Genetic Determinants of Type 2 Diabetes

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ABSTRACT

Hyperglycemia of type 2 diabetes mellitus (T2DM) results from a complex interplay of genetic and environmental factors that influence a number of intermediate traits (e.g., β-cell mass, insulin secretion, insulin action, fat distribution, obesity). The primary biochemical events leading to diabetes are still unknown in most cases. Although several monogenic forms of diabetes have been identified, T2DM seems to be a polygenic disorder in the majority of cases. T2DM is probably also multigenic, meaning that many different combinations of gene defects may exist among diabetic patients. Significant results were obtained in the identification of the genetic determinants of monogenic forms of diabetes with young age of onset. However, despite the evidence for a strong genetic background, little of the genetic risk factors for the more-common forms of polygenic T2DM are known to date. The goal of this chapter is to summarize and discuss the significant results of recent literature on the genetics of both the monogenic and polygenic forms of T2DM.

I. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous syndrome resulting from defects of both insulin secretion and action (DeFronzo, 1997). The precise molecular mechanisms leading to chronic hyperglycemia are largely unknown (Ferrannini, 1998). It is generally accepted that T2DM results from a complex interplay of genetic and environmental factors that influence a number of intermediate traits of relevance to the diabetic phenotype (e.g., β-cell mass, insulin secretion, insulin action, fat distribution, obesity). In fact, T2DM appears to be composed of subtypes whereby genetic susceptibility is strongly associated with environmental factors at one end of the spectrum and highly genetic forms at the other end. Although several monogenic forms of diabetes have been identified — such as maturity onset diabetes of the young (MODY) and maternally inherited diabetes and deafness (MIDD) (Froguel and Velho, 1999; van den Ouweland et al., 1994) — T2DM seems to be a polygenic disorder in the majority of cases. T2DM shows clear familial aggregation but does not segregate in classical Mendelian fashion. T2DM seems to result either from several combined gene defects or from the simultaneous action of several susceptibility alleles or else from combinations of

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frequent variants at several loci that may have deleterious effects when predisposing environmental factors are present. Type 2 diabetes is probably also multigenic, meaning that many different combinations of gene defects may exist among diabetic patients.

The primary biochemical events leading to diabetes are still unknown in most cases. Genetic and environmental factors may affect both insulin secretion and insulin action (DeFronzo, 1997). A variety of environmental factors can be implicated in the clinical expression of T2DM, such as the degree and type of obesity, sedentarity, malnutrition in fetal and perinatal periods, lifestyle, and different kinds of drugs (e.g, steroids, diuretics, antihypertensive agents). It is noteworthy that obesity, which is one of the so-called environmental determinants of T2DM, is also clearly under genetic control (Hager *et al.,* 1998). Both disorders are frequently associated and share many metabolic abnormalities, which suggests that they might also share susceptibility genes (Carmelli *et al.,* 1994). Moreover, retrospective studies showed that low birth weight was associated with insulin resistance and T2DM in adulthood (Hales *et al.,* 1991; Lithell *et al.,* 1996). It has been proposed that this association results from a metabolic adaptation to poor fetal nutrition (Barker, 1995). However, the identification of gene variants that contribute both to variation in fetal growth and to the susceptibility to T2DM suggests that this metabolic "programming" could be partly genetically determined (Hattersley and Tooke, 1999).

These complex interactions between genes and environment complicate the task of identifying any single genetic susceptibility factor to T2DM. Three general approaches have been adopted to search for genes underlying complex traits such as T2DM. The first approach is to focus on candidate genes — that is, genes selected as having a plausible role in the control of glucose homeostasis — on the basis of their known or presumed biological functions. Although this approach led to the identification of several susceptibility genes with small effects (see below), no genes with moderate or major effect on the polygenic forms of diabetes have been identified. Possible explanations for this failure to identify genes with a major effect include the possibility that they do not exist. It is also possible that our ignorance of the pathophysiological mechanisms of T2DM (and the genes that control them) has misled our choice of candidates. The second approach is to perform genome-wide scans for linkage in collections of nuclear families or sib-pairs with T2DM. This strategy requires no presumptions as to the function of the susceptibility loci. Although a large number of regions of presumed linkage have been mapped (Elbein *et al.,* 1999; Hanis *et al.,* 1996; Mahtani *et al.,* 1996; Pratley *et al.,* 1998), identification of the susceptibility genes is proceeding at very slow pace. The third approach to identify diabetes genes is to study spontaneous (Naggert *et al.,* 1995), bred (Gauguier *et al.,* 1996), or transgenic (Ahlgren *et al.,* 1998; Jonsson *et al.,* 1994; Kulkarni *et al.,* 1999; Naya *et al.,* 1997; Pontoglio *et al.,* 1998; Withers *et al.,* 1998) animal models of T2DM. The genes responsible

for diabetes in these models may not necessarily be major players in typical T2DM in humans. However, such studies provide the most-direct way to improve overall understanding of the molecular circuitry that maintains glucose homeostasis. Nevertheless, despite the evidence for a strong genetic background in T2DM, very few of the genetic risk factors for T2DM are known. Most of the available results were obtained by studying the highly familial and monogenic forms of diabetes with young age of onset.

II. Genetics of MODY

The well-defined mode of inheritance with high penetrance and the early age of onset of diabetes allows the collection of multigenerational pedigrees, making MODY an attractive model for genetic studies. MODY is a familial form of non-insulin dependent diabetes (NIDDM) with autosomal dominant inheritance. MODY usually develops at childhood, adolescence, or young adulthood and presents primary insulin-secretion defects (Froguel and Velho, 1999; Velho and Froguel, 1998). MODY is not a single entity but presents genetic, metabolic, and clinical heterogeneity. Mutations in six genes cause most of the MODY cases. These genes encode the enzyme glucokinase (MODY2/GCK) (Froguel *et al.,* 1992,1993; Velho *et al.,* 1997) and the transcription factors hepatocyte nuclear factor 4 alpha (HNF-4/MODY1) (Bell *et al.,* 1991; Yamagata *et al.,* 1996a), HNF 1 alpha (HNF-1/MODY3) (Vaxillaire *et al.,* 1995,1997; Yamagata *et al.,* 1996b), insulin promoter factor 1 (IPF-1/MODY4) (Stoffers *et al.,* 1997a,1997b), HNF 1 beta (HNF-1/MODY5) (Horikawa *et al.,* 1997), and NeuroD1/Beta2 (Malecki *et al.,* 1999). Moreover, additional MODY genes probably exist, since there are families in which MODY does not co-segregate with markers tightly linked to the known MODY loci (Chèvre *et al.,* 1998).

Although the prevalence of MODY is unknown, it has been estimated that 2 to 5 percent of patients with T2DM may, in fact, have MODY (Ledermann, 1995). The relative prevalence of the different subtypes of MODY has been shown to vary greatly in studies of British, French, German, and Spanish families (Chèvre *et al.,* 1998; Costa *et al.,* 2000; Frayling *et al.,* 1997; Lindner *et al.,* 1999). MODY2 represents from 8 to 63 percent of cases (the most-prevalent form in French families) and MODY3 from 21 to 64 percent of cases (the most-prevalent form in British families). The other MODY subtypes are rare disorders in all these populations, having been described only in a few families. Additional unknown MODY locus/loci (MODY-X) represent 16 to 45 percent of the cases of MODY (the most-prevalent form in German and Spanish families). These contrasting results may be due to differences in the genetic background of these populations or else may reflect, at least partially, ascertainment bias in the recruitment of families.

A. GLUCOKINASE MUTATIONS AND MODY2

Glucokinase (GCK) phosphorylates glucose to glucose-6-phosphate in pancreatic β cells and hepatocytes and plays a major role in the regulation and integration of glucose metabolism (Matschinsky, 1996). More than 80 different GCK mutations have been observed (Blanché et al., 1997; Velho et al., 1997). Expression studies have shown that the enzymatic activity of the mutant proteins was impaired (Gidh-Jain et al., 1993), resulting in decreased glycolytic flux in pancreatic β cells (Sturis et al., 1994). This defect translates in vivo as a glucose-sensing defect, leading to an increase in the blood glucose threshold that triggers insulin secretion (Velho et al., 1992) and a right shift in the dose-response curve of glucose-induced insulin secretion (Byrne et al., 1994). Decreased net accumulation of hepatic glycogen and augmented hepatic gluconeogenesis following meals were observed in GCK-deficient subjects and contribute to the post-prandial hyperglycemia of MODY2 (Velho et al., 1996b). Despite these multiple defects in the pancreas and the liver, the hyperglycemia associated with GCK mutations is often mild, with fewer than 50 percent of subjects presenting overt diabetes (Velho et al., 1997). There is a lower prevalence of diabetes microvascular complications (i.e., retinopathy and proteinuria) in MODY2 than in other subtypes of MODY and late-onset T2DM (Velho et al., 1996c,1997).

B. MUTATIONS IN TRANSCRIPTION FACTOR GENES

Positional cloning of MODY loci and studies in candidate genes have led to the identification of mutations in six transcription factors: HNF-1α, HNF-1β, HNF-4α, IPF1, and NeuroD1/Beta2 (Horikawa et al., 1997; Malecki et al., 1999; Stoffers et al., 1997a; Yamagata et al., 1996a,1996b). Gene targeting experiments in animals recently has demonstrated that many of these islet-expressed genes have a key role in fetal development, β-cell differentiation, proliferation, and neogenesis (Ahlgren et al., 1998; Jonsson et al., 1994; Naya et al., 1997). Mutations in HNF-1α account for most of the mutations associated with MODY identified in nuclear factors. More than 80 different mutations located in the coding regions or in the promoter were found in various populations (Boutin et al., 1997; Chèvre et al., 1997; Frayling et al., 1997; Glucksmann et al., 1997; Hansen et al., 1997; Kaisaki et al., 1997; Vaxillaire et al., 1997; Yamagata et al., 1996b). An insulin secretory defect in the absence of insulin resistance was observed in diabetic and nondiabetic carriers of MODY3 mutations (Byrne et al., 1996; Vaxillaire et al., 1999b), suggesting that HNF-1α is, indeed, implicated in pancreatic β-cell function. In contrast to the usually mild hyperglycemia due to glucokinase deficiency, MODY3 is a severe form of diabetes, often evolving to insulin requirement. Microvascular complications of diabetes are observed as frequently in MODY3 as in late age of onset T2DM subjects (Isomaa et al., 1998;

Velho *et al.*, 1996c). HNF-1α also is expressed in the kidney; a defect in the renal resorption of glucose often is associated with the pancreatic β-cell defect in MODY3 subjects (Menzel *et al.*, 1998; Velho *et al.*, 1998). Heterozygous knock-out mice lacking one copy of HNF-1α have a normal phenotype, while MODY3 subjects are all heterozygous for their mutations and fully express the diabetes phenotype (Pontoglio *et al.*, 1996,1998). This observation suggests that these mutations might have a dominant-negative effect. However, experimental data show that only the mutations located in the transactivation domain of HNF-1α have a dominant-negative effect on HNF-1α transactivation potential (Vaxillaire *et al.*, 1999a). Mutations located elsewhere in the protein do not interfere with the activity of the normal allele. The target genes associated with the β-cell defect of MODY3 remain unknown. Contrasting results were observed in studies of knock-out mice (Dukes *et al.*, 1998; Okita *et al.*, 1999; Pontoglio *et al.*, 1998; Wang *et al.*, 1998), notably regarding the role of the insulin gene.

MODY1 is much less prevalent than MODY2 and MODY3. Only a few kindred other than the large American RW family were found to carry an HNF-4α mutation (Bulman *et al.*, 1997; Furuta *et al.*, 1997; Lindner *et al.*, 1997; Yamagata *et al.*, 1996a). HNF-4α is a member of the steroid/thyroid hormone receptor superfamily and upstream regulator of HNF-1α expression. Interestingly, it was demonstrated that long-chain fatty acids directly modulate the transcriptional activity of HNF-4α by binding as acyl-CoA thioesters to the ligand binding domain of HNF-4α (Hertz *et al.*, 1998). This binding results in the activation or the inhibition of HNF-4α transcriptional activity as a function of chain length and the degree of saturation of the fatty acyl-CoA ligand (Hertz *et al.*, 1998). This observation contributes important data to the understanding of the role of dietary fats in the control of insulin secretion. Here, again, the target genes of HNF-4α associated with β-cell defect are not clearly determined (Stoffel and Duncan, 1997).

Mutations in HNF-1β recently were described in a few families with familial diabetes with early onset consistent with MODY (Horikawa *et al.*, 1997; Nishigori *et al.*, 1998). In these pedigrees, HNF-1β mutations were associated with diabetes and severe kidney disease that may appear before the impairment of glucose tolerance. Polycystic renal disease and/or particular histological abnormalities showing meganephrons were present in some subjects, suggesting that this gene could play a major role in kidney development and nephron differentiation. It is noteworthy that HNF-1β and HNF-1α can form heterodimers to bind DNA (Tronche and Yaniv, 1992).

All of these genetic defects in transcription factors lead to abnormalities of glucose homeostasis, thereby promoting the development of chronic hyperglyce-mia through alterations in insulin secretion and possibly in the development of

the pancreatic islets. In this regard, a deletion in the homeodomain transcription factor insulin promoter factor-1 (IPF-1 or IDX-1, STF-1, PDX-1) was found to co-segregate with MODY in a large kindred presenting a consanguineous link (Stoffers *et al.*, 1997a). This mutation results in a premature stop codon and a protein lacking a domain that is crucial for DNA binding. The phenotype of the subjects who are heterozygous for the mutation ranges from normal to impaired glucose tolerance to overt NIDDM. One child who is homozygous for the mutation was born with pancreatic agenesis and suffers from diabetes as well as exocrine insufficiency (Stoffers *et al.*, 1997b). IPF-1 is critically required for the embryonic development of the pancreatic islets as well as for transcriptional regulation of endocrine pancreatic tissue-specific genes in adults, such as the insulin, glucose transporter-2 (GLUT2), and glucokinase genes in β cells and the somatostatin gene in δ-cells. IPF-1 normally is expressed in all cells of the pancreatic bud. Its absence in mice arrests development at the bud stage, leading to pancreatic agenesis (Jonsson *et al.*, 1994).

The transcription factor NeuroD1 (also known as Beta 2) is involved in the regulation of endocrine pancreas development. In mice homozygous for a targeted disruption of NeuroD1, pancreatic islet morphogenesis is abnormal and hyperglycemia develops, due in part to inadequate expression of the insulin gene (Naya *et al.*, 1997). Recently, mutations in NeuroD1 were shown to co-segregate with T2DM of early age of onset and autosomal dominant-like transmission in two Caucasian kindreds (Malecki *et al.*, 1999). This observation suggests that NeuroD1 might play an important role in endocrine pancreas development and/or insulin gene expression in humans.

III. Mitochondrial Diabetes and Wolfram Syndrome

Mitochondria contain their own genetic information in the form of a circular DNA molecule of 16,569 base pairs that encodes 13 subunits of the oxidative phosphorylation complex, two ribosomal RNAs, and 22 transfer RNAs (tRNA) needed for mitochondrial protein synthesis. Several mitochondrial cytopathies and syndromes caused by point mutations, deletions, or duplications of mitochondrial DNA (mtDNA) and characterized by decreased oxidative phosphorylation are associated with diabetes (Gerbitz *et al.*, 1995; Luft and Landau, 1995). Moreover, about 40 point mutations of mtDNA have been identified in subjects and families having maternally inherited diabetes as the main phenotypic trait (Mathews and Berdanier, 1998). Only one of these mutations, an A-to-G transition in the mitochondrial tRNA[Leu(UUR)] gene at base-pair 3243, has been systematically tested and phenotypically characterized in several populations (Maassen and Kadowaki, 1996; Massin *et al.*, 1995; Van den Ouweland *et al.*, 1992,1994; Vialettes *et al.*, 1995,1997; Vionnet *et al.*, 1993). It co-segregates in families with diabetes and

sensorineural deafness of maternal transmission, a syndrome known as maternally inherited diabetes and deafness (MIDD). In some populations, MIDD might represent 1 to 3 percent of all cases of T2DM. The same mutation was observed in patients with MELAS — a syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes – that is often accompanied by diabetes and deafness (Ciafaloni *et al.*, 1992). The mechanisms underlying the different phenotypic expression (MIDD or MELAS) are unknown but might be related to the variable degree of heteroplasmy in different tissues.

Subjects with the 3243 mutation may present with variable clinical features, ranging from normal glucose tolerance to insulin-requiring diabetes. However, abnormalities in insulin secretion were found in all MIDD subjects that were tested, including those with normal glucose tolerance (Velho *et al.*, 1996a). The pathophysiological mechanisms leading to hyperglycemia and often to insulin-requiring diabetes in this syndrome are probably complex and multifactorial and might include defects in insulin production or glucose toxicity as well as insulin resistance. However, a defect of glucose-regulated insulin secretion is an early, possible primary abnormality in carriers of the mutation (Velho *et al.*, 1996a). This defect probably results from the progressive reduction of oxidative phosphorylation in β cells caused by the accumulation of mutant mitochondrial DNA (Luft and Landau, 1995; Maassen and Kadowaki, 1996).

Wolfram syndrome (or the acronym DIDMOAD) describes patients with diabetes insipidus, diabetes mellitus, optical atrophy, and deafness. Other endocrine and neurological abnormalities often are associated in this genetically and clinically heterogeneous syndrome. Wolfram syndrome is frequently transmitted as an autosomal recessive disorder by a locus mapped to the short arm of chromosome 4. This gene, named WFS1, recently was identified (Inoue *et al.*, 1998; Strom *et al.*, 1998). It encodes wolframin, a protein showing no perceptible homology to known DNA or protein sequences (Gerbitz, 1999). The physiological function of wolframin and its link to diabetes remain unclear. In contrast to this autosomal recessive transmission, a few cases of Wolfram syndrome were found to be associated with mitochondrial DNA mutations (Pilz *et al.*, 1994; Rotig *et al.*, 1993).

IV. Candidate Genes and Polygenic Forms of T2DM

The majority of the genes found to play a role in the common forms of T2DM have been identified by testing candidate genes, the most-used approach, up to now, to tackle the genetic determinants of T2DM. Reasons for candidacy are numerous: 1) known or presumed biological function in glucose homeostasis or energy balance in humans; 2) gene implicated in subtypes of diabetes (e.g., MODY); 3) gene associated with diabetes or associated traits in animal models;

4) gene responsible for an inherited disease that includes diabetes (e.g., mitochondrial cytopathies, Wolfram syndrome); or 5) product differentially expressed in diabetic and normal tissues.

For obvious reasons, the insulin gene was among the first genes to be studied. Mutations in the coding regions of the insulin gene (chromosome 11p) have been reported in less than 10 families but are not consistently associated with T2DM (Haneda *et al.*, 1984). However, mutations in the promoter region could affect the regulation of the insulin gene, leading to a decrease of transcription and absolute or relative hypoinsulinemia. A variant allele of the promoter was observed in about 5 percent of African Americans with T2DM and shown to be associated with decreased transcriptional activity (Olansky *et al.*, 1992). More recently, an association between T2DM and paternally transmitted class III alleles of the variable region upstream of the insulin gene (INS-VNTR) was observed in British families (Huxtable *et al.*, 2000). Interestingly, class III alleles also were found to be associated with increased length and weight at birth (Dunger *et al.*, 1998) and with a dominant protection against type 1 diabetes (Bennett *et al.*, 1995), as compared with type I alleles.

The role of the MODY genes and of other transcription factors in the development of the more-common forms of late-onset T2DM is still under investigation. Regarding the MODY genes, most studies have excluded a major role in the genetic determinants of T2DM. However, mutations in HNF-1α were identified in African-American and Japanese subjects with atypical non-autoimmune diabetes of acute onset (Boutin *et al.*, 1999; Iwasaki *et al.*, 1997). A common polymorphism in HNF-1α was found to be associated with mild insulin secretion defects (Urhammer *et al.*, 1997). Moreover, mutations in HNF-4α (Hani *et al.*, 1998b) and IPF1 (Hani *et al.*, 1999; Macfarlane *et al.*, 1999) were identified in a few families with late-onset T2DM. Several other transcription factors have been studied: a mutation in islet brain 1 (IB1) was found to be associated with diabetes in one family (Waeber *et al.*, 2000). IB1 is a homologue of the c-jun amino-terminal kinase interacting protein 1 (JIP-1), which plays a role in the modulation of apoptosis. IB1 is also a transactivator of the islet glucose transporter, GLUT2. The mutant IB1 was found to be unable to prevent apoptosis *in vitro*. It is thus possible that the abnormal function of this mutant IB1 may render β cells more susceptible to apoptotic stimuli, thus decreasing β-cell mass. As glucotoxicity and lipotoxicity are known to induce both apoptosis and transcription factor downregulation in pancreatic β cells, inherited or acquired limitations in IB1 activity could have deleterious effects in β-cell function.

On the other hand, mutations in peroxisome proliferator-activated receptor gamma (PPARγ) that severely decrease the transactivation potential were found to co-segregate with extreme insulin resistance, diabetes, and hypertension in two families with autosomal dominant inheritance (Barroso *et al.*, 1999). Interestingly,

given the proposed role of PPARγ in adipogenesis, all affected family members had no evidence of lipoatrophy or abnormal fat distribution. All together, these data suggest that mutations in transcription factors may contribute to the genetic risk to T2DM through various mechanisms: 1) dysregulation of target genes involved in glucose or lipid metabolism (i.e., HNFs, PPARγ, IPF1, IB1); 2) abnormal β-cell development and differentiation (i.e., IPF1, NeuroD1/Beta2); or 3) dysregulation of β-cell apoptosis (IB1). Deleterious mutations that significantly impair the transactivation activity of these transcription factors can be responsible in some families for monogenic-like forms of diabetes with late age of onset, which may represent an intermediary phenotype between MODY and the most-common forms of T2DM.

Other genes encoding key components of insulin secretion pathways were tested as potential candidates for a role in the genetic susceptibility of T2DM. The pancreatic β-cell ATP-sensitive potassium channel (IKATP) plays a central role in glucose-induced insulin secretion by linking signals derived from glucose metabolism to cell membrane depolarization and insulin exocytosis (Dukes and Philipson, 1996). IKATP is composed of two distinct subunits: an inwardly rectifying ion channel forming the pore (Kir6.2) and a regulatory subunit, a sulfonylurea receptor (SUR1) belonging to the ATP binding cassette (ABC) superfamily (Inagaki et al., 1995). The genes encoding these two subunits are located 4.5 kb apart on human chromosome 11p15.1. Mutations in each of these genes may result in familial persistent hyperinsulinemic hypoglycemia of infancy, demonstrating their role in the regulation of insulin secretion (Ashcroft and Gribble, 1999). Studies in various populations with different ethnic backgrounds provided evidence for associations of single nucleotide polymorphisms (SNPs) in these genes with T2DM ('t Hart et al., 1999; Hani et al.,1997,1998a; Hansen et al., 1998; Inoue et al., 1996; Ohta et al., 1998). However, sib-pair analyses in several populations indicated that the SUR1/Kir6.2 region is not a major diabetogenic locus (Hani et al., 1997; Iwasaki et al., 1996; Stirling et al., 1995).

Key components of the insulin-signaling pathways also were tested. They were at first thought to be important players in the context of the insulin resistance of T2DM. Several of these genes are expressed in pancreatic β cells. Recent results in knockout animals demonstrated that they also play an important role in the mechanisms of insulin secretion (Kulkarni et al., 1999; Withers et al., 1998). More than 50 different mutations have been found in the coding regions of the insulin receptor gene on chromosome 19p (Taylor, 1992). However, patients with these mutations seldom present with the common form of T2DM (Kan et al., 1995) but rather with syndromes of severe insulin resistance associated with leprechaunism or with acanthosis nigricans, hirsutism, or major hyperinsulinemia (Flier, 1992). Missense variants in the coding regions of the gene encoding the first substrate for the insulin receptor kinase (IRS-1) on chromosome 2q have been detected in

several populations (Almind *et al.*, 1993; Hager *et al.*, 1993; Hitman *et al.*, 1995; Ura *et al.*, 1996). However, association of these variants with diabetes was not observed in all these studies. Similarly, an association between polymorphisms of the muscle glycogen synthase gene (GSY1) on chromosome 19q and T2DM was observed in Finnish (Groop *et al.*, 1993) and in Japanese (Kuroyama *et al.*, 1994) subjects but not in French ones (Zouali *et al.*, 1993). Taken together, these results suggest that IRS-1 and GSY1 genes might act in some populations as minor susceptibility genes, which are neither necessary nor sufficient for disease expression but may nevertheless modulate the phenotype of patients.

Other genes were shown to be implicated in the genetic susceptibility to insulin resistance. Although they do not seem to be directly linked to or associated with T2DM, they could modulate the expression of diabetes. A common and widespread polymorphism at codon 905 of the gene encoding the glycogen-associated regulatory subunit of protein phosphatase-1 of the skeletal muscle was shown to be associated with insulin resistance and hypersecretion of insulin in Danish T2DM subjects (Hansen *et al.*, 1995). A missense mutation in the intestinal fatty acid binding protein 2 (FABP2) gene on chromosome 4q was found to be associated with increased fatty acid binding, increased fat oxidation, and insulin resistance in the Pima Indians of Arizona (Baier *et al.*, 1995), an ethnic group with the highest reported prevalence of T2DM and insulin resistance in the world. A point mutation in the gene encoding the beta-3 adrenergic receptor was found to be associated with an increased capacity to gain weight in a population of morbidly obese subjects (Clément *et al.*, 1995). This same mutation was associated with reduced metabolic rate and early onset of diabetes (Walston *et al.*, 1995) and with the development of upper-body obesity and insulin resistance (Widen *et al.*, 1995) in two T2DM populations.

V. Positional Cloning of T2DM Genes

The candidate gene approach presents limitations, as it is now clear that at least some T2DM susceptibility genes are likely to code for proteins of unknown function or a function not obviously implicated in glucose metabolism. The genome-wide linkage approach attempts to locate these unknown genes by a systematic search throughout the genome. This consists of genotyping the entire genome of affected sib-pairs or families with panels of 250-300 anonymous polymorphic markers to identify regions showing excess of allele sharing with the disease. This total genome approach has been successful in other multifactorial diseases such as type 1 diabetes (Davies *et al.*, 1994) and obesity (Hager *et al.*, 1998). More than 20 genome scans for T2DM are currently underway, involving thousands of pedigrees from different populations and ethnic groups. One of the limitations of the genome-scan approach is the relatively low power of the method, (i.e., inabilty to detect weak linkage signal), which is due to the low relative risk

for diabetes in siblings (about a 3- to 5-fold increase, compared to the general population). Working on large family collections, in homogenous ethnic groups, or in large pedigrees using quantitative traits instead of the dichotomous diabetes status could improve the efficiency to detect linkage. Moreover, because of the large number of markers that are tested, false-positive results are likely to occur. Thus, stringent criteria for linkage ($p < 10^{-5}$) need to be used to minimize the bias due to multiple testing.

Results of several genome scans already have been published. A locus for T2DM on chromosome 2q (*NIDDM1*) was localized in Mexican Americans (Hanis *et al.*, 1996). It showed that an interaction of this locus with a locus on chromosome 15 further increases the susceptibility to diabetes in this population (Cox *et al.*, 1999). Linkage was found at a locus near *MODY3* on chromosome 12q in Finnish T2DM families characterized by a predominant insulin secretion defect (Mahtani *et al.*, 1996). Evidence for an obesity-diabetes locus on chromosome 11q23-q25 (Norman *et al.*, 1998) and linkage of several chromosomal regions with pre-diabetic traits (Pratley *et al.*, 1998) were observed in Pima Indians, an ethnic group with a high prevalence of diabetes and obesity. A strong linkage between diabetes and chromosome 1q21-1q23 was reported in multigenerational families of northern European ancestry from Utah (Elbein *et al.*, 1999). Linkages with diabetes and with the age at onset of diabetes were found in a region on chromosome 10q in Mexican American families from San Antonio (Duggirala *et al.*, 1999). Evidence for the presence of one or more diabetes loci on chromosome 20 was found in different populations (Ji *et al.*, 1997; Zouali *et al.*, 1997). In these and other studies, many loci showing only suggestive or weak indication of linkage with diabetes-related traits have been reported, several of which fall in overlapping regions. Although many of these loci may represent false-positive results, some may harbor true diabetes-susceptibility genes. Comparisons of linkage result in different populations or family collections and/or meta-analysis of the data may help to guide positional cloning efforts. New statistical methods exploiting multiloci effects or analyzing quantitative traits should permit researchers to squeeze more power from genome-scan data.

These genome scans have mapped loci within large chromosomal regions containing 10-20 million nucleotides. Now, the challenge is to identify the diabetes-related genes within this interval. The classical approach that consisted of building a physical map of the region through contiguous artificial chromosomes spanning the entire region of linkage, followed by the cloning of the gene, is limited by size of the regions of linkage. An integrated genomic approach might be needed. It would combine linkage disequilibrium mapping, to define more-precise gene locations, with techniques to pick out the genes of these smaller regions (e.g., microarrays for the identification of genes differentially expressed in diabetic and nondiabetic subjects). These investigations will benefit from recent technological developments in SNP identification and genotyping. Moreover, the

results from the Human Genome Project — which include genomic DNA sequences, expressed sequences, and expression profile databanks — will certainly make easier the identification of T2DM-susceptibility genes by positional cloning. The recent identification by Graeme Bell and coworkers of NIDDM1 as the gene encoding calpain 10 (cAPN10), a nonlysosomal cysteine protease, demonstrates the feasibility of positional cloning of polygenic T2DM genes. Currently, it is believed that less than 15 percent of the genetic determinants of T2DM have been unveiled. It is likely that other genes contributing to the genetic risk of T2DM will be shortly discovered.

VI. Conclusions and Perspective

The identification of T2DM genes will improve our understanding of the molecular mechanisms that maintain glucose homeostasis and of the precise molecular defects leading to chronic hyperglycemia. A nosological classification of T2DM, based on primary pathophysiological mechanisms, will then be possible. This could lead to the development of more-specifically targeted antidiabetic drugs or even gene-based therapies. Moreover, pharmacogenetic testing might then be used to predict for each patient the therapeutic response to different classes of drugs. The identification of T2DM genes also will provide the tools for the timely identification of high-risk individuals who might benefit from early behavioral or medical intervention to prevent the development of diabetes. An important reduction in diabetes-related morbidity and mortality could be then expected, along with a reduction in the costs of treatment of diabetes and its complications.

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