphocytes of the 3 affected siblings. Sequencing the reversed transcribed RNA of a fragment starting at exon 1 and ending at exon 3 assured that exon 2 was missing with the expected frame shift resulting in a premature stop codon.

Conclusions: A novel and first splice site (IVS1-1G>A) mutation in GPR54 results in a severe IHH phenotype with failure to exhibit any pubertal maturation. Shorter transcripts were confirmed in cDNA sequencing. Carriers of the heterozygous mutation may manifest a subtle though a fertile phenotype. The subnormal gonadal response to hCG in patients may implicate a direct effect of GPR54 on gonadal function.

Nothing to Disclose: DHZ, MA-A, PR, SZ, HF, EL-L

P1-691

Mutational Analysis of *LIN28B* Gene in Children with Idiopathic Central Precocious Puberty.

AP Silveira-Neto Student¹, D Beneduzzi Undergraduate¹, LFG Silveira MD¹, VN Brito MD¹, P Cukier MD¹, IJP Arnhold MD¹, BB Mendonca MD¹ and AC Lãtronico MD¹.

¹Fac de Med da Univ de São Paulo Sao Paulo, Brazil.

Lin-28 was originally identified in *Caenorhabditis elegans* as a heterochronic gene that controls developmental timing (1). *LIN28B* encodes potent and specific regulators of processing of the let7 microRNAs family and regulates cell pluripotency and cancer growth. Deleterious mutations in *lin-28* produce an abnormal rapid tempo of development through larval stages to adult cuticle formation in *C. elegans* (2). Recently, independent genome-wide association studies revealed common genetic markers around the *LIN28B* locus associated with the age at menarche in women (2-5). **Aim:** To identify mutations or polymorphisms in the *LIN28B* gene in a cohort of children with idiopathic central precocious puberty. **Patients and Methods:** Eighty Brazilian children (76 girls) with sporadic or familial central precocious puberty were studied. All children had pubertal signals before 8 years old, advanced bone age, pubertal levels of basal and/or GnRH-stimulated LH levels and normal central nervous system evaluated by resonance magnetic. Mutations in *KISS1* and *KISS1R* were previously rule out in these children. A group of 100 individuals who had normal pubertal development was used as controls. Genomic DNA was extracted from peripheral leukocytes and the four exons of the *LIN28B* gene were amplified and automatically sequenced. **Results:** A novel heterozygous substitution of adenine by guanine at nucleotide 596 in exon 4 of the *LIN28B* was identified, resulting in the substitution of histidine by arginine at codon 199 (His199Arg) in the carboxy-terminal region. The His199Arg mutation was identified in a 4 yr old girl who had pubertal Tanner stage 3 and pubertal LH peak after GnRH-stimulation (17 U/L). Long term follow-up till 11 yr of age showed adequate response to GnRH analogs. Her father, carrier of the same mutation, had normal pubertal developmental. The His199Arg mutation was absent in all controls. Interestingly, the His199 is a highly conserved amino acid among mammals. No other allelic variants were identified in this cohort. **Conclusion:** A novel and rare variant of the *LIN28B* gene was identified in a girl with sporadic idiopathic central precocious puberty, suggesting that this genetic abnormality could impair the regulatory function of LIN28B and consequently to determine an acceleration of the puberty development in humans.

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- (3) Perry JR et al., Nature Genetics 2009; 41:648-650
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Sources of Research Support: FAPESP Grants 05/55745-4 and 08/55953-4.

Nothing to Disclose: APS-N, DB, LFGS, VNB, PC, IJPA, BBM, ACL

P1-692

Novel C617Y Mutation in the 7th Transmembrane Segment of Luteinizing Hormone/Choriogonadotropin Receptor in a Japanese Boy with Peripheral Precocious Puberty.

N Katsumata M.D.¹, K Nagasaki M.D.², Y Ogawa M.D.² and T Kikuchi M.D.².

¹Natl Res Inst for Child Hlth & Dev Tokyo, Japan and ²Niigata Grad Univ Sch Med & Dent Sci Niigata, Japan.

Background: Familial male-limited precocious puberty (FMPP), also known as testotoxicosis, is an autosomal dominant form of gonadotropin-independent precocious puberty caused by constitutively activating heterozygous mutations of the *LHCGR* gene encoding luteinizing hormone/choriogonadotropin receptor (LH/CGR). To date, only a limited number of *LHCGR* mutations causing FMPP have been reported, and none have been described in the 7th transmembrane segment of the receptor.

Patient: The patient is an 8-year-old Japanese boy who developed pubic hair at the age of 6 years. The father is 158 cm tall, and reported having had slightly early sexual maturation. The mother of the patient is 160 cm tall, and there is no history of sexual precocity or short adult height in her family. The patient had elevated serum testosterone levels, but a GnRH stimulation test revealed a prepubertal response of gonadotropins. Serum hCG was undetectable. No tumor lesions were found in thoracic and abdominal computerized tomography. Based on these clinical and laboratory findings, the patient was diagnosed as having peripheral precocious puberty.

Genetic analysis: Sequencing of the *LHCGR* gene revealed a novel heterozygous C617Y mutation in the patient and his mother, while his father had no mutations. The C617Y mutation is located in the 7th transmembrane segment of LH/CGR. Of note, a germ line heterozygous C672Y mutation in *TSHR* encoding thyrotropin receptor (TSHR) is known to cause constitutive activation of adenylyl cyclase, and eventually non-autoimmune autosomal dominant hyperthyroidism. TSHR is highly homologous to LH/CGR, and the C672Y mutation in TSHR corresponds to the C617Y mutation in LH/CGR. Therefore, it is very likely that the C617Y mutant LH/CGR also constitutively activates adenylyl cyclase to cause FMPP.

Conclusion: This report describes the first FMPP case with an *LHCGR* mutation in 7th transmembrane segment of the receptor, and adds to the repertoire of *LHCGR* mutations in FMPP.

Nothing to Disclose: NK, KN, YO, TK

P1-693

Mutational Analysis of *TAC3* and *TACR3* Genes in Children with Idiopathic Central Precocious Puberty.

C Tusset Student¹, E Gianetti MD², EB Trarbach PhD¹, LFG Silveira MD¹, P Cukier MD¹, UB Kaiser MD³, BB Mendonca MD¹, SB Seminara MD² and AC Latronico MD¹.

¹Hosp das Clins da Fac de Med da Univ de Sao Paulo Sao Paulo, Brazil ; ²Massachusetts Gen Hosp Boston, MA and ³Brigham and Women's Hosp and Harvard Med Sch Boston, MA.

Loss-of-function mutations of the genes that encode neurokinin B (*TAC3*) and its receptor NK3R (*TACR3*) were recently related to the pathogenesis of normosmic hypogonadotropic hypogonadism in humans. Several lines of evidence suggest that neurokinin B might have a role as a regulator of GnRH secretion. Neurokinin B is known to be highly expressed in hypothalamic neurons that also express kisspeptin, a potent stimulator of GnRH secretion. Whether activating mutations in *TAC3* and *TACR3* can play a role in the pathogenesis of idiopathic central precocious puberty remains unknown. **Aim:** To determine the presence of polymorphisms and mutations in the *TAC3* and *TACR3* genes in a large cohort of children with idiopathic central precocious puberty (CPP). **Patients and Methods:** One hundred and eight patients (105 girls and 3 boys) with sporadic or familial idiopathic central precocious puberty were studied. All CPP patients initiated puberty before eight years in girls and nine years in boys and had pubertal basal and/or GnRH-stimulated LH levels, advanced bone age and normal central nervous system magnetic resonance imaging. A group of 150 Brazilian individuals who had puberty at adequate age was used as controls. Genomic DNA was extracted from peripheral blood and the entire coding region of both *TAC3* and *TACR3* genes were amplified and automatically sequenced. **Results:** A new variant (p.A63P) in the neurokinin B was identified in a girl with CPP. This variant is located out of mature protein (proneurokinin B) and the alanine at position 63 is not a conserved residue among all species. The affected girl had pubertal onset at 7 yr of age. She had advanced bone age (11 year) and pubertal stage Tanner 3. Hormonal evaluation revealed basal LH level (IFMA) 1.2 U/L, LH after acute GnRH stimulation (IFMA) 17.9 U/L, and basal estrogen level (IFMA) 35.2 pg/mL. This variant was not found in any of the control subjects as well in a large cohort of patients with isolated hypogonadotropic hypogonadism previously studied. Familial segregation analysis was not available so far. In addition, two previously known polymorphisms in the NK3R, p.K286R and p.L291L, were identified in 1 and 3 patients with CPP, respectively. **Conclusion:** A new variant (p.A63P) was identified in a Brazilian girl with idiopathic central precocious puberty. The importance of this rare missense variant in the regulation of the *TAC3* gene transcription activity remains to be established.

Sources of Research Support: FAPESP # 2005/04726-0 and CAPES.

Nothing to Disclose: CT, EG, EBT, LFGS, PC, UBK, BBM, SBS, ACL

P1-694

A Novel *GPR54* Mutation in a Patient with Gonadotropin-Dependent Precocious Puberty.

ACS Reis MD¹, AC Moreira MD¹, M Castro MD¹ and SR Antonini MD¹.

¹Sch of Med of Ribeirao Preto, Univ of Sao Paulo Ribeirao Preto, Brazil.

Introduction: Gonadotropin-dependent precocious puberty (GDPP) results of a premature activation of the hypothalamic-pituitary-gonadal axis. This condition is more frequent in girls and its etiology is largely unknown. To date, the molecular basis of GDPP has been elucidated in a single patient harboring an autosomal dominant *GPR54* activating mutation. **Objective:** To investigate the presence of *GPR54* mutations in GDPP patients. **Patients and Methods:** 93 Brazilian patients (89F/4M) with idiopathic GDPP were evaluated. Mean age at start of puberty was 5.9 ± 2 yrs (0.1 to 7.9) in girls and 8 ± 1 yrs (7 to 9) in boys. In 11 patients (12%) familiar history of GDPP was present. *GPR54* gene entire coding and boundary regions were amplified by PCR and automated sequenced. **Results:** In one patient we found the heterozygous c.109C>T substitution in exon 1 (g.270C>T), resulting in the substitution of proline to serine at codon 37 (p.P37S). This genetic variation was not found in 100 control alleles from 50 matched controls with normal pubertal development and in the 184 alleles from the remaining 92 unrelated patients with GDPP. However, further functional studies are needed. Affected patient presented premature telarche since birth in 1988 and maintained breast development Tanner stage 3 since then. No family history of GDPP was present. Breast progression was very slow and no accelerated growth (height Z score from 2 to 6 yrs: 0.32 to -0.5) or advanced bone age were presented until 7 years old. From 2.5 to 7.5 yrs both basal LH (RIA: <3.9 to 12.2 ng/mL; NR: <15) and GnRH stimulated LH (RIA: 32 to 56.6 ng/mL; NR: <65) were considered to be within prepubertal range. At 7 yrs old, accelerated growth (8.2 cm/yr) and advanced bone age (BA: 9 yrs) were noted. Basal and GnRH stimulated LH were still prepubertal (RIA: 6.1 and 32 ng/mL, respectively). She was treated initially with medroxyprogesterone acetate and further with GnRH analog until 12.1 yrs. Menarche occurred at 12.2 yrs. Final height was within her target height (Z score: 0.69). She is now 21 yrs old and is a mother of a 2 yrs old girl, who also has premature telarche since birth and is under current investigation. **Conclusion:** We identified a novel mutation in *GPR54* (p.P37S) in a patient with GDPP presenting telarche since birth, very slow pubertal progression and near normal or mild elevated LH; this phenotype is remarkable similar to the single patient harboring an activating *GPR54* mutation described to date.

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Sources of Research Support: FAPESP and CAPES - Brazil.

Nothing to Disclose: ACSR, ACM, MC, SRA

P1-695

Familial Central Precocious Puberty: Prevalence, Segregation Analysis and Penetrance Rate.

P Cukier MD¹, LFG Silveira PHD¹, PA Otto PHD², IJP Arnhold PHD¹, BB Mendonca PHD¹, P Gagliardi MD³, T Pasqualini MD⁴, D Beckers⁵, AC Latronico PHD¹ and VN Brito PHD¹.

¹HCFMUSP Sao Paulo, Brazil ; ²Inst de Biociências da Univ de São Paulo Sao Paulo, Brazil ; ³Nemours Children's Clin Jacksonville, FL ; ⁴Hosp Italiano de Buenos Aires Buenos Aires, Argentina and ⁵Pediatric Endocrinology of the Univ of Leuven Leuven, Belgium.

Background: The variation in the timing of human puberty is highly correlated within racial/ethnic population groups, families and monozygotic twins, suggesting a genetic regulation for the onset of puberty. In 2004, de Vries et al (1) reported a prevalence of 27.5% of familial cases in a cohort of 156 Israeli children with idiopathic central precocious puberty (CPP). There are only few published descriptions of familial CPP so far. Aims: To establish the prevalence, mode of inheritance and penetrance rate of familial idiopathic CPP. Patients/Methods: 64 Brazilian patients with idiopathic CPP (60 girls and 4 boys), characterized by presence of secondary sexual characteristics before age 8 yr in girls and 9 yr in boys, pubertal basal or GnRH-stimulated LH levels and normal central nervous system MRI were selected for this study. Familial cases were diagnosed based on systematic anamnesis identifying at least one first-, second- or third-degree relative with a history of menarche before chronological age of 10 yr or, in males, history of shaving or full puberty before age 13 yr. Pedigrees composed by two or three generations were constructed from all families. A statistical method developed in the Department of Genetics of the Bioscience Institute of University of Sao Paulo was used to calculate the penetrance rate (k) of CPP in families with more than one affected member. For segregation analysis and penetrance rate estimation, three families, one from Belgium, one from USA and one from Argentina were also included. Therefore, the segregation analysis and penetrance rate were performed in a total of 14 families. Pre-pubertal and non-informative individuals were excluded from statistical analysis. Results: Of the 64 Brazilian patients, 11 children (10 girls and 1 boy) met the criteria for familial CPP, representing 18% from the Brazilian cohort. Autosomal dominant pattern of inheritance was observed in 9 pedigrees. In the other five, the mode of inheritance could not be determined. The calculated penetrance rate of familial CPP was 72% (CI 95%: 58 - 83%) in this cohort. Conclusions: The prevalence of familial CPP was 18% in the Brazilian cohort. Segregation analysis suggested autosomal dominant inheritance as the main pattern with incomplete penetrance, confirming previous data. When evaluating children with idiopathic CPP, a careful clinical investigation of other cases of CPP in the family is highly recommended, considering the potential genetic etiology.

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Sources of Research Support: FAPESP 06/56531-0, 06/52583-6.

Nothing to Disclose: PC, LFGS, PAO, IJPA, BBM, PG, TP, DB, ACL, VNB

P1-696

Allelic Variants of Enhanced at Puberty Gene (EAP1) Are Not Associated with Human Idiopathic Central Pubertal Disorders.

P Cukier MD¹, LFG Silveira PHD¹, MG Teles PHD¹, IJP Arnhold PHD¹, BB Mendonca PHD¹, AC Latronico PHD¹ and VN Brito PHD¹.

¹HCFMUSP Sao Paulo, Brazil.

Background: Gonadotropin-releasing hormone (GnRH) secretion is under a complex regulatory system that includes excitatory and inhibitory transsynaptic, as well as glial inputs. GnRH pulsatile secretion is essential for the pubertal development. A new regulatory transcription factor named enhanced at puberty (EAP1), previously known as C14orf4, is consistently more expressed at the time of puberty in the hypothalamic areas implicated with puberty onset in primates. EAP1 transactivates the GnRH promoter and represses the preproenkephalin promoter. Mice with silencing of EAP1 have delayed puberty, reduced reproductive capacity and short reproductive span. To date, no molecular analysis of EAP1 was performed in patients with central pubertal disorders. Aim: To identify molecular defects in EAP1 gene in a selected multicentric cohort of patients with idiopathic central pubertal disorders, including isolated hypogonadotropic hypogonadism (IHH) and central precocious puberty (CPP). Patients/Methods: 69 patients with idiopathic CPP (65 girls; 4 boys) and 47 patients (13 females; 34 males) with IHH were selected. CPP was diagnosed when pubertal signs appeared before 8 yr in girls and 9 yr in boys, associated to pubertal LH response and normal hypothalamic-pituitary MRI. IHH was diagnosed based on clinical signs of hypogonadism, prepubertal or low testosterone or estradiol levels for age, low or inappropriately normal gonadotropin levels, normal anterior pituitary function, absence of anosmia and normal hypothalamic-pituitary MRI. A control group with 50 individuals was included. Genomic DNA was extracted from peripheral leukocytes followed by amplification and automatic sequencing of the intronless EAP1 gene. Genotypic frequency was compared by chi-square test. Statistical significance was set at $p < 0.05$. Results: A novel silent variant p.C758C and eight known single nucleotide polymorphisms (SNPs): p.E87E, p.Q107Q, p.Q108del, p.Q109Q, p.Q114Q, p.Q116Q, p.A160del, p.A163A were identified in EAP1. These SNPs were identified in patients with idiopathic CPP and IHH. The genotypic frequency of all polymorphisms was not statistically different between the two groups or between patients and controls, when studying p.C758C variant ($p > 0.05$). Conclusion: Allelic variants in the EAP1 gene are not associated with central idiopathic pubertal disorders. However, involvement of somatic defects and/or expression abnormalities of EAP1 cannot be excluded by this study.

Sources of Research Support: FAPESP 06/56531-0, 06/52583-6.

Nothing to Disclose: PC, LFGS, MGT, IJPA, BBM, ACL, VNB

P1-697

Age at Menarche in Contemporary US Adolescents.

PA Lee MD, PhD¹, JW Frane PhD² and GM Bright MD³.

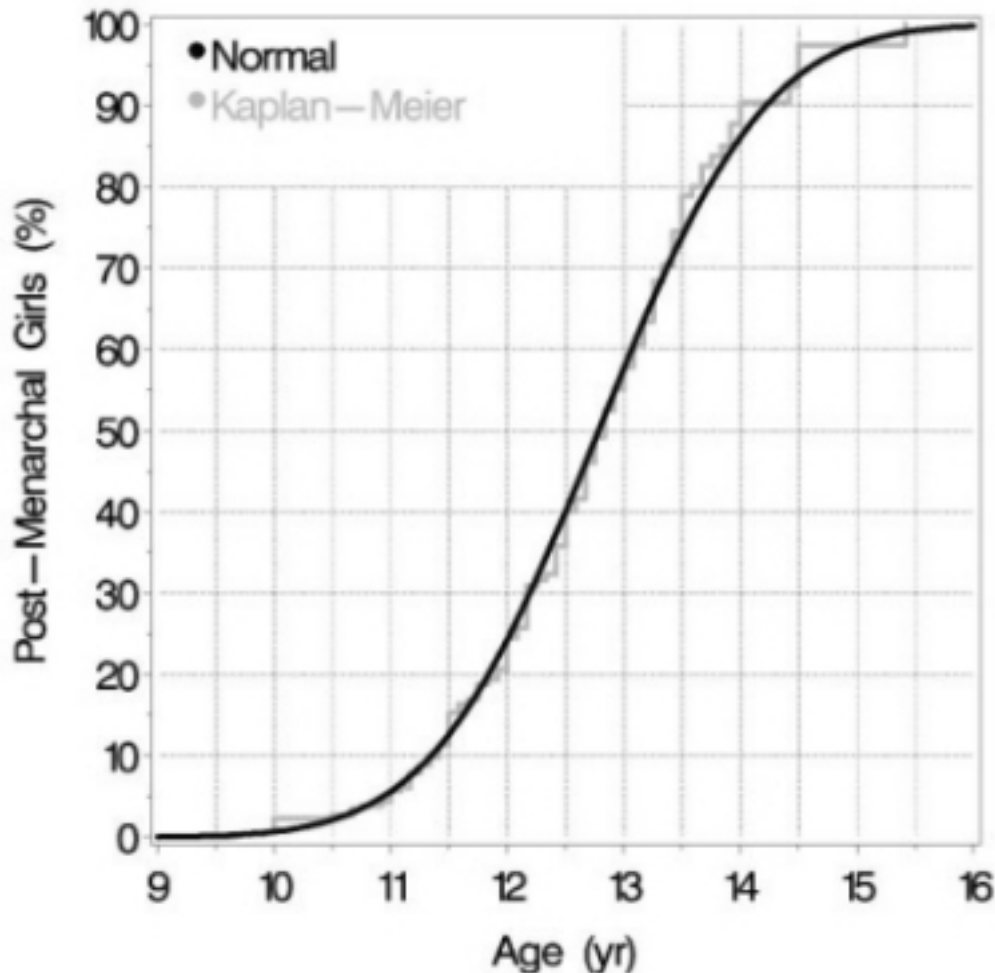
¹Penn State Coll of Med, The Milton S Hershey Med Ctr Hershey, PA ; ²Consultant Santa Monica, CA and ³Ipsen, US Brisbane, CA.

Introduction: A secular trend towards a younger age of menarche has occurred for over a century, although there have been few representative studies. This decline in age of menarche in the first half of the 20th century was 2 to 2.5 years. Since then, the mean reported age at menarche from the most representative North American surveys has remained fairly stable, although racial/ethnic differences have been documented (1-3). This study estimated the age at menarche in contemporary US adolescents.

Methods: 610 healthy females, ages 3.0 to 17.9 years (mean age, 11.4 years), from Arizona, Florida and Ohio, received physical examinations including Tanner staging. Date of menarche was established by the subject and parent. The statistical distribution of age at menarche was estimated using a normal time-to-event model and confirmed as appropriate by comparison with a Kaplan-Meier curve.

Results: 204 subjects reported menarche (age range at menarche, 9.0-15.4 years). There was no difference between sites in the mean age at menarche ($p=0.47$). African Americans experienced menarche an average of 0.6 years earlier than Non-Hispanic Caucasians ($p=0.0009$), however, there was no significant difference between Hispanics and Non-Hispanic Caucasians ($p=0.32$). The Kaplan-Meier ("step curve") and smoothed time-to-event normal curve for non-Hispanic Caucasians ($n=420$, 129 post-menarchal) are shown in Figure 1.

Figure 1. Age at Menarche for non-Hispanic Caucasians



The 3rd, 50th and 97th percentiles (SE) for age at menarche in this population were 10.7 (0.12), 12.8 (0.09) and 14.9 (0.17).

Discussion: The mean age at menarche for this cohort was similar to previous studies of white adolescents (12.6-12.88 years) (1-3). These data are in contrast to the secular trend for earlier age for breast development reported in this time period and suggest that the duration of pubertal maturation may have increased. There is no evidence for a secular trend in age at menarche in US non-Hispanic Caucasians in the past 5 decades.

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Sources of Research Support: Ipsen, US.

Disclosures: PAL: Consultant, Abbott Laboratories, Novo Nordisk; Coinvestigator, Abbott Laboratories; Clinical Researcher, Lilly USA, LLC, Novo Nordisk, Pfizer, Inc., Ipsen. JWF: Consultant, Ipsen, Genentech, Inc. GMB: Employee, Ipsen.

P1-698

Efficacy and Safety of Leuprolide Acetate 3 Month Depot 11.25 or 30 mg for the Treatment of Central Precocious Puberty.

PA Lee MD¹, K Klein MD², N Murras MD³, D Yang MS⁴, C Mattia-Goldberg MS⁴ and K Chwalisz MD, PhD⁴.

¹Penn State Coll of Med Hershey, PA ; ²Univ of California San Diego, CA ; ³Nemours Children's Hosp Jacksonville, FL and ⁴Abbott Labs Abbott Park, IL.

Introduction: Gonadotropin releasing hormone analogs (GnRHa) are the primary treatment for central precocious puberty (CPP). We evaluated the safety and efficacy of leuprolide acetate 3 month depot (LD) 11.25 mg and 30 mg in children with a clinical diagnosis of CPP.

Methods: This phase 3, randomized, open-label, 6 month study evaluated LD 11.25 mg (N=42) and 30 mg (N=42) formulations. Per treatment arm, 21 subjects were naïve to GnRHa treatment and 21 were previously GnRHa treated (pt). Subjects received 2 injections of either LD 11.25 mg or 30 mg IM at 3-month intervals. The primary endpoint was the percentage of subjects with suppression of peak stimulated LH (<4 IU/L) from month 2 through 6 using a life table method. LH was measured using ICMA and sex steroids (E₂ in females and T in males) were measured using HPLC Tandem Mass Spectrometry.

Results: 84 subjects were randomized, 76 female, mean age: 7.8 years (7.4 years naïve and 8.2 years pt). Mean±SD BMI was 19.7±3.5 kg/m² for females, 21.1±4.3 kg/m² for males. Peak stimulated LH levels were suppressed from month 2 through 6 in 78.1% of subjects in the 11.25 mg group and 95.2% of subjects in the 30 mg group (Table 1). 9 subjects in the 11.25 mg group were treatment failures (7 failed to be suppressed by month 2 and 2 escaped). In the 30 mg group, 2 subjects failed to be suppressed by month 2. At month 6 there was regression or no progression of breast development in 91% and 82% of females (11.25 and 30 mg, respectively) and no increase in testicular volume in 67% and 80% of males. In both groups, median basal E₂ decreased to pre-pubertal levels (<3 pg/mL) by month 1 and through month 6, and growth rate decreased by month 6 (Table 1). Adverse events (AEs) were similar between groups. The most common AE was injection site pain (19% in 11.25 mg and 26.2% in 30 mg), with 1 sterile abscess in the 11.25 mg group.

Conclusions: The LD 3-month preparations substantially suppress the GnRH axis in children with CPP. In this study, more subjects in the LD 30 mg group achieved and maintained effective suppression than those in the LD 11.25 mg group. Both LD doses had a similar safety profile.

Table 1.

| | LD 11.25 mg | | | LD 30 mg | | |
|---|---------------------|----------------------------------|---------------------|----------------------|----------------------|----------------------|
| | GnRHa Naïve | Previous GnRHa | Overall | GnRHa Naïve | Previous GnRHa | Overall |
| Suppression of peak stimulated LH, % (95% CI) | 76.2 (58.0%, 94.4%) | 80.0 (62.3%, 97.7%) | 78.1 (65.4%, 90.8%) | 90.5 (77.9%, 100.0%) | 100 (100.0%, 100.0%) | 95.2 (88.8%, 100.0%) |
| Peak stimulated LH at M6 (IU/L), mean (SD) | 1.94 (0.80) | 2.49 (2.58) | 2.23 (1.95) | 1.63 (1.02) | 1.42 (0.90) | 1.52 (0.94) |
| Basal LH (IU/L) at M6, mean (SD) | 0.56 (0.50) | 0.53 (0.49) | 0.54 (0.48) | 0.46 (0.39) | 0.48 (0.40) | 0.47 (0.39) |
| Growth Rate: Δ to M6 (cm/yr), mean (SD) | -2.51 (5.29) | -0.98 (2.24) | -1.63 (3.84) | -2.34 (4.22) | -0.91 (2.45) | -1.59 (3.43) |
| Basal estradiol ^a at M6 (pg/mL), median (min, max) | 1.00 (1.00, 5.00) | 2.30 ^b (1.00, 240.00) | 2.00 (1.00, 240.00) | 1.45 (1.00, 16.00) | 1.10 (1.00, 2.80) | 1.20 (1.00, 16.00) |

^aFemales

^bIncludes one subject with an elevated estradiol of 240 pg/mL.

Sources of Research Support: Abbott Laboratories.

Disclosures: PAL: Consultant, Abbott Laboratories, Novo Nordisk; Coinvestigator, Abbott Laboratories; Clinical Researcher, Lilly USA, LLC, Novo Nordisk, Pfizer, Inc., Ipsen. KK: Consultant, Abbott Laboratories. NM: Coinvestigator, Abbott Laboratories. DY: Employee, Abbott Laboratories. CM-G: Employee, Abbott Laboratories. KC: Employee, Abbott Laboratories.

P1-699

Pelvic Ultrasonography Findings in Girls with Precocious Puberty.

MH Jung M.D.¹, HJ Kang M.D.¹, WK Cho M.D.¹, KS Cho M.D.¹, SH Park M.D.¹, GY Lim M.D.¹, BK Suh M.D.¹ and BC Lee M.D.¹.

¹Coll of Med, The Catholic Univ of Korea Seoul, Republic of Korea.

Background: GnRH stimulation test that is used in the differentiation of central precocious puberty (CPP) from premature thelarche (PT) has high specificity, but low sensitivity and disagreement about the criteria limit its usefulness. We analyzed pelvic ultrasonography (USG) findings in girls with CPP, and assess the role of uterine and ovarian measurements in discriminating between girls with CPP and other pubertal conditions.

Methods: We enrolled 74 girls (chronological age 7.8 ± 0.5 years, bone age 9.9 ± 0.8 years) with precocious pubertal signs. Measurements of uterine and ovarian parameters by pelvic USG included anterior-posterior (A-P) diameter of uterine fundus and cervix, diameter of each ovary, number of follicles, and maximal diameter of largest follicle. The pelvic USG parameters were compared between girls with CPP (n=49) and atypical PT (n=25).

Results: Girls with CPP showed longer A-P diameter of uterine fundus (1.05 ± 0.34 vs 0.74 ± 0.78 cm, $P=0.001$), maximal ovarian diameter (2.13 ± 0.48 vs 1.84 ± 0.74 cm, $P=0.048$), mean ovarian area (2.31 ± 0.79 vs 1.69 ± 0.71 cm, $P=0.002$) compared to girls with atypical PT. For the diagnosis of CPP, the sensitivity and specificity of A-P diameter of uterine fundus (>0.9 cm) was 65.3% and 84.0%, the sensitivity and specificity of maximal ovarian diameter (>2.0 cm) was 55.1% and 76%, and the sensitivity and specificity of mean ovarian area (>2.0 cm²) was 62.9% and 80.0%.

Conclusions: Girls with CPP showed significantly higher ultrasonographic measurements of the uterus and ovaries compared to girls with atypical PT. Uterine parameters contributed more than ovarian parameters to the differentiation between CPP and atypical PT.

Nothing to Disclose: MHJ, HJK, WKC, KSC, SHP, GYL, BKS, BCL

P1-700

Transdermal Estrogen Patches for Pubertal Induction Are Easy To Administer and Dose: Modification of the Starting Dose Based on Population Pharmacokinetics.

Ensio Norjavaara MD, PhD¹ and Carina Ankarberg-Lindgren BSc, PhD¹.

¹Dept of Pediatrics, The Sahlgrenska Academy at Univ of Gothenbur Sweden.

Background: Transdermal matrix patches of 17 β -estradiol (Evorel® 25 μ g/24 hours Janssen-Cilag) is used off-label for induction of puberty in girls. The matrix formulation of Evorel® makes it possible to cut the patch into suitable size and thereby individualise the dose and to mimic the spontaneous levels as well as the diurnal pattern of serum 17 β -estradiol in early puberty (JCEM 2001: 86:3039). The aim of the study was to study how the recommended starting dose (0.08-0.12 μ g/kg), worked in out patients on serum 17 β -estradiol levels.

Methods: The patch is placed on the skin (glutea, superior lateral) in the evening at bedtime and removed the following morning when the girl has wakened. At the blood sampling, in the morning, the patch was removed after blood sampling to obtain the very highest serum 17 β -estradiol level. All samples used in this study are serum samples submitted for 17 β -estradiol analysis at the Tillväxtlab, The Queen Silvia Children's Hospital, Göteborg, Sweden as part of control of the start of pubertal induction therapy. After 17 β -estradiol determinations, identification markings were removed from all samples.

Results; The analysis consist of 115 observations in girls dosed with 0.05-0.15 μ g/kg. A linear correlation was found between the dose and morning serum 17 β -estradiol ($R=0.46$, $p<0.0001$). The early puberty range of morning serum 17 β -estradiol is 11-42 pM in girls and we found that the regression line is below 20 pM for girls doses up to 0.06 μ g/kg and below 30 pM for doses up to 0.10 μ g/kg. The recommendation for starting dose for pubertal induction will changed to 0.05 - 0.10 μ g/kg to reduce the risk for to high 17 β -estradiol level. For those clinics without possibility to control the morning serum 17 β -estradiol with sensitive estradiol determination we recommend the starting dose to be 0.05 - 0.08 μ g/kg to further reduce the risk for 17 β -estradiol levels above the early puberty range.

Conclusion; The starting dose for pubertal induction will changed to be 0.05 - 0.10 μ g/kg to reduce the risk for 17 β -estradiol levels above the early puberty range.

Nothing to Disclose: EN, CA-L

P1-701

***KISS1* Gene Analysis in Korean Girls with Central Precocious Puberty: A Polymorphism, p.P110T, Suggested To Exert a Protective Effect.**

Jung Min Ko M.D.¹, Hyo Sung Lee M.D.¹ and Jin Soon Hwang M.D., Ph.D.¹.

¹Ajou Univ Sch of Med Suwon, Korea.

Objective: Kisspeptin and its receptor, GPR54, have been suggested to play an important role in the onset of puberty by regulating the secretion of the gonadotropin-releasing hormone (GnRH) in the hypothalamic neurons. Mutations in the *GPR54* gene have already been identified as a cause of idiopathic hypogonadotropic hypogonadism and central precocious puberty (CPP) in certain patients. However, currently there is only a limited amount of data available regarding *KISS1* gene mutations or polymorphisms (1). The principal objective of this study was to identify *KISS1* gene mutations or polymorphisms in Korean girls with CPP.

Patients and Methods: 101 Korean girls with CPP were recruited as the patient group. The control group was composed of 51 healthy Korean female adults who had experienced menarche after the age of 12, all of whom evidenced a regular menstruation cycle. Genomic DNA was extracted from peripheral leukocytes, and all coding exons and exon-intron boundaries were amplified and sequenced automatically. The relationships between identified sequence variations and CPP were evaluated via the comparison of allele frequencies between the two groups. Different clinical characteristics were also compared between the subgroups with or without a certain variation in the patient group.

Results: Eight polymorphisms were identified in the *KISS1* gene. Although two of them were novel, those polymorphisms could not lead to amino acid changes. p.P110T was detected less frequently in CPP patients than in the controls ($P = 0.022$). Moreover, the CPP patients with p.P110T evidenced lower peak FSH values under GnRH stimulation than those without p.P110T ($P = 0.002$).

Conclusion: The allele frequencies of several polymorphisms in the Korean population were identified in this study. An infrequent polymorphism in the *KISS1* gene, p.P110T, appeared to be meaningful. This polymorphism was suggested to exert a protective effect on pubertal precocity, even though more evidence will be required to confirm the accurate function.

(1) Luan X et al., Eur J Endocrinol 2007;157:113

Nothing to Disclose: JMK, HSL, JSH

P1-702

Serum Levels of Anti-Mullerian Hormone (AMH) and Inhibin during Puberty in Healthy Girls Born Either Small for Gestational Age (SGA) or Appropriate for Gestational Age (AGA).

MI Hernandez MD¹, G Iniguez PhD¹, A Martinez-Aguayo MD³, V Pena MD², A Avila NS¹, T Salazar PhD¹, S Asenjo MD⁴ and V Mericq MD¹.

¹Univ of Chile Santiago, Chile ; ²Hosp Clin San Borja Arriaran Santiago, Chile ; ³Pontificia Univ Catolica de Chile Santiago, Chile and ⁴Univ de Concepcion Santiago, Chile.

Introduction: AMH and Inhibin are secreted in the ovary by the granulosa cells and both are accurate markers of the ovarian early antral follicle number and maturity. Women with PCOS have higher serum AMH levels and reduced fetal growth has been associated with adrenal and ovarian hyperandrogenism. Objective: The aim of this study was to evaluate serum levels of AMH (ELISA) and inhibin (ELISA) and the relationship with gonadal function in girls with different birth weights (BW) Methods: 65 girls (35 AGA/ 30 SGA) recruited from the community with normal BMI and Tanner II breast development were included. Girls were followed for three years with a complete physical exam, bone age, pelvic ultrasound and a leuprolide test (500ugr sc) with basal, 3 hr and 24 hrs sampling for gonadal hormonal assessment. Appropriate IRB clearance and informed consent were obtained. Longitudinal and transversal analysis was performed. Results: At recruitment mean chronological age (CA) was 9.9 ± 1.03 (7.8-12.5) years, similar BA/CA, BMI and height SDS. At the third year BMI SDS, BA, body composition and age of menarche was similar in both groups. During all the follow up period AMH levels were slightly higher in SGA girls ($p=ns$) and Inhibin-B was always similar between the groups. At second year higher number of follicles ($p<0.05$) and DHEAS, 17 OH Prog and androstendione were observed in SGA girls. By the third we did not find differences in ovarian morphology and androgen levels between groups. At the beginning AMH levels correlated positively with basal FSH, LH3-hours, ovarian volume and follicle n^o in all girls. In the SGA girls puberty progressed faster and AMH correlated positively with ovarian diameter ($r=0.605$ $p0.004$) and follicle n^o ($r=0.403$ $p0.0078$) and Inhibin-B with basal FSH ($r=0.412$ $p0.037$) and E224hr ($r=0.62$ $p0.001$). At the third year AMH correlated with LH 0, estradiol and ovarian volume. During the follow up AMH directly correlated with insulinogenic index (IIG). In all girls regardless of BW at second year AMH correlated with androgen levels. Conclusion: These results suggest slight differences in follicular development in SGA girls which are translated into a faster pubertal progression accompanied by transient increases in androgen and insulin levels but with no differences in gonadal morphology or androgens at the end.

Sources of Research Support: Fondecyt 1030610.

Nothing to Disclose: MIH, GI, AM-A, VP, AA, TS, SA, VM

P1-703

Detailed Ovarian and Uterine Ultrasonographic Features of Autonomous Ovarian Cysts in Young Girls.

MB McCloskey¹, S Milla² and B Shah².

¹NYU Sch of Med New York, NY and ²NYU Langone Med Ctr New York, NY.

Background: A small percentage of prepubertal ovarian cysts can develop transient autonomous activity, which may result in pseudoprecocious puberty. Prepubertal hyperfunctioning ovarian cysts are found frequently in McCune-Albright Syndrome (MAS). MAS represents a triad of polyostotic fibrous dysplasia, cafe-au-lait spots, and pseudoprecocious puberty that is due to a mutation in *GNAS* gene. The estrogen released from the autonomous ovarian cysts impacts uterine development and maturation. Uterine parameters during episodes of cyst activity is currently scant.

Objectives: To examine ovarian and uterine ultrasonographic features in young girls with autonomous ovarian cysts.

Methods: A retrospective chart review of girls, (Age 11 months-7 years) with isolated ovarian follicular cysts was performed. Clinical, biochemical and ultrasonographic features were reviewed during and after resolution of the ovarian cyst. Ultrasonographic data were studied at the presentation (A1), follow up at 4-9 weeks (A2) and after extended follow up period (E). Girls were studied until central precocious puberty was set in.

Results: There were 5 girls studied. They presented with either thelarche, vaginal discharge/ bleeding, 4/5 girls had cafe-au-lait spots. None had bony lesions.

(A1)-Data was available in 3 girls. Enlarged unilateral ovarian volume (OV) including cyst, ranged from 15-31.3 cc. Uterine length (UL), uterine volume (UV) and endometrial echo (EE) was increased in all the 3 girls. FSH and LH were low and estradiol was elevated in those when available.

(A2): All five girls were studied. OV declined to <1 cc in 3/5, other 2 girls ovarian cysts were still resolving. UL was still enlarged in 2/5 girls. However, UV and EE remained enlarged in 4/5 girls. The duration of each activity period was <1 wk-8weeks and all cysts resolved without any surgical interventions.

(E): 4/5 girls were studied (7 months -3 years duration) as one girl had early puberty and excluded. OV in all 4 girls was <1 cc. UL, UV, EE were still enlarged in 3/4 girls, (Only 1/3 girl had recurrent ovarian activity, the other 2/3 had no further activities).

Conclusion: Uterine parameters such as UL, UV and EE can be abnormal both during ovarian cyst activity and on longer interval follow up period, with or without recurrent ovarian activity. The ability to closely follow ovarian and uterine parameters during and after episodes of ovarian cyst represents a promising addition to the current armamentarium.

Nothing to Disclose: MBM, SM, BS

P1-704

Use of GnRH Agonist (GnRHag) Testing in the Diagnosis of Combined Primary and Secondary Hypogonadism; a Case Report.

BD Bordini MD¹ and RL Rosenfield MD¹.

¹Univ of Chicago Med Ctr Chicago, IL.

Background: Hypogonadism is a known complication of childhood cancer treatment. Primary ovarian failure may result from alkylating chemotherapy or irradiation, and gonadotropin deficiency may result from cranial irradiation. To our knowledge, the combination of primary and secondary hypogonadism has not been reported, and the criteria for diagnosing these simultaneous conditions are unknown. We report a 14.6 yr old female in remission from alkylating chemotherapy and craniospinal irradiation with arrested puberty, in whom GnRHag test results were compatible with both primary and secondary hypogonadism.

Clinical case: Metastatic pineoblastoma diagnosed at age 5.7 yr was treated with surgery, alkylating chemotherapy, and ≥ 36 Gy craniospinal irradiation. At age 9 yr she was in remission and had short stature due to isolated GH deficiency. On GH therapy, puberty began at 10.6 yr (pubarche)-11.3 yr (thelarche). However, puberty progressed slowly: by age 13.75 yr she was premenarcheal, with breast stage 3 and pubic hair stage 4. Breast size then decreased. At age 14 yr, daytime LH (1.5 U/L) and FSH (8.9 U/L) were pubertal, but estradiol was prepubertal at 9 pg/mL. To distinguish between primary and secondary hypogonadism, we performed a GnRHag test at age 14.6 yr and compared her results to early pubertal (PUB) 5th-95th reference ranges (Bordini, et al 2009).

Results: Her LH rose from 2.6 U/L at baseline (PUB \leq 0.15 - 5.0) to a peak of 18 U/L at 4 hr (4.8 - 98.0). FSH rose from 11.0 U/L at baseline (1.0 - 6.0) to a peak of 35.5 U/L at 8 hr (16.9 - 38.5). Estradiol did not respond from a baseline of 7.0 pg/mL (5.0 - 39), peaking at 8 pg/mL at 16 hr (44 - 376). Thus, her gonadotropins had an early pubertal response to GnRHag while estradiol remained prepubertal.

Conclusion: The diagnosis of primary hypogonadism was supported by prepubertal estradiol levels that did not increase in response to pubertal LH and FSH levels generated by GnRHag-stimulation. The diagnosis of secondary hypogonadism (partial gonadotropin deficiency) was supported by an FSH level that was marginally elevated at baseline but that did not hyperrespond to GnRHag. These results suggest that her arrested puberty resulted from a combination of primary ovarian failure that prevented her ovaries from responding to gonadotropin stimulation as well as partial gonadotropin deficiency that prevented her gonadotropins from responding appropriately to the primary ovarian failure.

Sources of Research Support: In part by USPHS grants U54-041859, RR-00055, and UL1RR024999.

Nothing to Disclose: BDB, RLR

P1-705

Pubertal Development and Precocious Puberty in Prader-Willi Syndrome.

CSY Cho fellow¹, PSW Park fellow¹, SYB Sohn fellow¹, KSJ Kim fellow¹ and JDK Jin professor¹.

¹Dept of Pediatrics Samsung Med Ctr, Sungkyunkwan Univ Sch of Med Seoul, Korea.

Introduction: Patients of Prader-Willi syndrome generally have delay in pubertal development due to central hypogonadism. Recently, however, there has been reports of cases in which Prader-Willi syndrome patients showed early onset of puberty or precocious puberty. The aim of our paper was to study such phenomenon.

Subject: Among the patients who made regular visits to our outpatient clinic for follow up, we chose girls who showed breast development before 9 years old and boys who showed increased testicular volume before 11 years old.

Methods: We analyzed the patients' medical record retrospectively.

Results: Out of 83 Prader-Willi syndrome patients (50 boys, 33 girls) who were followed since 1st of January 2006, 8 patients (7 boys, 1 girl) showed early onset puberty. Genetic test results revealed that two of these patients were uniparental disomy type and six patients had deletion 15q11-13. The girl presented with Tanner stage II breast development at the age of nine. At the time of diagnosis, her bone age was estimated to be 11 years old which was two years ahead of her chronological age. Her height was 0.65 SDS and weight was 1.85 SDS. As for the boys, their mean age was ten years old (8 years 10 months~11 years 9 months) at the time of diagnosis. Their average bone age was twelve years old (9~14 years old). Height was 1.06 ± 0.74 SDS, weight was 1.67 ± 0.76 SDS and BMI was 2.67 ± 1.44 SDS. One of the boys had unilateral undescended testis. The rest had bilateral undescended testis. All of them had orchiopey at the age of two. None of the boys had any past history of brain damage, neurologic symptoms such as convulsion or abnormalities on brain MRI. Out of the total eight children, three of them showed increased level of LH above 5 mIU/ml on GnRH stimulation test and increased bone age before 10 years old. The three were being treated with GnRH agonist.

Conclusion: Generally, patients of Prader-Willi syndrome show delay or incompleteness in pubertal development. Reported cases of precocious puberty in Prader-Willi syndrome until February, 2008 were 7 girls and 4 boys, which is extremely rare. Recently, we have observed patients from our clinic who has shown early onset puberty or precocious puberty. Their height SDS tended to be higher than patients who has not shown those symptoms. Further research is needed to reveal the mechanism by which precocious puberty is caused in these patients. Also, long term effects of GnRH agonist treatment need to be studied.

Nothing to Disclose: CSYC, PSWP, SYBS, KSJK, JDKJ

P1-706

Sterile Abscess Formation in Response to Two Separate Long-Acting Gonadotropin-Releasing Hormone Agonists, Supprelin LA® and Lupron Depot-Ped®.

BS Miller MD, PhD¹ and AR Shukla MD².

¹Univ of Minnesota Amplatz Children's Hosp Minneapolis, MN and ²Univ of Minnesota Med Ctr-Fairview Minneapolis, MN.

Background: Long-acting forms of gonadotropin releasing hormone (GnRH) agonists are commonly used for the treatment of central precocious puberty (CPP). Sterile abscess formation has been reported as a complication of Lupron Depot-Ped®, but not Supprelin LA®.

Methods: Chart review of child with sterile abscess formation following treatment with both long-acting forms of GnRH agonists.

Results: An 8 year old female with documented CPP developed a sterile abscess at the site of Lupron Depot-Ped® injection following the fifteen months of therapy. Because of this site reaction, a Supprelin LA® insert was placed. A similar reaction occurred two weeks after insert placement on two separate occasions in different arms. At the time of removal of the second insert, gram stain and swab culture of the purulent wound discharge was negative. The child subsequently tolerated intranasal nafarelin therapy.

Conclusion: Sterile abscess formation can be a complication of therapy with multiple different forms of long-acting GnRH agonist therapy in the same individual. Sterile abscess formation is likely a reaction to the polymers that deliver these medications. Clinicians and families need to be aware of this potential adverse reaction.

Carel JC et al., Pediatrics 2009; 123:e752.

Disclosures: BSM: Study Investigator, Abbott Laboratories; Speaker, Abbott Laboratories.

Nothing to Disclose: ARS

P1-707

LH Surge Induction in Pre-Menarcheal Girls: Positive Neuroendocrine Feedback Occurs Early in Puberty.

Julia G Keltz BA¹, Barbara Isaac RN¹, Cary Dicken MD¹, Marie Menke MD, MS¹, Cheryl Hickmon BS¹, Sangita Jindal PhD¹, Beatrice Babbs BA², Andy Bradford PhD², Alex J Polotsky MD, MS¹, Staci Pollack MD¹ and Nanette Santoro MD².

¹Albert Einstein Coll of Med/Montefiore Med Ctr Bronx, NY and ²Univ of Colorado Denver, CO.

Background:

The development of a positive feedback signal from estradiol is mandatory for luteinizing hormone release and for ovulation. It is widely believed to be a late feature of pubertal maturation that is not consistently operational until after menarche. We have previously observed spontaneous LH surges in premenarcheal girls (1). We therefore hypothesized that the positive neuroendocrine feedback response of the hypothalamic-pituitary axis to an estradiol challenge would be present prior to menarche. We present an interim analysis of the experiment in progress.

Methods:

The experimental group to date consists of 5 premenarcheal girls aged 9-11 who were administered 300mcg of transdermal estradiol for 7 days. The control group consisted of 8 women aged 19-33 with regular menstrual cycles who were administered the same dose and duration of transdermal estradiol starting on day 3 of their cycle. Daily, first morning voided urine was collected for both groups. Demographic information including age, BMI, and Tanner staging for the pubertal girls was recorded. Urinary excretion of LH and FSH (Immulite; SIEMENS Healthcare Diagnostics, Deerfield, IL), and estradiol (E1c), and progesterone (Pdg) metabolites (in-house ELISA) are measured. Statistical comparisons of data were performed using a two-tailed, two-group t test, as assumptions of normality of data distribution were not violated. A p value of <0.05 was considered statistically significant. Urinary LH results are reported herein.

Results:

| | Pubertal, n=5 | Adult, n=8 | P value |
|--------------------------|---------------|------------|---------|
| Age, yrs | 10.4 ± 0.9 | 23.6 ± 4.7 | <0.01 |
| BMI, kg/m ² | 20.5 ± 4.1 | 24.0 ± 2.9 | 0.10 |
| Mean LH, IU/L | 7.1 ± 1.0 | 8.0 ± 3.8 | 0.79 |
| LH surge amplitude, IU/L | 4.0 ± 1.4 | 7.9 ± 5.3 | 0.39 |
| Days to surge | 4.5 ± 2.1 | 5.8 ± 1.0 | 0.34 |

Data shown as mean ± standard deviation

There is no significant difference between the mean LH level and the LH surge and surge characteristics in premenarcheal girls and the regularly cycling controls. The only significant difference is age.

Conclusion:

Our results show that premenarcheal girls given an exogenous estradiol challenge are able to consistently mount an LH surge that is similar to regularly cycling adult women in amplitude and timing. These findings support the notion that positive neuroendocrine feedback mechanisms are present in girls prior to menarche and can be stimulated exogenously.

(1) Zhang K, et al, JCEM 2008; 93:1186

Sources of Research Support: K24 HD041978 to NS.

Nothing to Disclose: JGK, BI, CD, MM, CH, SJ, BB, AB, AJP, SP, NS

P1-708

Genistein Levels in Korean Girls with Precocious Puberty.

MJ Park M.D.¹, SH Kim M.D.¹ and K Huh M.D.².

¹Sanggye Paik Hosp, Inje Univ Coll of Med Seoul, Korea and ²Kwangmyung Sung-Ae Hosp Kwangmyung, Korea.

Background: The prevalence of idiopathic precocious puberty (PP) is rapidly increasing in Korea. This trend may be related with high phytoestrogen levels in diet. Possible adverse effects of phytoestrogens in soy products on sexual maturation in children has been raised. Genistein and daidzein, the principal isoflavones of soy-based foods, may be related with PP. Nevertheless, studies on the levels of genistein or daidzein among the children with PP are limited.

Objective: This study assessed the serum and urine levels of genistein and daidzein in Korean girls diagnosed with idiopathic central PP and compared them with those of the age-matched normal control group.

Method: Anthropometry (height, weight, body composition analysis), bone age, Gonadotropin Releasing Hormone (GnRH)-stimulation test were conducted in 111 girls with PP and 43 girls with age-matched controls. Serum LH, FSH, Estradiol level were analyzed by radioimmunoassay. Serum and urinary genistein and daidzein levels, and urinary equol (metabolite of daidzein) levels were analyzed by gas chromatography/mass spectrometry method.

Result: Serum genistein levels were significantly higher in PP than in control groups (4.9 ± 7.2 vs. 3.9 ± 6.2 ng/ml, $P < 0.05$). Urinary genistein levels did not show a significant difference between PP and control groups (177.0 ± 248.5 vs. 158.3 ± 274.5 ng/ml). Serum genistein level was correlated with urine genistein level ($r = 0.24$, $P < 0.0003$). Serum genistein level was correlated with serum daidzein levels ($r = 0.17$, $p < 0.05$). Urine genistein level was correlated with urine daidzein levels ($r = 0.82$, $P < 0.0001$) and urine equol levels ($r = 0.26$, $P = 0.01$). Serum or urinary genistein levels did not correlate with serum estradiol levels, GnRH stimulated LH, FSH levels, and body fat percent.

Conclusion; The serum genistein levels were higher in PP than in controls, which suggests that genistein may be associated with the development of idiopathic PP. To clarify the effect of genistein on PP, further longitudinal studies including dietary isoflavone intake analysis are needed.

Nothing to Disclose: MJP, SHK, KH

P1-709

High Prevalence of Cardiovascular Abnormalities in Turner Syndrome Evaluated by Magnetic Resonance Imaging.

MA Czepielewski PhD¹ and BF Pereira PhD¹.

¹Hosp Clins De Porto Alegre Porto Alegre, Brazil.

Objective: To evaluate the incidence of cardiovascular malformations in 33 patients with Turner's syndrome followed up in the Hospital de Clínicas of Porto Alegre. **Methodology:** A transversal study in which a Cardiac and Thoracic Magnetic Resonance Imaging (MRI) with focus on the evaluation of the aorta was performed in 33 patients. **Results:** The patients had average age of 20 years and 10 months and height of 138,7 cm. Approximately 42,42% of the patients presented karyotype 45,X and 33,33% webbed neck. Through MRI 54,54% of patients showed anomalies; the bicuspid aortic valve was the most frequent found present in 24,24% of patients. Through MRI, cardiovascular malformations were found in 42,42%, and elongation of the transverse arch was present in 27,27% of patients. Aortic dilatation was found in 66,66% of patients, and it was considered severe in 12,12%. The most frequent place of dilatation was in the aortic root and in the tubular portion of the ascending thoracic aorta. **Conclusion:** The results of present study corroborate with the literature that says cardiovascular anomalies are common in Turner's syndrome, especially diagnosed through magnetic resonance imaging. Aortic dilatation, most prominent in the ascending aorta, is very frequent in Turner's syndrome and that predicts high risk for acute aortic events such as dissection, potentially fatal.

Nothing to Disclose: MAC, BFP

P1-710

Classical Turner Syndrome (TS) and Microdeletion of Short Arm of Chromosome 3 (3p14.1) Associated with Severe Behavioral Problems.

L Iglesias M.D¹, M Angulo M.D¹ and M Castro-Magana M.D¹.

¹Winthrop Univ Hosp Mineola, NY.

Background: Turner syndrome (TS) is the second most frequent form sex chromosome aneuploidy with an incidence of approximately 1:2500. Classical form of TS associated with 45,X occurs in about 50% of affected girls. Clinical features of TS are highly variable and differ depending on the age of the child at the time of diagnosis and paternal versus maternal origin of the X chromosome. Aortic coarctation and bicuspid aortic valve seem to be the most common clinically significant anatomic defects in live born individuals with this condition. Severe behavioral problems are uncommon in TS.

Clinical case: We present a 5.6 year old girl with classical TS (45,X) diagnosed prenatally in amniocytes and postnatally in peripheral blood lymphocytes. She was the product of IVF conception, 36 week gestation born to a 35 years old mother and delivered by cesarean section due to breech presentation. Physical examination after birth revealed a female infant with weight of 4 lbs 6 oz and length of 18 inches. Clinical picture included short webbed neck, micrognathia, low set ears, broad shield-like chest and lymphedema of hands and feet. She had normal renal sonogram. Cardiovascular evaluation revealed pulmonary venous anomalous return without evidence of aortic anomalies. MRI of the brain showed Dandy Walker malformation. She was a very irritable infant and by age 2, she was started on growth hormone therapy. She had delayed speech. By age 4 she was noticed to have impaired social adjustment, short attention span and severe behavioral problems. Diagnosis of attention- deficit hyperactive disorder was made at age 5 years. A large right knee ganglion cyst was surgically removed. Due to severe behavioral problems and delayed speech by age 4 unusual for TS, further genetic evaluation was recommended. She had normal DNA testing for fragile X, serum amino acids and urine organic acids. Microarray based comparative genomic hybridization (CGH) revealed a *de novo* microdeletion (1.4 Mb) on the short arm of chromosome 3 (3p14.1). Both parents had normal CGH studies. Loss of heterozygosity (LOH) at this break point (3p14.1) has been reported in sporadic ovarian and renal cancers.

Conclusion: The phenotype and developmental abnormalities beyond the typical TS could be the result of this *de novo* unbalanced genomic alteration involving 3p microdeletion. In spite of not having any renal abnormalities, further renal sonograms are recommended to monitor kidneys structural changes.

Nothing to Disclose: LI, MA, MC-M

P1-711

An Unusual Presentation of Autoimmune Polyglandular Syndrome Type 1 (APS1) in a Preadolescent Male.

B Ergun-Longmire MD¹, C Plowgian MD¹ and C Fenton MD¹.

¹Akron Children's Hosp Akron, OH.

APS1 is a rare autosomal recessive disorder. We present a 10 year old previously healthy white boy who was brought to our hospital after having tonic clonic seizure lasting 2-3 minutes at home. Prior to this episode, there was no history of illness or taking any medications. His past medical history was unremarkable. Family history was significant for type 2 diabetes, hypothyroidism and hypertension in maternal grandmother, dermatomyositis and celiac disease in maternal aunt, hypothyroidism in maternal uncle. On admission, he was in moderate distress due to headache. His vitals signs were stable and physical exam was unremarkable. Fingertick glucose was 149. His total calcium was 4.4 mg/dl, sodium was 132 mEq/L, potassium was 4.8 mEq/L, CO₂ was 26 mEq/L, and phosphorus was 11.3 mg/dl. Hemoglobin and hematocrit was low at 9.6 g/dL and 28.1 % respectively. EKG showed normal sinus rhythm with a ventricular rate of 100 bpm and QTc of 570 ms. CT of the head showed subtle hyperdensity in the frontal region, which could either be acute hemorrhages or calcific areas. He was started on calcium gluconate. PTH intact was <3 pg/ml (reference range 10-65) when serum calcium was 6.4 mg/dl, confirming hypoparathyroidism. Despite IVF, hyponatremia continued. On second day of admission, his ACTH stimulation confirmed the diagnosis of adrenal insufficiency. He was started on hydrocortisone. The 21 hydroxylase antibody was positive. He had positive GAD65 antibodies. Ophthalmologic exam showed bilateral papilledema which then resolved. An MRI of brain showed multifocal areas of ischemic change/infarction of right posterior parietal, temporal, and occipital regions. After correction of calcium, QTC was normalized. Hypercoagulable studies, septic work up and celiac screening were negative. Vitamin D panel, thyroid studies were normal.

AIRE gene mutation confirmed the diagnosis of APS-1. He was heterozygote for the R257X and the 967-979del13bp mutations. The patient developed candida onychosis after 6 months of his diagnosis of APS-1. Anemia was corrected by iron supplement.

The clinical diagnosis of APS1 is based on the presence of two of the three main clinical manifestations: chronic mucocutaneous candidiasis, primary hypoparathyroidism, and primary adrenocortical insufficiency. Although candidiasis is the first clinical presentation in majority of cases, the diagnosis of APS1 should be suspected in any child with severe hypocalcemia, hyponatremia and positive GAD65 antibody.

Nothing to Disclose: BE-L, CP, CF

P1-712

Autoimmune Polyglandular Syndrome Type 3 with Autoimmune Hepatitis.

J.K. Duggal MD¹, B Theckedath MD¹, P Butler MD¹ and S.P Singh MD¹.

¹Rosalind Franklin Univ of Med and Scis North Chicago, IL.

Background:

Constellation of multiple endocrine gland failures associated with diseases of nonendocrine organs comprise the autoimmune polyglandular syndrome.(APS) These polyglandular insufficiencies are often underrecognised in clinical practice.We describe an interesting case of APS type III associated with autoimmune hepatitis.APS III revolves around thyrogastric autoimmunities and is characterised by autoimmune thyroiditis in association with type 1 diabetes,pernicious anemia ,vitiligo,alopecia and/or other organ specific autoimmunity .(1,2)

Clinical case:

A 16 year old girl who presented with abdominal pain and jaundice of 2 weeks duration to ER . She got diagnosed with autoimmune hepatitis with high anti-LKM antibody titres.(table1) She was initially treated with azathioprine and prednisone. 5 months later she developed polydipsia/polyuria and a swelling in the neck.Thyroid gland was firm, 50gm, nontender and uniformly enlarged. TSH was >100, FT4-0.52, TPO antibody positive and HbA1c-10.7%. Diabetes was considered to be secondary to prednisone, she was started on NPH and regular insulin twice a day but even after the tapering of steroid dose, blood glucose remained elevated. Subsequently anti-glutamic acid decarboxylase GAD-65 antibody and islet cell antibody were high.

Autoantibody results

| ANTIBODY | RESULTS |
|------------------------|-----------------------------|
| Anti-LKM antibody | positive -61.6 Units (high) |
| GAD-65 antibody | >30.0 U/ml (high) |
| Islet cell antibody | 10240 Units (very high) |
| Thyroglobulin antibody | positive |
| Antiparietal antibody | positive |

For hypothyroidism secondary to thyroiditis,treatment initiated with levothyroxine 0.1 mg p.o daily.Her glycemic control improved with basal-bolus regimen of lantus and aspart insulin.She is doing well on azathioprine. Her siblings have hypothyroidism and father has diabetes.Interestingly,her parietal antibody is also positive but there is no clinical evidence of pernicious anemia.She has also developed alopecia now without any vitiligo.The cosyntropin stimulation test done was normal. Conclusion: Cases of APS with protean clinical manifestations have been reported.(3,4) This is a rare combination of APS with sequential involvement of autoimmune hepatitis followed by development of autoimmune thyroiditis and type1 diabetes after 4 years.She doesn't manifest adrenal insufficiency now but the APS disorder is complex and variable with several other clinical features that might unfold over time.

1.Neufeld M,et al.Polyglandular autoimmune disease.Autoimmune aspects of endocrine disorders. London, UK: Academic Press; 1980:357-65.

2.DeGroot et al:Endocrinology.4th edition:587-589.

3.Oki K et al.A case of polyglandular autoimmune syndrome type III complicated with autoimmune hepatitis. Endocr J. Oct 2006;53(5):705-9.

4.Lubinska M et al. Acquired von Willebrand's syndrome in a patient with severe primary hypothyroidism associated with myasthenia gravis in the course of autoimmune polyglandular syndrome type 3. Haemophilia 2007.

Nothing to Disclose: JKD, BT, PB, SPS

P1-713

Familial Hypocalciuric Hypercalcemia in a Child with Kabuki Syndrome and Crohn Disease.

J Ho MD¹, D Fox¹, M Innes MD¹, R McLeod MD¹, D Butzner MD¹, N Johnson MD¹, C Trevenen MD¹, V Kendrick MD¹ and D E C Cole MD².

¹Univ of Calgary Calgary, Canada and ²Univ of Toronto Toronto, Canada.

Background: Familial hypocalciuric hypercalcemia (FHH) is a rare condition resulting from mutations of the calcium sensing receptor (*CASR*) gene. We describe a child with FHH, Kabuki syndrome, and Crohn disease.

Case: This 27 week gestation male infant had neonatal complications including: transient tachypnea of the newborn, bronchopulmonary dysplasia, sepsis and necrotizing enterocolitis. Transient nephrocalcinosis was present; and likely related to prematurity. FHH was considered because of persistently elevated serum Ca with low urine Ca excretion and a family history of asymptomatic hypercalcemia. At 1 month, total serum Ca was 2.76 mmol/L (N < 2.54), phosphate (PO₄) 1.55 mmol/L (N: 0.80–1.50), alkaline phosphatase (alkP) 866 U/L (N < 117), and Ca/creatinine clearance ratio 0.019 (N > 0.008). At 6 months, Ca was 3.79, PO₄ 1.53 and parathyroid hormone (PTH) 29 ng/L (N: 10–55). Based on classic clinical dysmorphic features at 6 years, Kabuki syndrome was diagnosed. At 9 years, he developed abdominal pain, weight loss and diarrhea. Colonic biopsies revealed segments of chronic colitis with poorly formed granulomata interspersed with normal mucosa consistent with a Crohn-like autoimmune disease. There was no evidence of pancreatitis. At 10 years, he was diagnosed with arthropathy. At 11 years, he was seen for short stature with delayed bone age, normal thyroid function, normal IGF-1 and IGF-BP3. Ca was 3.30, PO₄ 0.97, PTH 38 ng/L, alkP 287 U/L (N: 55–480), vitamin 25OHD 64 nmol/L (N: 80–200), vitamin 1,25-OH₂D 152 pmol/L (N: 55–190) and Ca/creatinine clearance ratio 0.00067.

Molecular Analysis: Genomic DNA was screened for *CASR* mutations by denaturing HPLC. Heterozygosity was observed in exon 3, which encodes a portion of the extra-cellular domain. Sequencing revealed a n.476T>G nucleotide transversion, predicting a non-conservative substitution of arginine for leucine at codon 159 (p.L159R). The leucine-159 codon in the *CASR* gene is conserved throughout vertebrate evolution. Based on the SIFT computer algorithm, its replacement by arginine is predicted to have a deleterious effect on protein structure and function.

Conclusion: Kabuki syndrome has been associated with other autoimmune disorders and Crohn disease occurs more frequently in some genetic conditions (eg. 45, X syndrome), but neither has been described with FHH. Whether this concurrence is due to chance or a common underlying predisposition remains to be seen.

Nothing to Disclose: JH, DF, MI, RM, DB, NJ, CT, VK, DECC

P1-714

Early Onset Mandibuloacral Dysplasia Due to Compound Heterozygous Mutations in *ZMPSTE24*.

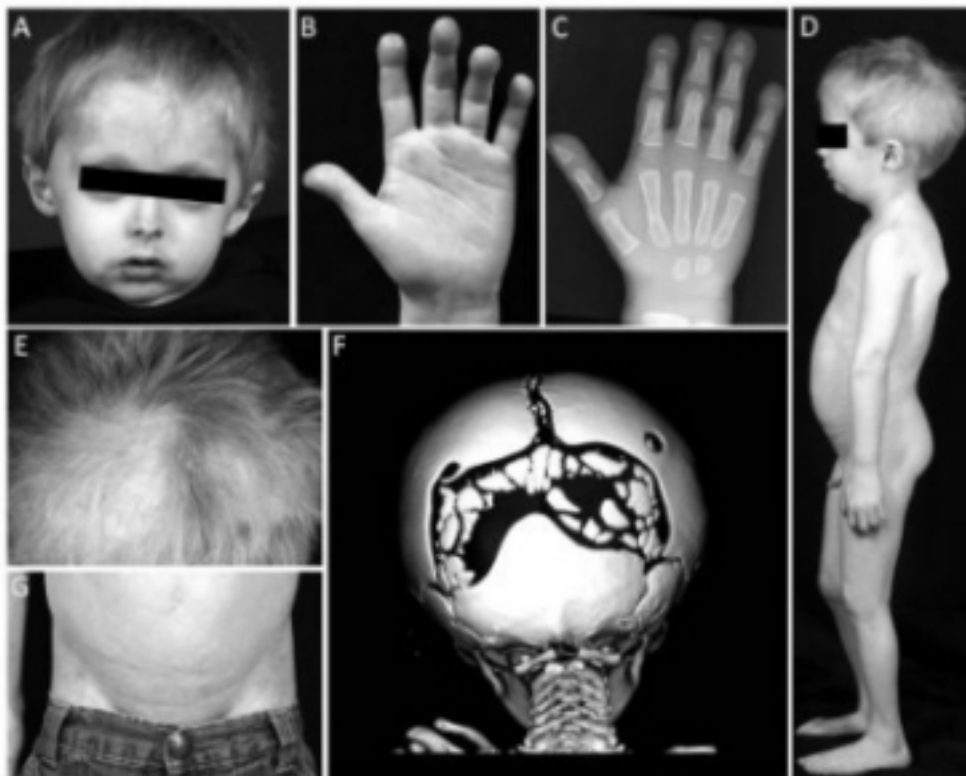
Z Ahmad MD¹, L Medne MS, CGC², E Zackai MD² and A Garg MD¹.

¹Univ of Texas Southwestern Med Ctr Dallas, TX and ²The Childrens Hosp Philadelphia Philadelphia, PA.

Context: Mandibuloacral dysplasia (MAD) is an autosomal recessive disorder characterized by hypoplasia of the mandible and clavicles, acro-osteolysis and lipodystrophy due to mutations in *LMNA* or *ZMPSTE24*. Only limited phenotypic data on 6 patients are available for MAD due to *ZMPSTE24* mutations.

Objective: To report two brothers (4 years and 8 month old) with early onset MAD due to compound heterozygous *ZMPSTE24* mutations.

Patients: Thin skin was noted as early as 5 months of age in each child. Both had micrognathia, mottled hyperpigmentation, small pinched noses, enlarged fontanelles and Wormian bones but had no lipodystrophy. There was no delay of mental development. The older brother showed stunted growth, joint stiffness, and multiple fractures. There was no evidence of renal disease. Both were compound heterozygotes harboring a previously reported missense *ZMPSTE24* mutation (Pro248Leu) and a novel null mutation (Trp450stop).



Patient 1 (MAD4700.3) A, small beaked nose with telangiectasias, prominent superficial veins, low-set ears, micrognathia, and slight double chin. B, bulbous appearance to distal fingers consistent with acro-osteolysis. C, Radiograph of hand at 18 months showing irregularity of the tufts of all digits, consistent with acro-osteolysis. D, Lateral view of the patient showing near normal body fat distribution and lack of lipodystrophy. E, dry, straight, bright red hair with areas of hair thinning and alopecia particularly on the back of scalp and on the left parietal and frontal region. F, CT head at 18 months with 3-D reformations showing multiple wormian bones in both lambdoid sutures. G, mottled pigmentation in the abdomen and inguinal areas.

Clinical features of two types of MAD due to *ZMPSTE24* or *LMNA* mutations

| Clinical Feature | <i>ZMPSTE24</i> MAD cases (n=8) | <i>LMNA</i> MAD cases (n=28) |
|------------------------------------|---------------------------------|------------------------------|
| Birth \leq 33 wk gestation | 3/7 (43%) | 0 |
| Post-natal growth retardation | 4/5 (80%) | 20/24 (83%) |
| Age of onset of symptoms | 4 months | 7 years |
| Dental overcrowding | 5/5 (100%) | 21/23 (91%) |
| Delayed closure of cranial sutures | 6/6 (100%) | 16/20 (80%) |
| Sparse/absent hair or alopecia | 4/5 (80%) | 13/21 (62%) |
| Mottled hyperpigmentation | 7/7 (100%) | 22/23 (96%) |
| Acanthosis nigricans | 0/4 (0%) | 15/17 (88%) |
| Calcified skin nodules | 2/4 (50%) | 0 |

| | | |
|-------------------------------------|-----------|--------------|
| Acro-osteolysis | 6/7 (86%) | 25/26 (96%) |
| Dysplastic/hypoplastic clavicles | 5/7 (71%) | 24/27 (89%) |
| Joint stiffness or contractures | 6/7 (86%) | 25/27 (93%) |
| Fractures (skull, long bones) | 3/4 (75%) | 3/7 (43%) |
| Focal sclerosing glomerulosclerosis | 2/4 (50%) | 0 |
| Partial lipodystrophy | 5/7 (71%) | 27/27 (100%) |

Micrognathia and Wormian bones were present in all reported cases.

Conclusion: MAD due to *ZMPSTE24* mutations manifests earlier in life than MAD due to *LMNA* mutations. Other distinguishing features may include premature birth, renal disease, calcified skin nodules, and lack of acanthosis nigricans.

Nothing to Disclose: ZA, LM, EZ, AG

P1-715

Vitamin D Excess in a Child: A Result of Taking a Foreign Vitamin Supplement That Is Readily Available in the United States.

V Auyeung MD¹, A Chartoff RN, APN, C, CDE¹, R Rothenberg RN, BSN, MAS, CPN¹ and J Aisenberg MD¹

¹Hackensack Univ Med Ctr, Joseph M Sanzari Children's Hosp Hackensack, NJ.

We present a case of a 9-year-old girl who initially presented to us with hirsutism and precocious puberty. She also had Rett syndrome, global developmental delay, seizure disorder, and was wheelchair bound. In addition to her evaluation for precocious puberty, we obtained a vitamin D level due to her sedentary state. Bloodwork showed 25-hydroxyvitamin D level of 124 ng/ml.

We contacted the patient's mother to inquire about any vitamin supplements that the patient may have been receiving. Her mother reported that a physician in the Dominican Republic had recommended that she give her daughter a vitamin supplement. We advised her to stop the vitamin supplement and to bring the bottle for us to see. The full name of the vitamin was Soladek-N (Indo-Pharma, S.A., Santo Domingo, Dominican Republic). Each three milliliters of the supplement contained 360,000 IU of vitamin D; 172,000 IU of vitamin A; and 3 mg of vitamin E. The patient's mother had given her two of the 3ml bottles, approximately 1 week apart. Repeat 25-hydroxyvitamin D level, after stopping the vitamin, revealed a normal 25-hydroxyvitamin D level of 52.6 ng/ml.

Another case of vitamin D toxicity also involving Soladek-N was reported in 2008. This 60-year-old woman obtained a 5-ml bottle of the supplement from a grocery store in New York that carried products from the Dominican Republic. The 5-ml bottle contained 600,000 IU of vitamin D. (1)

A third case of vitamin D toxicity due to Soladek was reported in 2006 in a 4-year-old boy, also from New York. He presented with a 1-week history of increasing weakness, nausea, and vomiting. (2)

An internet search for Soladek produced results advertising its effects on boosting the immune system as well as its use as a painkiller. It was also prevalent on muscle-building websites for athletes. It is apparently a well-known supplement in the Dominican Republic and discussed and recommended in many blog sites.

Although Soladek is found mainly in the Dominican Republic, it is readily available without a prescription in the United States. It contains a much higher dose of vitamin D than is standard in over-the-counter vitamins produced in the United States. Even though the first case of vitamin D toxicity was reported 4 years ago, this supplement is still readily available in the United States without a prescription as well as in the Dominican Republic. This indicates that there is a need for international surveillance of vitamin supplements.

(1) Leu JP et al., *Endocrine Practice* 2008; 14(9):1188-1190

(2) Schwaner RA et al., *Clinical Toxicology*, 44:625-783, 2006

Nothing to Disclose: VA, AC, RR, JA

P1-716

Unmasking of Partial Diabetes Insipidus (DI) during Stress but Not Maintenance Dosing of Glucocorticoids (GCs) in an Infant with Septo-Optic Dysplasia (SOD).

A Azam MD¹, JR Law MD¹, C Constantacos MD¹, M Puri MD¹ and KJ Loechner MD. PhD¹.

¹Univ of North Carolina Chapel Hill, NC.

The unmasking of DI once GC replacement is initiated is an expected sequence in the treatment of hypopituitarism including septo-optic dysplasia (SOD). We now present an infant in whom DI was not present during maintenance GC replacement but was unveiled during times of acute stress GC dosing.

Case: A 2mo old female with SOD presented with hypothermia and hypernatremia (Na= 155 mmol/L after receiving stress doses of GC, (normal range 35-145), Vasopressin <0.6 pg/mL). She was not found to have DI prior to admission. After routine treatment for sepsis, her temperature stabilized and she returned to her maintenance GC dose (8 mg/m²/day) while continued on levothyroxine (37.5 mcg). Of note, her serum Na normalized (136-142).

A modified water deprivation test was performed and at 15 hours her Na was still normal at 141 and urine specific gravity (USG) was 1.018, arguing against fulminant DI. A subsequent febrile episode occurred during her hospitalization and her Na increased to a peak of 163 on stress (triple) dose GCs. At that time, her USG was 1.005, supporting DI. DDAVP was initiated and titration proved that only 0.25 mcg of IN prep given orally (effective dose of 2.5 mcg) was required to keep her Na in the 145-155 range without episodes of hyponatremia. Again, once stress dosing was discontinued, her Na returned to normal levels.

Discussion: The "unmasking of DI" upon institution of GC replacement is an anticipated event that typically occurs during initial hormonal replacement in children with pituitary insufficiency. However, the exact mechanisms still remain unclear and may include both central (1, AVP release), and nephrogenic (2, AVP action) mechanisms. We now present an infant in whom DI was unmasked only during episodes where stress (triple) dose GCs were required, suggesting a very mild/partial DI. Moreover, this case supports that in children who are not found to have DI during initial evaluation for hypopituitarism, that re-screening of serum Na levels with a concomitant USG during acute stress and its associated increased GC dosing could unveil a partial DI that requires transient courses of DDAVP treatment.

(1)Yamada et al.,J Clin Endocrinol Metab. 1989 Aug;69(2):396-401

(2)Linas et al.,Kidney Int. 1980 Jul;18(1):58-67.

Nothing to Disclose: AA, JRL, CC, MP, KJL

P1-717

A Case of Neurofibromatosis Type1 with Renovascular Stenosis Causing Severe Hypertension.

M Ahmed MD¹ and AE Hazmi MD¹.

¹King Faisal Specialist Hosp & Res Ctr Riyadh, Saudi Arabia.

Background Information:

Neurofibromatosis type 1 (NF I) is an autosomal dominant neurocutaneous genetic disorder. About one-half cases are familial; the remainder are new mutations. NF1 gene is a large gene, spanning over 350 kb containing 60 exons & is mapped to ch 17q11.2. Gene mutations result in a loss of functional protein Neurofibrin, a tumor suppressor gene and account for a highly variable phenotypic expression. The hallmarks of NF1 are the multiple café-au-lait spots (CALs), and cutaneous neurofibromas. The diagnostic criteria developed by the NIH Consensus Conference are highly specific & sensitive. According to these criteria, at least 2/7 features must be evident for the Dx. We describe a case that fulfilled the diagnostic criteria, had renovascular stenosis causing HTN but had no evidence of Pheochromocytoma, a much less cause of HTN in NF1.

Clinical Case: A 19-year old asymptomatic man was referred for evaluation of HTN (BP 177/118). He had café-au-lait spots (cals), axillary freckling & Lesch nodules (iris hamartomas). Of the 7 full, & 16 half-sibs & parents there was no history of similar skin lesions or HTN. There was a prominent left flank bruit. **Lab Findings:** hypokalemic metabolic alkalosis K⁺ 3.2 mmol/l, CO₂ 31mmol/l, plasma Renin was 815 mu/l, (RR: 4.4-46.1) Pheochromocytoma was ruled out with normal 24 h urinary meta/neo-manephrines & MIBG scan. MR angiogram showed severe ostial stenosis at left renal artery take-off at Oorta. CT & MRI showed a midline soft tissue sacral 3.5x2.7 cm lesion at S1-2 level, compatible with a neurofibroma. CT brain & orbits were normal for intracranial tumors. Renal artery angioplasty failed. Pt. received Lisinopril 40 mg & Amlodipine 10 mg/D with sustained normalization of BP & renal functions.

Conclusions:

NF1 pts. must undergo comprehensive workup to define phenotypic features and regular BP monitoring. Should they develop HTN, evaluation for renal artery stenosis should be done & renal angioplasty be undertaken. If this procedure fails, ACE inhibitor therapy is a reasonable choice. For pts. with no evidence of RAS, evaluation for pheochromocytoma should be done.

Nothing to Disclose: MA, AEH

P1-718

Abdominal Pain as an Unusual Presentation of Adult Glycogen Storage Disease.

THANH D. Hoang D.O.¹, HUONG D. Nguyen M.D.¹, VINH Q. MAI D.O.¹, PATRICK W. Clyde M.D.¹, BABETTE C. Glister M.D.¹ and K.M. MOHAMED Shakir M.D.¹.

¹Natl Naval Med Ctr Bethesda, MD.

Background: Glycogen Storage Diseases (GSDs) are inherited metabolic disorders of abnormal glycogen storage due to gene mutations that affect glycogen synthesis, degradation, or regulation. GSDs have largely been categorized numerically according to the chronology of recognition of the responsible enzyme defect. Age of onset of GSDs varies from birth to adulthood. Major manifestations of GSDs are hypoglycemia, myalgia, cramps, exercise intolerance, easy fatigability, progressive weakness, and myoglobinuria. Long-term complications may include renal failure, hepatic adenomata, and hepatic carcinoma. Genetic testing can be performed to identify the precise abnormalities that cause the specific enzyme impairments of various GSDs.

Clinical case: A 32-year-old white female presented with abdominal discomfort. CT scan showed approximately 25 cm hepatomegaly extending into the pelvis.

Laboratory: AST 29, ALT 29, total bilirubin 0.3, alkaline phosphatase normal, alpha-1 antitrypsin normal, ceruloplasmin 26, ferritin 57, iron saturation 31%, negative HBV, HCV, AMA, ASMA, and ANA. Liver biopsy showed hepatocellular cytoplasmic glycogen accumulation consistent with possible glycogen storage disease. Patient also reported a history of fasting hypoglycemia. During a 72 hour fast (see Table1), she experienced hypoglycemia with adrenergic symptoms.

72 hour fast at the nadir of blood glucose concentration.

| Tests | Values | Reference |
|-------------------------------|---------------|-----------|
| Glucose (mg/dL) | 42 | 74-106 |
| Insulin (mIU/mL) | 0.2 | 2.6-24.9 |
| Proinsulin (pmol/L) | 8.1 | < 18.8 |
| C-peptide (ng/mL) | 0.3 | 0.8-3.1 |
| Beta hydroxybutyrate (mmol/L) | 5.56 | < 0.28 |
| Lactate (mmol/L) | 1.7 | 0.7-2.1 |
| Serum Sulfonylurea (ng/mL) | none detected | |
| Uric acid (mg/dL) | 2.8 | 2.6 - 6.0 |

Review of systems revealed myalgia, arthralgia, easy bruising, and 15 lb weight loss. Physical exam was unremarkable, except for hepatomegaly.

Genetic PYGL sequencing testing was negative for type VI. Testing for type IX gene is pending.

Conclusion: Abdominal pain in a patient with a history of hypoglycemia, myalgia and hepatomegaly may indicate the possibility of GSDs. Advances in genetics have led to identifying abnormalities of specific enzyme impairments in GSDs, although in our patient testing was negative for Type VI. Early diagnosis and initiation of dietary therapy can prevent hypoglycemia, improve biochemical abnormalities, decrease liver size, normalize physical growth and development especially in childhood.

Wolfsdorf JI, Holm IA, Weinstein DA. Glycogen storage diseases. Phenotypic, genetic, and biochemical characteristics, and therapy. *Endocrinol Metab Clin North Am* 1999; 28:801-823.

Wolfsdorf JI, Weinstein DA. Glycogen storage diseases. *Reviews in Endocrine & Metab Disorders* 2003; 4:95-102.

Nothing to Disclose: TDH, HDN, VQM, PWC, BCG, KMMS

P1-719

Case Report: Autoimmune Polyendocrine Syndrome Type II (APS II). A New Class II HLA Polymorphism?

S Mallea-Gil MD¹, C Ballarino MD¹, C Ridruejo MD¹, K Bertini MD¹, M Aparicio MD¹, S Gimenez MC¹, P Galarza PhD², A Perusco PhD², S Roveto PhD² and D Rimoldi MD².

¹Hosp Militar Central Buenos Aires, Argentina and ²Inst de Invest Médicas Lanari Buenos Aires, Argentina.

APS is the association of autoimmune endocrine diseases with other non-endocrine autoimmune disorders. Type II APS is defined by occurrence of Addison's disease with thyroid autoimmune disease and/or type 1 diabetes mellitus. The prevalence of APS II has been estimated at 1.4 to 2/100,000; women are affected three times more often than men. Clinical case: A 46-year man was hospitalized because he presented asthenia, adynamia, hyporexia, severe loss of weight (50 kg in 2 years); dizziness and vomits for a 15-day period.

Personal background: obesity 120 kg (BMI 36.13), diabetes mellitus diagnosed 3 years earlier, treated with metformine and rosiglitazone. When 45 years, insulin therapy was initiated.

Family background: mother and sister with thyroid pathology, father died of autoimmune hepatopathy.

Physical examination: weight: 56 kg (BMI 20), BP 90-60 mm Hg, darkening of the skin and oral mucosa. Laboratory: Hematocrit 28 %, glycemia 128 mg%, sodium 132 mEq/l, potassium 4.5 mEq/l. Because of persisting symptoms, hyponatremia and frequent hypoglycemia in spite of decreasing insulin dose, hormonal tests were performed: TSH: 24.25 mUI/ml (0.27 to 4.7); T4: 4.51 ug/dl (4.5 to 12), TPO antibody >1000 UI/ml (0 to 12); Cortisol <0.24 ug/dl (6.2 to 10.4); ACTH: 10.7 pg/ml (7.2 to 63.3); FSH: 10.37 mUI/ml (1.5 to 12.4); LH: 5.24 mUI/ml (2 to 9); PRL: 83 ng/ml (4.6 to 21.4); Testosterone: 3 ng/ml (3 to 9 ng/ml); glycemia 122 mg/dl and HbA1c 10.1 (4 to 6.5 %). Normal PTH, calcium and phosphorous levels. Serum autoantibodies: GAD: 76 U/ml (0 to 1); 21-hydroxylase: 810 U/ml (0 to 1) and TSH receptor: 64% (0 to 10%) (RSR). Anti nuclear factor (ANF), anti smooth-muscle (ASMA), Antigliadin and anti endomisium: negative. Treatment was initiated with hydrocortisone, fludrocortisone and later with levothyroxine, insulin was adjusted. Genetic studies: Class II HLA typification was performed by PCR-SSOP (Innolipa): HLA DRB1* 0804, DRB1* 0804 and DQB1* 0302, DQB1* 0402.

Conclusions:

1- HLA DRB1 *08 found in our patient has not been described in APS II or in any other autoimmune disorders. He also has HLA DQB1*0302 described in previous reports related to APS II.

2- In type 1 diabetic patients whose insulin requirement decreases, it would be convenient to rule out adrenal insufficiency, a component of APS II, as etiologic factor in spite of its low prevalence.

3- In diabetic obese patients (mainly young) who lose weight without a defined cause, type 1 diabetes mellitus should be excluded.

Nothing to Disclose: SM-G, CB, CR, KB, MA, SG, PG, AP, SR, DR

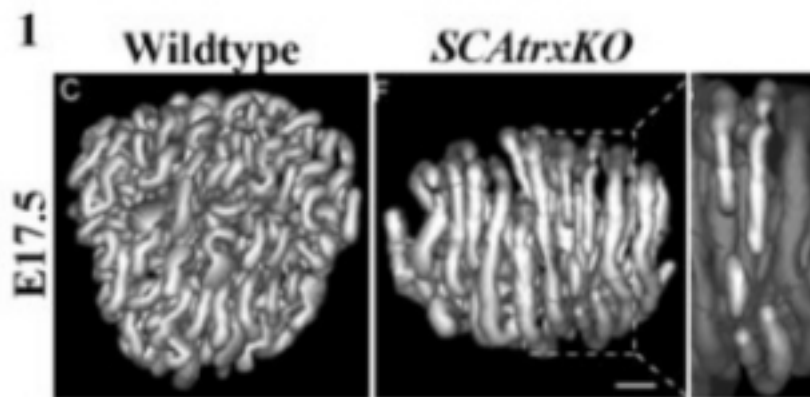
P1-723

ATRX Is a Sertoli Cell Survival Factor and Regulator of Spermatogenesis Via Interaction with Androgen Receptor.

S Bagheri-Fam PhD¹, A Argentaro PhD¹, T Svingen PhD², A Combes PhD², P Koopman PhD² and VR Harley PhD¹.

¹Prince Henry's Inst of Med Res Melbourne, Australia and ²The Univ of Queensland Brisbane, Australia.

The *ATR-X* syndrome, (α -thalassemia mental retardation, X-linked) is a developmental disorder affecting males. *ATR-X* syndrome individuals display a range of genital abnormalities, varying from small testes and hypospadias to XY male-to-female sex reversal. ATRX is a large protein (280kDa) displaying nucleosome remodeling activity in vitro, suggesting an involvement in gene regulation. However, no target genes of ATRX have been defined to date. We show that in testes of fetal and adult mice, ATRX is strongly expressed in the Sertoli cells. Sertoli cells play key roles 1) in testis differentiation in the fetal gonad, leading to male sexual differentiation and 2) during spermatogenesis in the adult testis. To understand the gonadal role of ATRX, we inactivated *Atrx* specifically in Sertoli cells (*ScAtrxKO* mice) from embryonic day 14.5.



At E17.5 testis cord volume in *ScAtrxKO* mice is ~30% of wildtype, with discontinuous or isolated testis cords apparent (Figure 1, red arrows). While Sertoli cell apoptosis is increased by 10-fold when compared to wildtype, no difference in Sertoli cell proliferation was observed. ATRX is required for fetal Sertoli cell survival and elongation and branching of fetal testis cords.

Adult *ScAtrxKO* testes weigh ~25% of wildtype and a third of tubules contain germ cells arrested in late meiosis or at the round spermatid stage. Testicular Sertoli cells, upon androgen stimulation of the androgen receptor (AR) transcribe genes necessary for the completion of germ cell meiosis, such as *Rhox5*. While gene expression of AR itself was unchanged in *ScAtrxKO* testes, expression of three AR-dependent genes including *Rhox5* was significantly down-regulated. Moreover, ATRX and AR proteins interact in the TM4 Sertoli cell line and co-operatively activate the *Rhox5* promoter.

In summary, ATRX plays an important role in Sertoli cell survival during fetal testis development and in adult testis function, where ATRX protein interacts with AR to regulate the transcription of AR-dependent genes that control spermatogenesis, such as *Rhox5*, the first ATRX target gene identified.

Sources of Research Support: National Health & Medical Research Council (NHMRC) Program Grant 334314; NHMRC Fellowship 441102 awarded to VRH.

Nothing to Disclose: SB-F, AA, TS, AC, PK, VRH

P1-724

Deletion of Nuclear Receptor Corepressor Protein (NCoR) in Adipose Tissue Improves Systemic Insulin Resistance in Diet-Induced Obese Mice.

Pingping Li PhD¹, Jianfeng Xu PhD¹, Min Lu PhD¹, Dorothy D Sears PhD¹, David Patsouris PhD¹, Saswata Talukdar PhD¹, Jachelle M Ofrecio Bachelor Degree¹, Sarah Nalbandian Bachelor Degree¹ and Jerrold M Olefsky MD¹.

¹Univ of California San Diego, CA.

Nuclear receptor corepressor protein (NCoR) serves as a corepressor for nuclear receptors and other factors and recent evidence suggests that NCoR can be a regulator of metabolism. NCoR represses PPAR γ (peroxisome proliferator-activator receptor gamma) mediated transcriptional activity in 3T3-L1 adipocytes in vitro, but its role in vivo remains unclear. We generated adipocyte specific NCoR1 knock-out (NCoR-ap2) mice by crossing NCoR f/f with ap2-Cre mice, to investigate the function of NCoR in insulin sensitivity. Our data show that NCoR-ap2 mice gain more weight on a 60% high fat diet (HFD) with increased accumulation of subcutaneous and visceral fat. The KO mice also display reduced plasma free fatty acid (FFA) and insulin levels. Insulin tolerance and glucose tolerance tests showed that NCoR-ap2 mice are more glucose tolerant compared with wt HFD mice. Hyperinsulinemic-euglycemic clamp studies demonstrate that the NCoR-ap2 mice have higher insulin stimulated glucose disposal rate (IS-GDR), suppression of hepatic glucose production (HGP) and FFA suppression, showing that NCoR deletion in adipocytes leads to improved systemic insulin sensitivity in all three major insulin target tissues (liver, muscle and fat). Hepatic triglyceride content and steatosis were reduced in the KO mice, while in adipose tissue, there was less macrophage infiltration, reduced inflammatory gene expression (including, IL-1 β , iNOS and IL-12p40), but greater adipogenic gene expression (including, SCD1, FAS, ACC and SREBP-1c) in the KOs. The effects of rosiglitazone treatment to improve in vivo insulin sensitivity were blunted in the NCoR-ap2 mice, indicating that NCoR-ap2 mice are relatively refractory to PPAR γ stimulation. Together, these results demonstrate that NCoR-dependent transrepression is a key determinant of the adipogenic set point as well as an integrator of glucose metabolism and whole-body metabolic homeostasis.

Sources of Research Support: DK033651.

Nothing to Disclose: PL, JX, ML, DDS, DP, ST, JMO, SN, JMO

P1-725

ACTH Ameliorates Glucocorticoid-Induced Osteonecrosis of Bone.

M Zaidi MD, PhD¹, L Sun MD.PhD¹, LJ Robinson², IL Tourkova², L Liu², Y Wang², LL Zhu¹, X Liu¹, JH Li¹, YZ Peng¹, J Iqbal¹, BB Yaroslavskiy², A Levine¹, A Kirschenbaum¹, C Isales³ and HC Blair².

¹Mount Sinai Sch of Med New York, NY ; ²Univ of Pittsburgh Sch of Med and the Pittsburgh Veterans Affairs Med Ctr Pittsburgh, PA and ³Med Coll of Georgia Augusta, GA.

The long-term use of corticosteroids has given rise not only to a variety of metabolic complications, mainly diabetes and osteoporosis, but also to a painful debilitating condition, osteonecrosis, most commonly involving the femoral head. Osteonecrosis almost invariably requires surgical debridement of dead bone, and contributes to ~10% of the 120,000 hip replacements annually in the US. We report that the anterior pituitary hormone ACTH protects against osteonecrosis of the femoral head induced by depot methylprednisolone acetate (depomedrol), in essence, testifying to its direct effect on the skeleton. Depomedrol (4 mg/kg, daily, s.c.) induced dramatic regional necrosis of the femoral head and suppressed pituitary-derived ACTH in rabbits. This osteonecrosis, visualized as deep tetracycline labeling, was reduced by ~65% when synthetic ACTH was co-administered daily (0.05 µg/kg). This dramatic therapeutic response was associated with an equally profound local up-regulation of VEGF mRNA. In keeping, ACTH also induced VEGF production from mineralizing human osteoblasts in culture. The VEGF up-regulation was associated with a significant increase in osteoblast maturation, seen as an elevation in the marker genes Runx2, osterix, alkaline phosphatase and bone sialoprotein. That the osteoblast maturation induced by ACTH was VEGF-dependent was tested in VEGF shRNA-transfected cells, in which the effect of ACTH was abolished. Furthermore, ACTH withdrawal from osteoblast cultures containing dexamethasone and ACTH, leaving cells in dexamethasone alone for 36 hours, caused a marked increase in annexin V-positivity, proving that ACTH was critical for cell survival. VEGF production was also induced by co-culturing human osteoblasts with CD14⁺ osteoclast precursors. Glucocorticoids abolished this effect, showing that endogenous VEGF in bone was sensitive to glucocorticoid inhibition, and was perhaps causal to the osteonecrosis. Finally, a modest stimulation of early osteoclastogenesis was noted in RAW264.7 cells and murine osteoclast precursors, with little or no effects on bone resorption by mature osteoclasts. The results not only substantiate our view regarding a novel pituitary-bone axis, in which stimulating hormones, such as ACTH, FSH and TSH, bypass traditional endocrine targets to effect the skeleton directly, but also, on a broader clinical front, call for studies to examine the efficacy of ACTH in preventing human osteonecrosis.

Nothing to Disclose: MZ, LS, LJR, ILT, LL, YW, LLZ, XL, YZP, JI, BBY, AL, AK, CI, HCB

P1-726

RAD1901, a Novel Selective Estrogen Receptor Modulator (SERM), Demonstrates Evidence of Efficacy on Postmenopausal Hot Flashes in an Early Phase Human Study.

L.St.L O'Dea MD¹, D McCarthy¹, J Guerriero MBA¹, C Miller PhD¹, G Hattersley PhD¹ and CR Lyttle PhD¹

¹Radius Hlth, Inc Cambridge, MA.

RAD1901 is a novel non-steroidal SERM with specific activity in the central nervous system (CNS). Preclinical studies have established that RAD1901 functions as an estrogen agonist in bone while being an antagonist in MCF-7 cells and uterine tissue. CNS efficacy has been established in a preclinical hot flash model and behavioral effects have been demonstrated in a partner preference model. In early human studies, RAD1901 was safe at doses up to 200mg for 7 days, with a half-life of approximately 30 hours. More recently, a proof of concept (POC) study was undertaken in otherwise healthy postmenopausal women experiencing at least 50 moderate/severe (M/S) hot flashes (HF) per week to investigate safety and efficacy of RAD1901 for relief of symptoms in this population. 100 patients with a mean of 64 M/S HF/week were randomized to RAD1901 10, 25, 50, 100mg, or matching placebo, administered orally once daily for 28 days.

Overall, the greatest clinical benefit was demonstrated by RAD1901 10mg, with a 50, 69, 76 and 77% reduction in M/S HF frequency at weeks 1, 2, 3 and 4, respectively. When assessed by dose following 28 days of RAD1901, HF frequency was reduced by 54, 77, 52, 54, and 67% for M/S and by 48, 70, 51, 47 and 61% for overall HF frequency in placebo, 10mg, 25mg, 50mg and 100mg dose groups, respectively. Composite score (freq x severity), was reduced by 56, 76, 55, 49, and 62%, respectively. While not a classical dose response, these results are not atypical of the response to CNS-active drugs with mixed agonist/antagonist effects. Also, while not statistically powered, the RAD1901 10mg dose nonetheless showed statistical difference ($p < 0.05$) from placebo in HF frequency, both M/S and overall, or composite score, at either the 3- or 4-week time point. Further, mean severity score was reduced by 28% after 4 weeks in the 10mg group, and 90% of the patients treated with RAD1901 10mg also demonstrated a 50% or greater reduction in HF frequency by week 4.

Overall, RAD1901 was well tolerated. No serious adverse events occurred. Treatment-related adverse events were reported in 38% of patients and were not dose-related. No change in endometrial thickness was detected.

In this POC study, RAD1901, a novel SERM, shows early evidence of potential benefit on menopausal symptoms, specifically at the 10mg dose level. Further larger human studies are planned to confirm this finding and to further elucidate the general and tissue-specific safety profile of RAD1901.

Sources of Research Support: Radius Health, Inc.

Disclosures: LSLO: Employee, Radius Health, Inc. DM: Employee, Radius Health, Inc. JG: Employee, Radius Health, Inc. CM: Employee, Radius Health, Inc. GH: Employee, Radius Health, Inc. CRL: Employee, Radius Health, Inc.

P1-727

FGF21 Action in the Brain Increases Energy Expenditure and Insulin Sensitivity in Obese Rats.

DA Sarruf PhD¹, JP Thaler MD, PhD¹, GJ Morton PhD¹, J German BS¹, JD Fischer BS¹, K Ogimoto PhD¹ and MW Schwartz MD¹.

¹Univ of Washington Seattle, WA.

The hormone fibroblast growth factor 21 (FGF21) exerts diverse, beneficial effects on energy balance and insulin sensitivity when administered systemically to rodents with diet-induced obesity (DIO). The current studies investigate whether central FGF21 treatment recapitulates these effects. Research Design and Methods: After preliminary dose-finding studies, either saline vehicle or recombinant human FGF21 (0.4 µg/d) was infused continuously for 2 wk into the lateral cerebral ventricle of male Wistar rats rendered obese by high-fat feeding. Study endpoints included measures of energy balance (body weight, body composition, food intake, energy expenditure and circulating and hepatic lipids), glucose metabolism (insulin tolerance test, euglycemic-hyperinsulinemic clamp and hepatic expression of genes involved in glucose metabolism) and expression of liver and adipocyte genes involved in lipid metabolism and mitochondrial function. Results: Compared to vehicle, continuous intracerebroventricular (icv) infusion of FGF21 increased both food intake and energy expenditure (accompanied by increased UCP1 mRNA expression in WAT) in rats with DIO, such that neither body weight nor body composition was altered. Despite unchanged body fat content, rats treated with icv FGF21 displayed a robust increase of insulin sensitivity due to increased insulin-induced suppression of both hepatic glucose production and gluconeogenic gene expression, with no change of glucose utilization. Despite no effects on liver fat accumulation, icv FGF21 decreased the expression of genes involved in lipid storage and increased the expression of genes involved in lipid oxidation relative to veh. Conclusions: FGF21 action in the brain increases hepatic insulin sensitivity and metabolic rate in rats with DIO. These findings identify the CNS as a potentially important target for the beneficial effects of FGF21 in the treatment of diabetes and obesity.

Nothing to Disclose: DAS, JPT, GJM, JG, JDF, KO, MWS

P1-728

Role of the Orphan Nuclear Receptor SHP in Diet-Induced Obesity and Diabetes.

YK Lee Ph.D.¹, YJ Park M.D., Ph.D.² and DD Moore Ph.D.³.

¹Northeastern Ohio Univs Colls of Med Rootstown, OH ; ²Seoul Natl Univ Bundang Hosp Seongnam, Korea and ³Baylor Coll of Med Houston, TX.

The orphan nuclear receptor SHP is associated with human obesity and diabetes. An earlier study with mixed background SHP^{-/-} revealed that they were protected from diet-induced obesity (DIO), glucose and insulin resistance, and hepatic steatosis (1). These phenotypes were attributed to increased whole body energy expenditure by brown adipose tissue, where major genes involved energy expenditure were up-regulated due to loss of SHP. Congenic SHP^{-/-} mice on C57Bl/6 background fed the same western diet (WestD) were also resistant to DIO, but the expression of these brown adipose genes was not altered. Surprisingly, the congenic SHP^{-/-} mice became more glucose resistant even though they were leaner. Several other distinctive metabolic phenotypes were manifested under the WestD regimen: they showed hepatic insulin resistance with higher β -oxidation; they maintained a higher respiratory quotient (RQ), which did not decrease even after WestD feeding; and they displayed islet dysfunction and pancreatic exocrine insufficiency. These results suggest that SHP plays a pivotal role as a master metabolic switch in response to energy-laden WestD while its failure results in dysregulated lipid metabolism, hepatic insulin resistance, and pancreatic dysfunction.

Nothing to Disclose: YKL, YJP, DDM

P1-729

Characterizing the *In Vivo* Physiological Roles for ER α AF-1 and AF-2 Using a Mutant AF-2 Knock-In Mouse Line.

Y Arao PhD¹, K Hamilton¹, M Ray PhD¹, G Scott¹ and KS Korach PhD¹.

¹Natl Inst of Environmental Hlth Scis Research Triangle Park, NC.

Estrogen (E) has many biological actions in different tissues involving gene expression regulated by estrogen receptors (ER) α and β as ligand-dependent transcription factors, composed of functional domains: DNA binding domain, ligand binding domain (LBD), ligand-dependent transcription activation domain (AF2) and ligand-independent transcription activation domain (AF1). AF1 and AF2 have been shown *in vitro* cell lines to have different functionality and receptor agonist responsiveness. ER ligands bind to the LBD that contains the AF2 and induces a conformational change of this domain. Helix 12, a part of AF2, plays a crucial role in determining interactions with coactivators and corepressors for transcriptional regulation. ER α knock-out (α ERKO) mice have revealed the various physiological phenomena involving ER α , however, this model is unable to further identify the selective functionality of AF1 or AF2 *in vivo*. To evaluate the physiological AF1 and AF2 functions, a non-functional AF2 ER α knock-in (AF2ERKI) mouse model was generated. AF2ERKI mice have two point mutations of leucines 543 and 544 to alanines in helix 12 (AF2ER). Studies *in vitro* suggest AF2ER mutant binds DNA sequences and hormone with the same affinity as wild-type (WT) ER α . However, even with E binding AF2ER is not transcriptionally activated due to the failure to recruit transcriptional coactivators. In addition, AF2ER changes the properties of the antiestrogen, ICI182780 (ICI) to a full agonist involving AF1. AF2ERKI homozygote females had hypoplastic uterine tissue and rudimentary mammary gland ductal trees similar to α ERKO mice. Females were infertile due to anovulation from hemorrhagic cystic ovaries, involving elevated serum LH and E levels. Western blot and IHC for ER α revealed the same uterine ER α protein level in AF2ERKI homozygote as WT. The AF2ERKI phenotype confirms that the disruption of ligand-dependent AF2-mediated transactivation is essential for maintaining many female α ERKO phenotypes. The agonist functionality of ICI was tested in AF2ERKI mice with stimulation of an ER α regulated uterine gene *Igf1*. *Igf1* was stimulated by ICI in AF2ERKI mice but not WT, while WT mice responded to E but not ICI, showing the unique properties of this mutant AF2ER is functional *in vivo* for hormonal gene expression. Future studies of other phenotypes in this model will allow *in vivo* evaluation of ER α AF1-mediated biological pathways and responses.

Nothing to Disclose: YA, KH, MR, GS, KSK