



Journal of Applied Sciences

ISSN 1812-5654

science
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Type 2 Diabetes: Genetic Advance and Aetiology

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Abstract: The aim of this review was to discuss the current understanding of Type 2 Diabetes Mellitus (T2DM) genetic advance and aetiology. The T2DM is a genetically heterogeneous disease, with several relatively rare monogenic forms and a number of more common forms resulting from a complex interaction of genetic and environmental factors. Earlier studies using a candidate gene approach, family linkage studies and gene expression profiling uncovered a number of T2DM genes, but the genetic basis of common T2DM remained unknown. Recent years have seen a tremendous surge in our understanding of the genetics of T2DM by Genome-Wide Association Study (GWAS). Approximately 20 genes consistently associated with T2DM mainly implicate pancreatic β -cell function in the pathogenesis of T2DM.

Key words: Type 2 Diabetes Mellitus (T2DM), susceptibility gene, aetiology

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a complex heterogeneous group of metabolic condition characterized by elevated levels of serum glucose, caused mainly by impairment in both insulin action and insulin secretion. T2DM is a complex trait where common genetic variants having modest individual effects act together and interact with environmental factors to modulate the risk of the disease. Traditional genetic research, including twin, adoption, and family studies, consistently supports that T2DM has a genetic component. Two broad approaches have been used to define the genetic predisposition of T2DM. First, molecular events in T2DM pathogenesis have been examined directly by testing the role of sequence variants of specific candidate genes. The candidate gene approach focuses on the search for an association between T2DM and sequence variants in or near biologically defined candidate genes which have been chosen based on their known physiological function. To overcome the shortcomings of the candidate gene studies, investigators have applied a genome-wide linkage scan strategy in which regularly spaced markers are traced in families and sibling pairs for segregation with T2DM. No prior knowledge of gene or gene effects is necessary, but the genetic locus must have sufficient impact on the disease susceptibility to be detectable. During the past decades, extensive efforts have been made to detect the underlying genetic structure for T2DM. However, until very recently, the genes involved have been poorly understood. Using a new and powerful

technology in the form of a genome-wide chip that genotypes up to hundreds of thousands of SNPs, Genome-Wide Association Studies (GWAS) have recently led to the discovery of a group of novel genes that were reproducibly associated with T2DM risk. These studies have increased our understanding of the genetic aetiology of T2DM and provided invaluable insights into the way genetic studies should be conducted. The present review discusses the current understanding of T2DM genetic advance and aetiology.

EVIDENCE FOR THE GENETIC COMPONENT OF T2DM

The genetic component of T2DM comes from ethnic group differences in prevalence rates. These differences in prevalence range from 1% in Mapuche Indian tribes or Chinese population living in rural areas in mainland China, to extremely high levels found in Nauru and Pima Indians in Arizona (King and Rewers, 1993). Furthermore, the prevalence is higher in full-blooded Nauruan and Pima Indians than in those with admixture (Knowler *et al.*, 1988; Serjeantson *et al.*, 1983). Another source of evidence for genetic contribution in T2DM is familial aggregation. Lifetime risk of T2DM development is 40% in offspring of one T2DM parent and increases up to 70% if both parents have T2DM (Groop and Tuomi, 1997). In addition, evidence shows a greater likelihood of T2DM in offspring of affected mothers than affected fathers, indicating an excess maternal transmission of the disease (Thomas *et al.*, 1994; De Silva *et al.*, 2002; Arfa *et al.*,

2007). Family, history of T2DM has a significant, independent and graded association with the prevalence of T2DM. In twins studies, very high concordance (55 to 100%) for T2DM has been reported among monozygotic (MZ) twins (Newman *et al.*, 1987; Barnett *et al.*, 1981).

Mutations in some genes cause rare forms of T2DM, giving additional support for the genetic roles in the aetiology of the disease. For example, genes *KCNJ11* and *ABCC8* carry rare mutations that cause a Mendelian form of neonatal diabetes (Babenko *et al.*, 2006; Gloyn *et al.*, 2004). Specific mutation in the mitochondrial genome was found to cause maternally inherited diabetes and deafness (Van Ouweland *et al.*, 1994). Maturity-onset Diabetes of the Young (MODY), which is characterized by high penetrance, early age at onset of hyperglycemia and defective function of β -cells in the pancreas, accounts for 1-5% of all T2DM cases. Mutations in *HNF4A*, *GCK*, *HNF1A*, *IPF1*, *HNF1B* (*TCF2*) and *NEUROD1* cause different subtypes of MODY1-6 (Fajans *et al.*, 2001).

SEARCH FOR T2DM SUSCEPTIBILITY GENES

Susceptibility genes identified by linkage studies: The first T2DM gene identified by linkage is *CAPN10* that belonged to the family of calcium activated non-lysosomal thiol proteinases. Horikawa *et al.* (2000) used relatively sparse Linkage Disequilibrium (LD) map of SNPs in the region of linkage on chromosome 2q to identify 3 common intronic variants of a previously unknown gene. Both a single intronic variant (UCSNP-43: G to A) and a specific haplotype combination defined by three polymorphisms (UCSNP-43, -19 and -63) were associated with T2DM in Mexican Americans, with lesser evidence of an association in a Northern European population from the Botnia region of Finland. Surprisingly, individuals with a combination of two different haplotypes were at the highest risk of T2DM (Horikawa *et al.*, 2000; Wang *et al.*, 2002). Baier *et al.* (2000) showed altered gene transcription and reduced muscle mRNA levels in muscle biopsies from Pima Indians with T2DM. *CAPN10* SNP-44 (rs2975760 g4841T/C), located in intron 3 and 11 bp from SNP-43 (rs3792267 A/G), was independently associated with T2DM in several populations and was in LD with the missense mutation Thr504Ala and two 5'-UTR variants (SNP-134 and SNP-135). An initial meta-analysis also supported an association with T2DM (Weedon *et al.*, 2003). Furthermore, *CAPN10* variants were valuable along with traditional risk factors in predicting the onset of T2DM in a prospective study of Botnian Finnish individuals (Lyssenko *et al.*, 2005).

The *TCF7L2* gene encodes for an enteroendocrine transcription factor that has a role in the Wnt signaling

pathway, which is one of the key developmental and growth regulatory mechanisms of the cell. Reynisdottir *et al.* (2003) found suggestive linkage of T2DM to chromosome 10q in the Icelandic population. Fine-mapping with 228 microsatellite markers in Icelandic individuals with T2DM and controls throughout a 10.5 Mb interval on 10q identified one microsatellite marker, DG10S478 in intron 3 of the *TCF7L2* gene, as being strongly associated with T2DM ($p = 2.1 \times 10^{-9}$) (Grant *et al.*, 2006). This association was replicated in both a Danish cohort ($p = 4.8 \times 10^{-3}$) and a US cohort ($p = 3.3 \times 10^{-9}$). Replications have appeared from analysis in subjects of Amish (Dancott *et al.*, 2006), Finnish (Scott *et al.*, 2006), UK samples (Groves *et al.*, 2006), French (Cauchi *et al.*, 2006), US (Zhang *et al.*, 2006; Saxena *et al.*, 2006), German KORA 500 K study population (Herder *et al.*, 2008), population-based study of Caucasian (Van Hoek *et al.*, 2008) and Chinese population (Ng *et al.*, 2007; Chang *et al.*, 2007). A meta-analysis of 28 original published association studies included 29,195 control subjects and 17,202 cases confirmed the association between the *TCF7L2* rs7903146 polymorphism and susceptibility to T2DM (OR = 1.46 95% CI 1.42-1.51; $p = 5.4 \times 10^{-140}$). Compared with any other gene variants previously confirmed by meta-analysis, *TCF7L2* can be distinguished by its tremendous reproducibility of association with T2DM and its OR twice as high (Cauchi *et al.*, 2007). Very recently, a large meta-analysis summarizes the strong evidence for an association between *TCF7L2* gene and T2DM both overall and in Caucasians, North Europeans, East Asians, Indians, and Africans and suggested a potential multiplicative genetic model for all the four polymorphisms (rs7903146, rs7901695, rs12255372, rs11196205) of *TCF7L2* gene among different ethnic populations except for Africans, where additive genetic mode is suggested for rs7903146 polymorphism, as well as suggests the *TCF7L2* gene involved in near 1/5 of all T2DM (Tong *et al.*, 2009). The increase in risk of T2DM associated with the *TCF7L2* variant alleles so far identified is substantially greater than that associated with variants in the other confirmed T2DM-susceptibility genes (*PPAR* (γ), *KCNJ11*). The strongest signal for T2DM in GWAS was found for *TCF7L2* rs7903146 with p-values down to $<10^{-48}$ and increase in the odds for the disease of 37% (Scott *et al.*, 2007; Saxena *et al.*, 2007). In the prediction study, *TCF7L2* rs7903146 was significantly associated with the risk of future T2DM (OR = 1.30; $p = 9.5 \times 10^{-13}$) and predicted progression from normal glucose tolerance to T2DM (OR = 1.27; $p = 2.7 \times 10^{-7}$) in the MPP cohort (Lyssenko *et al.*, 2008).

The locus at 18p was originally found to be linked to T2DM in families from Finland and Southern Sweden (Parker *et al.*, 2001) and was confirmed in a second series derived from the Southwest of the Netherlands (Van Tilburg *et al.*, 2003). The strongest evidence was obtained for marker D18S63 (LOD score 2.3, nominal $p = 0.0006$). In all three studies, the effect of the 18p region was strongest in the obese subpopulation. One of the human homologs of mouse *Lpin1* gene, *LPIN2*, is located in the 18p11 region. *LPIN2* is ubiquitously expressed in different tissues, including skeletal muscle (Zhou and Young, 2005). The *LPIN2* gene is responsible for the lipodystrophy in the fld line, which, among other traits, is characterized by severe insulin resistance. Fld mice, in which *Lpin1* is deleted, have diminished adipose tissue mass and multiple pathologies, including insulin resistance, fatty liver and progressive neuropathy of peripheral nerves (Shimomura *et al.*, 1999; Péterfy *et al.*, 2001). The protein Lipin is required upstream of $PPAR\gamma$ for normal adipocyte differentiation (Phan *et al.*, 2004). The rs3745012 SNP of the *LPIN2* gene is associated with T2DM and fat distribution (Aulchenko *et al.*, 2007).

The human *PTPNI* gene maps on chromosome 20q13.13 which showed evidence of linkage with early onset T2DM in a subset of 55 French families (Zouali *et al.*, 1997). Multiple noncoding SNPs in the *PTPNI* gene were implicated in T2DM in Caucasian and Mexican-American populations (Bento *et al.*, 2004; Palmer *et al.*, 2004), with the most consistent evidence for association occurring with SNPs spanning the 3' end of intron 1 of *PTPNI* through intron 8 ($p = 0.043-0.002$). All of the associated SNPs were present in a single 100 kb haplotype block that encompassed the *PTPNI* gene. Haplotype frequencies were significantly different between T2DM case and control subjects ($p = 0.0035-0.0056$), with a single common haplotype (36%) contributing strongly to the evidence for association, with $OR = 1.3$. Furthermore, the same haplotypes were associated with glucose homeostasis measures in Hispanic subjects (Bento *et al.*, 2004). However, Florez *et al.* (2005) failed to replicate the findings in a large Caucasian study.

Adiponectin (*APMI*) is a strong candidate for T2DM given the clear role of plasma adiponectin levels in insulin sensitivity and the fall in adiponectin levels with obesity and T2DM. Adiponectin was mapped to the region on chromosome 3q27 which found to have positive linkage to metabolic traits and T2DM (Vionnet *et al.*, 2000; Hegele *et al.*, 1999; Kissebah *et al.*, 2000). Multiple studies found evidence that promoter variants in the *APMI* gene were associated with T2DM in French and Swedish Caucasian and Japanese populations. However,

APMI variants did not appear to account for the 3q27 linkage in French families (Gibson and Froguel, 2004). *APMI* variation at positions 45 (G allele) in exon 2 and 276 in intron 2 (T allele) were associated with a 4.5 fold increased risk of converting from impaired glucose tolerance to T2DM in the STOP-NIDDM trial (Zacharova *et al.*, 2005). A meta-analysis of nine association studies included 2379 subjects confirmed significant association between the SNP45TG+GG and SNP276GG polymorphisms of *APMI* with T2DM in Chinese populations ($p = 0.05$) (Li *et al.*, 2008). The search for T2DM susceptibility genes on most other chromosomes (1q21.3-23, 2q37.3, 3p24.1, 3q28, 10q26.13, 12q24.31, and 18p11.22) is ongoing.

Susceptibility genes discovered by candidate gene association: Variants in genes encoding proteins that play a role in pathways involved in insulin control and glucose homeostasis are excellent candidates for T2DM. The candidate gene approach focuses on the search for an association between T2DM and sequence variants in or near biologically defined candidate genes which have been chosen based on their known physiological function. The importance of these variants is tested by comparing the frequency in T2DM subjects and control individuals. Numerous variants within several genes that confer an increased susceptibility to T2DM have been identified by candidate genes studies but only a small number have been identified as strong candidates for T2DM such as *PPAR* (and *KCNJ11* genes, which they were confirmed by GWAS (Zeggini *et al.*, 2007; Sladek *et al.*, 2007).

The $PPAR\gamma$ is a member of nuclear hormone receptor superfamily of transcription factors and the target of the widely used class of insulin sensitizers, the thiazolidinediones. Multiple studies have examined a Pro12Ala polymorphism in the *PPAR* (2 isoform. The rare Ala allele is seen in about 15% of Europeans and was shown to be associated with increased transcriptional activity, increased insulin sensitivity and protection against T2DM in an initial study (Deeb *et al.*, 1998). Subsequently, smaller studies provided inconsistent results. Larger studies have shown a consistently protective effect of the Ala12 allele when compared with the common Pro12 allele. The common Pro12 allele increases T2DM risk by an OR of 12.5 in a meta-analysis in cross sectional studies (Altshuler *et al.*, 2000). Several, but not all studies suggest that the rare Ala12 allele protects against insulin resistance and obesity, but the association with obesity has been inconsistent. A meta-analysis of 41 published and 2 unpublished studies included 42,910 subjects (Asia, Europe and North

America) demonstrated that the reduced risk of T2DM in Ala12 carriers is not homogeneous (Ludovico *et al.*, 2007). Recent replication studies reported significant associations between rs17036328, rs11709077, rs1801282 in *PPAR*2 and T2DM in the German KORA 500 K study population (Herder *et al.*, 2008). *PPAR*2 Pro12 allele was associated with T2DM in Hubei Han Chinese population (Dehwah *et al.*, 2008). In the prediction study, *PPAR*2 SNP rs1801282 was significantly associated with the risk of future T2DM (OR = 1.20; $p = 4.0 \times 10^{-4}$) and predicted progression from normal glucose tolerance to T2DM (OR = 1.15; $p = 0.03$) in the MPP cohort (Lyssenko *et al.*, 2008).

KCNJ11 encodes a subunit of an inwardly rectifying ATP-sensitive potassium channel I (KATP). I (KATP) channels are crucial for the regulation of glucose-induced insulin secretion in pancreatic β -cell. The β -cell potassium channel comprises two subunits, the potassium channel encoded by the gene *KCNJ11* and the regulatory subunit (SUR1 encoded by *ABCC8*) that binds sulfonylureas and ATP. Variants in both subunits have been associated with T2DM. Two variants in the *ABCC8* gene were initially associated in smaller studies (Inoue *et al.*, 1996; Hart *et al.*, 1999; Meirhaeghe *et al.*, 2001) and larger subsequent studies appeared to confirm the association (Barroso *et al.*, 2003; Hani *et al.*, 1997). However, the other studies have not confirmed the association (Gloyn *et al.*, 2003; Florez *et al.*, 2004). The association between these variants and altered insulin secretion was also observed (Elbein *et al.*, 2001; Hansen *et al.*, 1998). The adjacent *KCNJ11* gene was initially noted to have multiple nonsynonymous coding variants (Inoue *et al.*, 1997), but none were associated with T2DM (Altshuler *et al.*, 2000; Sakura *et al.*, 1996). However, a E23K polymorphism was shown to lower the sensitivity of the potassium channel to ATP and thus reduce insulin secretion *in vitro* (Schwanstecher *et al.*, 2002) and to be associated with T2DM (Barroso *et al.*, 2003; Love-Gregory *et al.*, 2003; Yokoi *et al.*, 2006). The result of Meta-analysis confirmed the association between the E23K variant and susceptibility to T2DM in UK cohort (K allele OR = 1.23; $p = 1.5 \times 10^{-5}$; KK genotype OR = 1.65; $p = 2 \times 10^{-6}$) (Gloyn *et al.*, 2003) and white case-control subjects ($n = 2,824$, OR = 1.49, $p = 2.2 \times 10^{-4}$) (Nielsen *et al.*, 2003). Subsequently, Florez *et al.* (2004) thoroughly examined *KCNJ11* and *ABCC8* and tested sufficient variants to completely tag all variation in this region. Again, the E23K variant was associated with T2DM in 3,413 subjects and the association was confirmed in a meta-analysis that included over 5000 T2DM subjects and 4747 controls ($p < 10^{-5}$, OR = 1.15). Recent replication studies reported significant associations between common variants in the

KCNJ11 genes and T2DM in a Japanese population (Omori *et al.*, 2008).

Evidence for a role of the *HNF4A* gene in T2DM predisposition is also mounting (Barroso *et al.*, 2003; Love-Gregory *et al.*, 2004; Silander *et al.*, 2004; Zhu *et al.*, 2003). *HNF4A* regulates genes involved in glucose and fatty acid metabolism, as well as insulin secretion, and is therefore critical for maintaining lipid and glucose homeostasis. The K121Q variant of the *ENPP1* gene has been best studied and shown to be associated with obesity but not T2DM in Caucasian and African American subjects ascertained from the New York Cancer Project (Matsuoka *et al.*, 2006). Other investigators found an association of K121Q with earlier onset T2DM and coronary disease among T2DM patients (Bacci *et al.*, 2005). The association of K121Q with T2DM was replicated in three relatively small populations, two of South Asian ancestry and one Caucasian (Abate *et al.*, 2001).

Minton *et al.* (2002) sequenced DNA from 29 patients and uncovered 12 SNPs of *WFS1*. The most abundant genetic variant alters the amino acid at position 611 from a histidine to an arginine (H611R). The arginine variant was present in 40% of diabetic cases and 45% of controls ($p < 0.02$), suggesting that the variant was protective from T2DM. Through large-scale candidate-pathway study, 1536 SNPs in 84 candidate genes were studied for association with T2DM, only (rs10010131, rs6446482, rs752854, rs734312) SNPs in *WFS1* were associated with T2DM in UK and an Ashkenazi Jewish population (OR = 0.92 95% CI 0.88-0.95, $p < 10^{-4}$ - 10^{-7}) (Sandhu *et al.*, 2007). The result was then replicated in northern Swedish populations, the minor allele (G) at SNP rs752854 was statistically associated with reduced risk of T2DM (OR = 0.85 95% CI 0.75-0.96, $p = 0.01$), while borderline statistical significance was observed for the other three SNPs (rs10010131, rs6446482, rs734312) (Franks *et al.*, 2008). Recent replication studies reported significant associations between *WFS1* rs10012946 (proxy for rs10010131, $r^2 = 1.0$) and T2DM in population-based study of Caucasian (Van Hoek *et al.*, 2008). A meta-analysis of 11 association studies included 12,979 cases subjects and 14,937 controls was robustly confirmed the association of *WFS1* rs10010131 with risk of T2DM (OR = 0.89 95% CI 0.86-0.92; $p = 4.9 \times 10^{-11}$) (Franks *et al.*, 2008). In the prediction study, *WFS1* SNP rs10010131 was significantly associated with the risk of future T2DM (OR = 1.12; $p = 0.001$) and predicted progression from normal glucose tolerance to T2DM (OR = 1.13; $p = 0.004$) in the MPP cohort (Lyssenko *et al.*, 2008).

The *HNF1B* (also known as *TCF2*) gene is located on chromosome 17cen-q21.3, a region linked to T2DM

(Demenais et al., 2003). Although *HNF1B* is important for the development of the pancreas, mutations in *HNF1B* show other more characteristic phenotypes like cystic kidney disease, liver dysfunction and abnormal urogenital tract development (Nishigori et al., 1998; Lindner et al., 1999; Bingham et al., 2000). Polymorphisms in *HNF1B* were reported to be associated with T2DM in Caucasians (Bonnycastle et al., 2006; Winckler et al., 2007; Gudmundsson et al., 2007). However, the previously T2DM associated SNPs in *HNF1B* is not associated with T2DM in cohorts of 2,293 individuals from the Botnia study (Finland) and in 15,538 individuals from the Malmö Preventive Project (Sweden) (Holmkvist et al., 2008).

Novel susceptibility genes from genome wide association studies: Using a candidate gene approach, family linkage studies and gene expression profiling uncovered a number of T2DM susceptibility genes (Huang et al., 2006). Since, 2007, a new window has opened on defining potential T2DM genes through genome-wide SNP association (GWA) studies of very large populations of individuals with T2DM. GWAS revealed new susceptibility loci for T2DM and validated some of the known candidates (Table 1, Fig. 1). To date, the total of the twelve update GWA scans for T2DM have been published (Table 2). Six of these represent high-density scans (i.e., at least 300 000 SNPs, offering genome-wide coverage >65%), in samples of Northern European descent (Sladek et al., 2007; Saxena et al., 2007;

Scott et al., 2007; Steinthorsdottir et al., 2007; Zeggini et al., 2007; Salonen et al., 2007). Another four studies featured a wider array of ethnic groups including Native American (Hanson et al., 2007), Hispanic (Hayes et al., 2007) and populations of European descent (Rampersaud et al., 2007; Florez et al., 2007), but were less extensive with respect to both sample size and SNP density (all used the Affymetrix 100K array). Two recent studies in East Asian subjects were on a smaller scale (featuring between 82 000 and 207 000 typed SNPs in a few hundred cases only) (Yasuda et al., 2008; Unoki et al., 2008).

The first GWAS covered 392,935 SNPs (passing quality control) and identified four novel loci including *SLC30A8*, *LOC387761*, *IDE-KIF11-HHEX* and *EXT2-ALX4* (Sladek et al., 2007). Subsequence three GWAS analyzed 386,731, 393,453, and 315,635 SNPs respectively (Zeggini et al., 2007; Saxena et al., 2007; Scott et al., 2007). Common variants in *CDKAL1*, *IGF2BP2*, *CDKN2A/B* genes were significantly associated with T2DM risk, with the allele-associated odds ratios ranging from 1.07 to 1.48. These studies also confirmed the T2DM effects of *SLC30A8* and *HHEX*. In another GWAS, Steinthorsdottir et al. (2007) found that variant rs7756992 in the *CDKAL1* gene was significantly associated with T2DM risk in individuals of European ancestry (allele-specific OR = 1.20 95% CI 1.13-1.27) and Han Chinese ancestry (OR = 1.25 95% CI 1.11-1.40), but not in those of African ancestry. Salonen et al. (2007)

Table 1: T2DM susceptibility loci for which there is genome-wide significant evidence for association*

Locus (nearest gene)	Ch	Year of discovery	Approach	Probable mechanism	variants	Effect size**
PPARγ	3	2000	Candidate	Insulin action	rs1801282	1.14
KCNJ11	11	2003	Candidate	β-cell dysfunction	rs5215	1.14
TCF7L2	10	2006	Linkage scan	β-cell dysfunction	rs7901695	1.37
FTO	16	2007	GWA	Altered BMI	rs8050136	1.17
HHEX/IDE	10	2007	GWA	β-cell dysfunction	rs1111875	1.15
SLC30A8	8	2007	GWA	β-cell dysfunction	rs13266634	1.15
CDKAL1	6	2007	GWA	β-cell dysfunction	rs10946398	1.14
CDKN2A/2B	9	2007	GWA	β-cell dysfunction	rs10811661	1.20
IGF2BP2	3	2007	GWA	β-cell dysfunction	rs4402960	1.14
HNF-1β	17	2007	Large-scale candidate-pathway	β-cell dysfunction	rs4430796	1.10
WFS1	4	2007	Large-scale candidate-pathway	Unknown	rs10010131	1.12
JAZF1	7	2008	GWA	β-cell dysfunction	rs864745	1.10
CDC123/CAMK1D	10	2008	GWA	β-cell dysfunction	rs12779790	1.11
TSPAN8/LGR5	12	2008	GWA	β-cell dysfunction	rs7961581	1.09
THADA	2	2008	GWA	Unknown	rs7578597	1.15
ADAMTS9	3	2008	GWA	Unknown	rs4607103	1.09
NOTCH2	1	2008	GWA	Unknown	rs10923931	1.13
KCNQ1	11	2008	GWA	β-cell dysfunction	rs2237892	1.29
MTNR1B	11	2008	GWA	β-cell dysfunction	rs10830963	1.09

*ADAMTS9: ADAM metalloproteinase with thrombospondin type 1 motif 9; CAMK1D: Calcium/calmodulin-dependent protein kinase 1D; CDC123: Cell division cycle 123 homologue (*Saccharomyces cerevisiae*); CDKAL1: CDK5 regulatory subunit-associated protein1-like1; CDKN2A/2B: Cyclin-dependent kinase inhibitor 2A/2B; FTO: Fat mass and obesity associated; HHEX: Haematopoietically expressed homeobox; HNF1B: Hepatocyte nuclear factor 1 homeobox B; IDE: Insulin degrading enzyme; IGF2BP2: Insulinlike growth factor 2 mRNA binding protein 2; JAZF1: Juxtaposed with another zinc finger gene 1; KCNJ11: Potassium inwardly rectifying channel, subfamily J, member 11; KCNQ1: Potassium voltage-gated channel, KQT-like subfamily, member 1; LGR5: Leucine-rich repeat-containing G-protein coupled; NOTCH2: Notch homologue (*Drosophila*); PPARγ: Peroxisome proliferator-activated receptor gamma; SLC30A8: Solute carrier family 30 (zinc transporter), member 8; TCF7L2: Transcription factor 7 like 2; THADA: Thyroid adenoma associated; TSPAN8: Tetraspanin 8; WFS1: Wolfram syndrome1; MTNR1B: **Melatonin receptor 1B. Estimates of effect size (given as per-allele odds ratios) reported for European descent populations based on available data (Fig. 1)

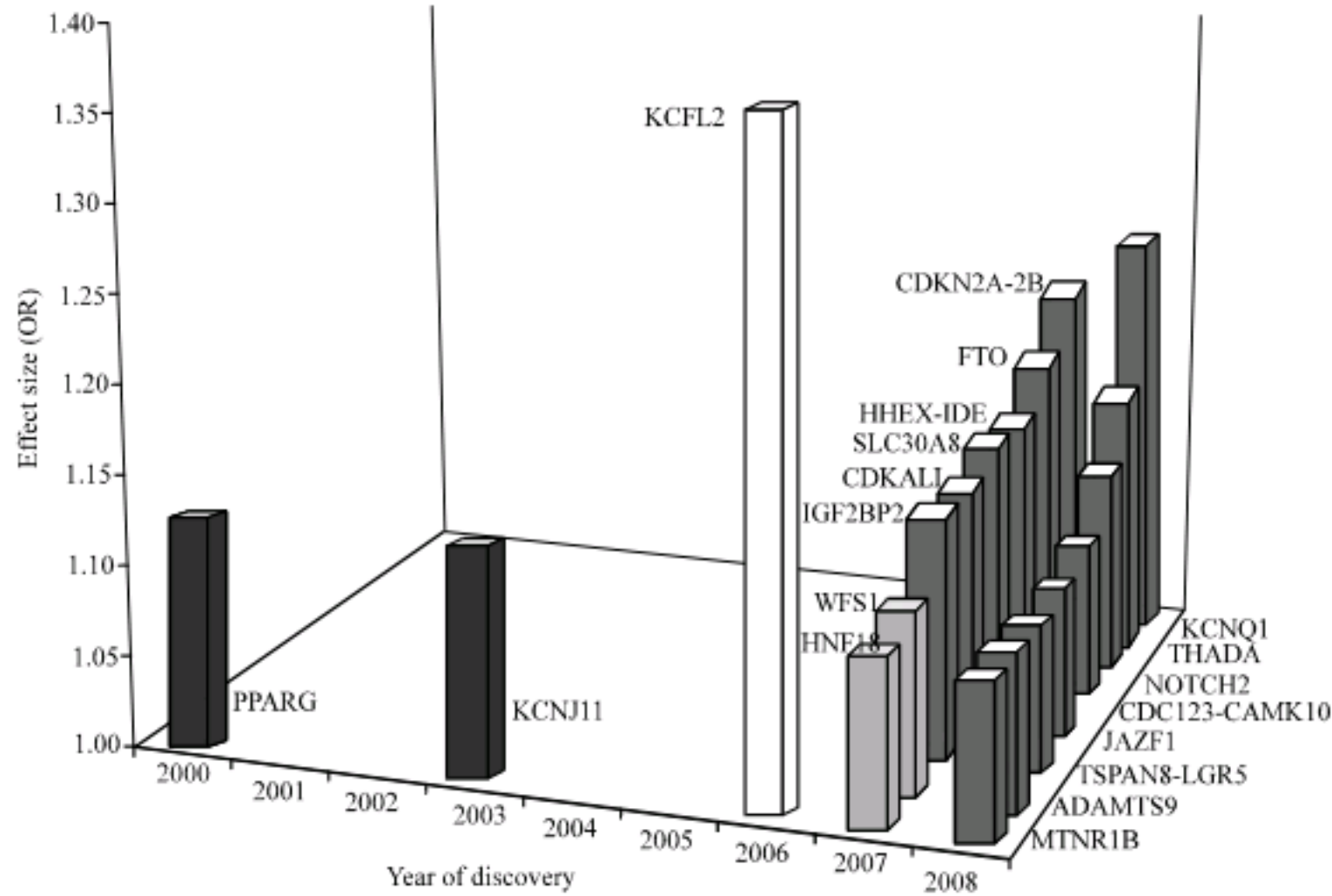


Fig. 1: Effect sizes of 19 common T2DM susceptibility loci. The x axis gives the year that published evidence reached the levels of statistical confidence that are now accepted as necessary for genetic association studies. The y-axis gives the per-allele odds ratio (estimated for European-descent samples) for each locus listed on the y-axis. Loci are sorted by descending order of per-allele effect size from TCF7L2 (1.37) to ADAMTS9 (1.09) (Table 1). Loci shown in white and dark grey are those identified by GWA approaches and by Linkage scan respectively, whereas those identified by candidate-gene approaches and by large-scale candidate-pathway studies are shown in black and light grey, respectively. Odds ratios are as in Refs (Zeggini *et al.*, 2007; Scott *et al.*, 2007; Frayling *et al.*, 2007; Franks *et al.*, 2008; Zeggini *et al.*, 2008; Yasuda *et al.*, 2008; Prokopenko *et al.*, 2009)

Table 2: GWA scans for type 2 diabetes*

Study	No. of cases	No. of controls	Sample source	Genotyping array	T2DM phenotype	References
Diabetes gene group	694	645	France	Illumina 300K +Illumina 100K	Family history of T2DM, AAO <45 years, BMI <30 kg m ⁻²	Sladek <i>et al.</i> discovery (2007)
Diabetes Genetics Initiative (DGI)	1464	1467	Finland, Sweden	Affymetrix 500K	Partial enrichment for family history and lean T2DM	Saxena <i>et al.</i> (2007)
Wellcome Trust Case Control Consortium (WTCCC)	1924	2938	UK	Affymetrix 500K	Enrichment for family history of T2DM, AAO <65 year	Zeggini <i>et al.</i> (2007)
Finland-US Investigation of NIDDM Genetics (FUSION)	1161	1174	Finland	Illumina 300K	Partial enrichment for family history	Scott <i>et al.</i> (2007)
deCODE Genetics	1399	5275	Iceland	Illumina 300K	No specific enrichment for family history, young AAO or BMI	Steinthorsdottir <i>et al.</i> (2007)
DiaGen	500	497	East Finland, Germany, UK, Ashkenazi	Illumina 300K	Enrichment for family history, AAO<60 years	Salonen <i>et al.</i> (2007)
Pima	300	334	Pima Indians	Affymetrix 100K	AAO <25 years, enrichment for family history	Hanson <i>et al.</i> (2007)
Starr County, Texas	281	280	Mexican Americans	Affymetrix 100K	Controlled for admixture	Hayes <i>et al.</i> (2007)
Framingham Health Study	91	1087	Massachusetts	Affymetrix 100K	Incident T2DM cases	Florez <i>et al.</i> (2007)
Old Order Amish	124	295	Amish	Affymetrix 100K	Enrichment for family history	Rampersaud <i>et al.</i> (2007)
Japanese multi-disease collaborative genome scan	187	1504	Japanese	JSNP Genome Scan 100K SNPs	T2DM cases	Yasuda <i>et al.</i> (2008)
BioBank Japan	194	1556	Japanese	Custom set of~ 268K SNPs	Enrichment for T2DM cases with retinopathy	Unoki <i>et al.</i> (2008)

*AAO: Age at onset

analyzed 315,917 HapMap-derived tagging SNPs in a two-stage study of 3,073 T2DM cases and 3,273 healthy controls. SNPs in the *AHII-LOC441171* region were found to confer ~30% increased risk. Other three GWAS genotyping 100 k SNPs in the Framingham Heart Study (Florez *et al.*, 2007), Amish (Rampersaud *et al.*, 2007) and American Indians (Hanson *et al.*, 2007) reported some genetic variants related to the risk of T2DM. However, these associations were not univocally observed. Yasuda *et al.* (2008) reported that the C allele of SNP rs2237892 in the *KCNQ1* gene is associated with an increased risk for developing T2DM in Japanese population. It is surprising that rs2237892 was associated with T2DM not only in the original Japanese set of cases and controls, but also in Chinese, Korean and European samples. At the same time Unoki *et al.* (2008) found similar evidence for SNPs closely related to rs2237892 (rs2237895 and rs2237897) in the *KCNQ1* gene were strongly associated with T2DM in the Singaporean population of East Asian descent and the Danish population of European descent. These studies have demonstrated how studies in non-European descent populations can reveal novel susceptibility loci.

Strong signals for T2DM were observed for *FTO*, which is also highly associated with fat mass and obesity (Dina *et al.*, 2007). The effect of *FTO* variants on T2DM risk has been replicated and seems to be mediated entirely by their marked effect on adiposity (Frayling *et al.*, 2007). Melatonin receptor 1B (*MTNR1B*) gene is located on human chromosome 11q21-q22 and encodes one of two high affinity forms of a receptor for melatonin, the primary hormone secreted by the pineal gland. This gene product is an integral membrane protein that is a G-protein coupled 7-transmembrane receptor, its predominant expression in retina and brain (Reppert *et al.*, 1995). GWAS have shown that variation in *MTNR1B* is associated with insulin and glucose concentrations. One of the strongest signals for glucose-stimulated insulin secretion in the DGI scan emanated from a SNP (rs10830963) in *MTNR1B* ($p = 7 \times 10^{-4}$, rank order 595) (Saxena *et al.*, 2007). Given that the melatonin pathway had previously been suggested to be involved in pathogenesis of T2DM, the *MTNR1B* gene was a prime candidate gene for T2DM. The strong signal was observed at rs10830963, where each G allele was associated with an increase of (OR = 0.07 95% CI 0.06-0.08) mmol L⁻¹ in fasting glucose concentrations ($p = 3.2 \times 10^{-50}$) and reduced β -cell function as measured by homeostasis model assessment in ~24,000 participants from the ten studies (HOMA-B, $p = 1.1 \times 10^{-15}$). The same allele was associated with an increased risk of T2DM (OR = 1.09 95% CI 1.05-1.12; per G allele $p = 3.3 \times 10^{-7}$) in large-scale meta-analysis of 13

case-control studies including data from GWAS, totaling 18,236 cases and 64,453 controls (Prokopenko *et al.*, 2009). The rs10830963 variants in *MTNR1B* seem to have a more marked effect on risk of T2DM, the effect size being comparable in magnitude (OR = 1.09 95% CI 1.05-1.12) to several other T2DM susceptibility genes recently identified in GWAS. Lyssenko *et al.* (2009) provided strong support for a role of melatonin and its receptor *MTNR1B* in the pathogenesis of T2DM. A common variant in the *MTNR1B* receptor was associated with an increase in fasting glucose over time and predicted future T2DM, most likely through impairment of insulin secretion from the pancreatic β -cell function. The result of GWA meta-analysis for fasting plasma glucose indicated that the *MTNR1B* rs1387153 strongly modulates fasting plasma glucose in the European population ($\beta = 0.06$ mmol L⁻¹, $p = 7.6 \times 10^{-29}$, $n = 16,094$) and increases the risk for T2DM (OR = 1.15 95% CI 1.08-1.22; $p = 6.3 \times 10^{-5}$, cases $n = 6,332$) (Bouatia-Naji *et al.*, 2009). Effects of melatonin are mediated by two distinct receptors, *MTNR1A* and *MTNR1B* (Pandi-Perumal *et al.*, 2008), which are members of the G-protein coupled receptor family, specifically inhibitory G-proteins (Gi). Both receptors have been found to be expressed in human and rodent islets (Muhlbauer and Peschke, 2007), with *MTNR1A* predominating, especially in glucagon-producing α -cells (Ramracheya *et al.*, 2008). There is some evidence that melatonin may exert an effect on insulin secretion, in that acute effects exerted by cAMP-elevating agents are inhibited by melatonin, whereas prolonged effects of the hormone may be stimulatory (Peschke, 2008).

The recent replication studies confirmed significant associations between SNPs within the *HHEX*, *CDKN2A/B*, *CDKAL1* and *KCNQ1* genes and T2DM in the Korean population (Lee *et al.*, 2008), *HHEX/KIF11/IDE*, *CDKN2A/B* and *IGF2BP2* loci in Danish subjects (Grarup *et al.*, 2007), *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX* and *SLC30A8* genes and T2DM in a Japanese population (Omori *et al.*, 2008), *CDKAL1*, *IGF2BP2*, *HHEX* and *FTO* genes and T2DM in German KORA 500 K study population (Herder *et al.*, 2008), *ADAMTS9*, *CDKAL1*, *CDKN2A/B*, *FTO*, *IGF2BP2*, *JAZF1* and *SLC30A8* in population-based study of Caucasian (Van Hoek *et al.*, 2008), *CDKAL1*, *CDKN2A/B*, *IGF2BP2*, *SLC30A8* and *KCNQ1* genes independently or additively contribute to T2DM risk in the Chinese Han population (Wu *et al.*, 2008; Hu *et al.*, 2009; Liu *et al.*, 2008, 2009), *TCF7L2* (rs12255372), *CDKAL1* (rs7756992, rs7754840), *HHEX* (rs7923837), *IGF2BP2* (rs4402960 and rs1470579), *CDKN2A/B* (rs10811661), and *SLC30A8* (rs13266634) were significantly associated with T2DM in Japanese population (Tabara *et al.*, 2009). The association of

SLC30A8, *HHEX*, *CDKALI*, *CDKN2A/CDKN2B*, *IGF2BP2* and *FTO* with risk for T2DM was confirmed, in the recent large-scale study of Asian ancestry from Hong Kong and Korea, with odds ratios ranging from 1.13 to 1.35 ($1.3 \times 10^{-12} < p_{\text{unadjusted}} < 0.016$) (Ng *et al.*, 2008). It's very recently reported that the common variants in the *CDKALI*, *SLC30A8*, *HHEX*, *EXT2*, *IGF2BP2*, *LOC387761* and *CDKN2B* genes did not confer a significant risk for T2DM in Pima Indians (Rong *et al.*, 2009). In the prediction study, common variants in *TCF7L2* (rs7903146), *PPAR γ* (rs1801282), *FTO* (rs9939609), *KCNJ11* (rs5219), *NOTCH2* (rs10923931), *WFS1* (rs10010131), *CDKALI* (rs7754840), *IGF2BP2* (rs4402960), *SLC30A8* (rs13266634), *JAZF1* (rs864745), *HHEX* (rs1111875) were significantly associated with risk of future T2DM (OR = 1.30; $p = 9.5 \times 10^{-13}$, OR = 1.20; $p = 4.0 \times 10^{-4}$, OR = 1.14; $p = 9.2 \times 10^{-5}$, OR = 1.13; $p = 3.6 \times 10^{-4}$, OR = 1.13; $p = 0.02$, OR = 1.12; $p = 0.001$, OR = 1.11; $p = 0.004$, OR = 1.10; $p = 0.008$, OR = 1.10; $p = 0.008$, OR = 1.08; $p = 0.03$ and OR = 1.07; $p = 0.03$, respectively) in the MPP cohort (Lyssenko *et al.*, 2008).

Additional loci from Meta analysis: Efforts to find additional T2DM susceptibility loci have to contend with the modest effect sizes anticipated and the stringent significance thresholds required when many hundreds of thousands of SNPs are tested in parallel. The obvious solution is to increase sample size and the most effective strategy for this involves combining existing GWAS data through meta-analysis. The Diabetes Genetics Replication And Meta-analysis (DIAGRAM) consortium integrated data from three previously published GWAS (Saxena *et al.*, 2007; Scott *et al.*, 2007; Zeggini *et al.*, 2007), thereby doubling the sample size compared to the largest of the individual studies to ~4500 cases and 5500 controls. The consortium also used novel imputation approaches (Marchini *et al.*, 2007) to infer genotypes at additional SNPs that were not directly typed on the commercial arrays used for the original GWAS, thereby extending the analysis to a total of ~2.2 million SNPs across the genome. In this study, 69 signals showing the strongest associations in the GWAS meta-analysis were genotyped in an initial replication set of 22426 individuals and the top eleven signals emerging from this second analysis were then evaluated in ~57 000 further subjects. After integrating data from all study subjects, six signals reached combined levels of significance, including the *JAZF1* (rs864745) ($p = 5.0 \times 10^{-14}$), *CDC123-CAMKID* (rs12779790) ($p = 1.2 \times 10^{-10}$), *TSPAN8-LGR5* (rs7961581) ($p = 1.1 \times 10^{-9}$), *THADA* (rs7578597) ($p = 1.1 \times 10^{-9}$), *ADAMTS9* (rs4607103) ($p = 1.2 \times 10^{-8}$) and *NOTCH2* (rs10923931) ($p = 4.1 \times 10^{-8}$) (Zeggini *et al.*, 2008).

However, the replication study of these six SNPs in Khatri Sikh diabetics of North India, only *CDC123/CAMKID* (rs12779790) revealed a significant evidence of association with T2DM ($p_{\text{dominant}} = 0.031$) (Sanghera *et al.*, 2009).

NEW GENES NEW AETIOLOGY

The strongest signal for T2DM in GWAS was found for *TCF7L2* with p-values down to $< 10^{-48}$ increase in the odds for the disease of 37% (Scott *et al.*, 2007; Saxena *et al.*, 2007), the *TCF7L2* rs7901695, increases disease risk with an odds ratio of 1.37 (Table 1; Fig. 1). The expression of *TCF7L2* was related to genotype and metabolic parameters in human islets, the risk T allele was associated with impaired insulin secretion, in cretin effects, and enhanced rate of hepatic glucose production. Over expression of *TCF7L2* in human islets reduced glucose-stimulated insulin secretion (Lyssenko *et al.*, 2007). The *HHEX* region which also harbors *IDE* encoding insulin-degrading enzyme which has been implicated in both insulin signal and islet function. T2DM risk allele for *HHEX/IDE* gene associated with decreased pancreatic β -cell function, including decreased β -cell glucose sensitivity that relates insulin secretion to plasma glucose concentration (Pascoe *et al.*, 2007). Both *HHEX* and *IDE* are critical for ventral pancreas development and are powerful biological candidates for T2DM.

A central theme for many of the recently discovered genes is that many of them seem to be involved in insulin secretion, pin-pointing the pivotal role of β -cell function in the pathogenesis of T2DM. These genes include *TCF7L2*, *KCNJ11*, *HHEX*, *SLC30A8*, *CDKALI*, *CDKN2A/2B*, *IGF2BP2* and *KCNQ1* (Steinthorsdottir *et al.*, 2007; Grarup *et al.*, 2007; Pascoe *et al.*, 2007; Staiger *et al.*, 2007, 2008; Yasuda *et al.*, 2008; Unoki *et al.*, 2008). The loci seem particularly to be associated with an increased risk of developing T2DM through a reduced insulin-secretory capacity. A further highly interesting finding was the fat mass and obesity associated *FTO* gene which predisposes to T2DM by altering Body Mass Index (BMI). It is expressed in hypothalamus (Frayling *et al.*, 2007) which is the key brain region for influencing appetite. Interestingly, the GWAS have identified a separate set of SNPs that seem to represent the strongest common genetic risk factor for heart disease (myocardial infarction) (Zeggini *et al.*, 2007; Helgadottir *et al.*, 2007; McPherson *et al.*, 2007). There is no correlation between the T2DM signal and the heart disease signal, but the latter does fall closer to the *CDKN2* genes, which

encodes p16INK4a. Over expression of p16INK4a results in a decreased islet proliferation and β -cell dysfunction in ageing mice (Krishnamurthy *et al.*, 2006), the initial human physiology studies have not provided any evidence that the risk alleles alter insulin secretion, but the mouse phenotype strongly implicates β -cell dysfunction. *CDKAL1* is highly expressed in human islets (Zeggini *et al.*, 2007). *CDKAL1* shares homology with the *CDK5RAP1* gene, a known inhibitor of CDK5 activation. CDK5 is implicated in reduced β -cell function, through the formation of p35-CDK5 complexes, which down regulate insulin expression (Ubeda *et al.*, 2006; Wei *et al.*, 2005). T2DM risk allele for *CDKAL1* gene associated with decreased pancreatic β -cell function, including decreased β -cell glucose sensitivity that relates insulin secretion to plasma glucose concentration (Pascoe *et al.*, 2007). The IGF2BP2 binds to the key growth and insulin signaling molecule insulin-like growth factor 2 (IGFII) and is also expressed in the pancreatic islet (Zeggini *et al.*, 2007). T2DM risk for *CDKAL1*, *SLC30A8*, *IGF2BP2*, and *LOC387761* is specifically mediated through defects in insulin secretion (Palmer *et al.*, 2008). The product of *KCNQ1* gene can form hetero multimers with two other potassium channel proteins, KCNE1 and KCNE3. The SNP rs2237892 in *KCNQ1* has known roles in cardiac muscle, it is not yet known how this gene could affect the risk for T2DM, but there is evidence that it is turned on in the pancreas (Yasuda *et al.*, 2008).

Curiously, some of the recent studies suggest a possible explanation for previous much debated epidemiological observations that men with T2DM are less likely to develop prostate cancer. The same allele in *HNF1B* that predisposes to T2DM was protective of prostate cancer (Gudmundsson *et al.*, 2007). Moreover, different variants in *JAZF1* are associated with T2DM and with prostate cancer (Saxena *et al.*, 2007; Zeggini *et al.*, 2007; Scott *et al.*, 2007; Thomas *et al.*, 2008). In keeping with an effect on development and transcriptional processes these findings may not come as a surprise although the exact causal relationships remain to be investigated (Frayling *et al.*, 2008).

Moreover, carriers of novel T2DM risk alleles within *JAZF1*, *CDC123/CAMK1D*, and *TSPAN8* it's suggested an impaired pancreatic β -cell function in glucose-tolerant regions in the cohort of middle-aged people (Grarup *et al.*, 2008). *CDC123* is regulated by nutrient availability in *S. cerevisiae* and has a role in cell cycle regulation. Taken together, evidence from GWAS implicating variants in or near *CDKAL1*, *CDKN2A/B*, *CDC123* and *CAMK1D* suggests that cell cycle dysregulation may be a common pathogenetic mechanism in T2DM (Ridderstråle and

Groop, 2009). Notch homologue 2, *Drosophila* (*NOTCH2*) is known to be involved in pancreatic development, but the mechanisms involved for the *ADAMTS9* and *THADA* genes remain unclear.

MTNR1B is thought to participate in light-dependent functions in the retina and in melatonin's neuronal regulation of circadian rhythmicity and sleep cycles. As certain sleep disorders, such as obstructive sleep apnea, result from obesity and are associated with insulin resistance (De Sousa *et al.*, 2008; Kashyap and DeFronzo, 2007). Very recently, three new studies identified and reported extremely strong, incontrovertible evidence that the *MTNR1B* is associated with high fasting glucose levels and increased risk of T2DM (Prokopenko *et al.*, 2009; Bouatia-Naji *et al.*, 2009; Lyssenko *et al.*, 2009). *MTNR1B* could represent a new interesting candidate gene linking sleep disorders with T2DM. These new data implicate an association between the sleep-wake rhythm, the so-called circadian rhythm, and fasting glucose levels, T2DM, which was not known previously. The greatest benefit of T2DM genetic study is likely to come from new and better therapies derived from an improved understanding of the aetiology of the disease.

PREDICTION OF RISK OF T2DM

One of objectives for identification of T2DM susceptibility genes is to predict T2DM risk and identify high-risk subjects. To predict the risk of T2DM for a healthy individual we need to know and be able to measure risk factors, their effect sizes and how they interact. Although, prediction of total risk is an ultimate goal, prediction of genetic risk that can be attributed to inherited genetic variants is an important component. Generally, the predictive value of genetic testing for prediction of T2DM is unclear. Several empirical studies on the predictive value of genetic polymorphisms have been conducted before the GWAS data were available (Lyssenko *et al.*, 2005; Weedon *et al.*, 2006). In a case-control study, combining the information of three polymorphisms improved disease prediction, albeit to a limited extent (Weedon *et al.*, 2006). The predictive value was low compared with clinical characteristics (Vaxillaire *et al.*, 2008). The complex disease is caused by multiple genetic variants, the predictive testing based on a single genetic marker will be of limited value. GWAS have dramatically increased the number of common genetic variants that are robustly associated with T2DM. The predictive value could be improved by combining multiple common low-risk variants, but all showed limited predictive value so far (Janssens *et al.*, 2006, 2007;

Yang *et al.*, 2003; Wray *et al.*, 2007). The currently known and replicated genetic variants found in GWAS contributed modestly to the prediction of T2DM in population-based setting and marginally improved the risk prediction beyond clinical characteristics (Van Hoek *et al.*, 2008). Individuals carrying more risk alleles had a higher risk of T2DM and the common risk variants for T2DM do not provide strong predictive value at a population level (Lango *et al.*, 2008). Similarly Lin *et al.* (2009) constructed an additive genetic score using the most replicated SNPs within 15 T2DM susceptibility genes and reported that the weighted 15 SNP-based genetic score provides additional information over clinical predictors of prevalent T2DM. However, the clinical benefit of this genetic information is limited. The prediction model for T2DM may not be so useful but has some value and incorporation of data from additional risk loci is most likely to increase the predictive power (Miyake *et al.*, 2009). New gene discoveries from GWAS will certainly identify novel etiological pathways and novel intermediate biomarkers, which consequently may be stronger predictors of disease than the genetic variant that led to its identification. But it is likely that the complexity of complex diseases may ultimately limit the opportunities for accurate prediction of disease in asymptomatic individuals as unraveling their complete causal pathways may be impossible (Janssens and van Duijn, 2008). More extensive studies are needed to assess the usefulness of combining information from multiple variants.

CONCLUSION

Studies in human genetics have made tremendous strides in discovering T2DM genes especially through GWAS. Identification of the causal variants responsible for the association signals uncovered will provide valuable clues to understanding disease predisposition. GWAS is only the beginning in establishing the role of genetic variants in disease etiology. This would be followed by the fine-mapping of the susceptibility region through deep sequencing in large population and the validation of causality for genetic variants in experimental settings.

- Fine-map the new T2DM gene regions. That will involve deep sequencing and further rounds of genotyping to build up a full picture of all the possible common variation that might explain the association signals. This should include efforts to define Copy Number Variants (CNVs) such as

duplications and deletions, and should also attempt to define independent associations in the same gene regions

- Additional association studies of the new variants are needed. Investigators will need to assess their role in other populations, especially populations with a high prevalence of T2DM. Further studies of the role of risk alleles in the general population are also important
- The predictive value of genetic testing for prediction of T2DM is unclear. Additional studies are needed to identify and replicate new genetic susceptibility variants and gene-gene and gene-environment interactions to approach levels of discriminative accuracy that enable the identification of at-risk groups and to assess whether individuals with extreme numbers of risk alleles may benefit from genetic testing
- The mechanisms whereby a given DNA change leads to an increased risk of T2DM need to be reconstructed. We need to know whether they influence T2DM predisposition through primary effects on β -cell function, through insulin action, or by some other mechanism. The ultimate objective is, of course, to understand how genetic findings can translate into advances in clinical management. In principle, genetic testing might offer insights into disease risk and predict response to the various therapeutic and preventative options available, but much work will be required to understand how to deploy such tests in clinically effective ways. A route is to apply the insights gained into the mechanisms of disease predisposition to identify new targets for drug development. In this respect, genes of small effect are likely to provide clues just as valuable as those of large effect

Looking ahead, new methodologies and approaches may be needed to discover the remaining as yet unidentified genetic contributors to disease risk. At associated loci, fine mapping can help narrow down the list of possible causal variants and simplify future functional studies. Additional GWAS, in larger samples and multiple ethnicities, will almost certainly lead to new discoveries and incremental gains in the amount of risk accounted for by identified genetic variants. Exploration of these novel loci will very likely uncover additional alleles, both common and rare, that explain additional variance in phenotype, help pinpoint which gene(s) are responsible for the association and provide better clinical and molecular tools for assessing function and mechanism of disease.

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