Are differences in methylation in cord blood DNA associated with prenatal exposure to alcohol?

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Outline

• Background
  – Rationale
  – Objectives
  – Relevance and importance

• Methylation experiments
  – Population
  – Study design
  – Results

• Discussion
  – Replication / validation
  – Future work
Background

Prenatal alcohol exposure and neurodevelopment

• Heavy drinking causes FAS and FASD
• Moderate maternal drinking even suggested protective
• Safe threshold?  →  Mixed messages

Need for causal analyses and elucidation of pathways
DNA methylation and prenatal alcohol exposure

Prenatal alcohol exposure → Epigenetic effects - DNA methylation → Neuro-development

- Moderate levels of exposure
- Early gestation – comparable to before pregnancy recognition in humans
Objectives

To investigate cord-blood DNA methylation profiles of offspring differentially exposed to alcohol *in utero*:

1. Across the genome

2. In candidate genes in pathways likely to be targeted/disrupted by early ethanol exposure

• With emphasis on causal associations
Relevance and importance

- Behavioural Risk Factors Surveillance System US
- Binge drinking: 5+ drinks/occasion in past 30 days
ALSPAC
Avon Longitudinal Study of Parents and Children

- 14,541 pregnancies in 1991-92
- Prospective q.aires – alcohol exposure and confounders
- biobank – GWAS, DNA methylation
- Clinical data collections + record linkage

Two complementary experiments

- Lower self-reported maternal alcohol use
- Higher self-reported maternal alcohol use

- Maternal ADH1B*2 genotype
- Maternal ADH1B*1 genotype

Offspring (Cord blood) DNA methylation
- 450k CpGs genome-wide
- 96 CpGs in candidate genes

N = 914
N = 49
N = 49
N = 272

!! Confounding !!
No confounding

N = 49
1. Epigenome-wide association study: Exposure based on maternal self-reports
ARIES is a BBSRC-funded resource to generate epigenomic information on a range of human tissues at multiple timepoints across the lifecourse. The epigenetic data will be integrated with genetic and transcriptomic data and made available to the scientific community, with the facility to link data to both exposure and phenotypic data.

ARIES will use both Illumina Infinium 450k methylation arrays and BS-sequencing approaches to generate epigenetic data samples from the ALSPAC cohort. In addition, genome-wide DNA methylation analysis of four human tissues in cohort members and matched peripheral blood samples will also be performed using Illumina Infinium 450k methylation arrays to enable comparison of methylation levels between tissues.
EWAS – methylation across 450k CpG

Linear regressions of probes surviving extensive QC ~250k CpGs

‘Hits’ surviving FDR<0.05 corrections
EWAS – methylation

Adjusted for:

**maternal** age, social class, education, depression, smoking, parity, ethnicity

**child** sex, ethnicity
2. Mendelian randomization: Minimising confounding

Maternal genotype → Fetal exposure → Fetal outcome

Confounders
Alcohol metabolism and ADH1B variant

Choice of candidate CpG sites

- 96-probe custom Illumina VeraCode array (Beadxpress)

- 50 Candidate genes (cost limitations)
  - Evidence from mice differentially exposed to alcohol *in utero* (genome-wide studies)
  - Genes with role in features of FASD (cognition, growth) + imprinted genes
  - Examples: IGF2, BDNF, CRF/CHRH1, GABRA ... also DNMTs

- ~2 CpG sites per gene
  - Included in Illumina Infinium 450k
  - Avoid allele-specific methylation sites / SNPs (reduced power)
  - In CpG islands/shores extending to promoter regions (500bp from TSS) or enhancers
  - Evidence of DMR across individuals in cord blood DNA, and in fetal brain (HEA)
MR - Methylation differences across 94 CpG

Model including random batch effects and robust standard errors

Survives multiple testing correction:

cg01534217 (FOXP1)
p = 0.00016
Beta = -0.005
Se = 0.001
Associations between maternal alcohol consumption and offspring cord blood methylation: selected probes

Associations between maternal alcohol consumption and offspring cord blood methylation: random probes
Discussion – results

- EWAS of prenatal alcohol exposure based on maternal q.aire yields 34 differentially methylated probes
  - But only for one of the definitions of prenatal alcohol exposure and results dramatically affected by (measured) confounders

- Mendelian randomization analysis highlighted a CpG in FOXP1, amongst a set of 96 highly selected probes based on priors
  - Associated with mental retardation
  - Same direction of association (hypermethylated by alcohol)
  - Expressed in fetal brain (GO : in utero fetal development)
Discussion – inference

• Replication
  • Direction of effect of CpG in FOXP1 replicated in largely independent cohort
• Validation
  • VeraCode Vs 450k – Spearman rank (to do...)
• Causality
  • Mendelian randomization minimises confounding
• Specificity
  • Association of alcohol with candidate CpGs Vs random CpGs
• Relevance for ‘target’ tissue
  • Probes showing variation in methylation levels in fetal brain (Human Epigenome Atlas)
Future directions

- Still a lot of work!
- Validation of VeraCode readings against Infinium 450k
- Causal exploration of EWAS signals
- Replication in independent cohort
- Correlation with methylation signatures in fetal brain
- Persistence of signals into childhood and adolescence
- Full mediation analysis prenatal alcohol exposure – ND outcomes
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ALSPAC cohort

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