Type-2 diabetes: a cocktail of genetic discovery

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Diabetes is one of the most challenging health problems of the 21st century with an alarming increase in the prevalence of type-2 diabetes mellitus (T2DM) and associated conditions such as hypertension, dyslipidemias and obesity. T2DM is a complex genetic disease comprised of many metabolic disorders with a common phenotype of glucose intolerance. Patients with T2DM would have inherited a variety of different genetic factors that together with environmental factors combine as the primary cause. This complicates the genetic study of the disease and means that different methodological approaches are needed if we hope to identify susceptibility genes and genetic variants. The biochemical and physiological processes that underpin T2DM are still unclear although most certainly involve impairment in insulin secretion and insulin action. In this review, we will discuss the most exciting advances in understanding the genetics of T2DM by looking at recent discoveries employing human association studies and candidate genes arising from animal models.

INTRODUCTION

Approximately, 150 million people worldwide are affected by type-2 diabetes mellitus (T2DM), and this figure is expected to double in the next 20 years. Until recently, T2DM was considered to be a disease confined to adulthood, rarely observed in individuals under the age of 50, but clinically based reports and regional studies suggests that T2DM in children and adolescents, although still rare, is more frequently being diagnosed (1). This is reflective of the growing number of children entering adulthood with unprecedented levels of obesity. Diabetes leads to a reduced life expectancy and quality of life, as well as a greater risk of heart disease, stroke, peripheral neuropathy, renal disease, blindness and amputation (2,3).

Currently, treatment of T2DM is limited to medical therapies that ameliorate the condition and do not aim to restore normal glucose metabolism. This leaves patients open to the risks of life-threatening complications (4). Understanding the basis of the genetic traits of T2DM could lead to the identification of new therapeutic targets, which currently represents one of the most promising strategies for long-term treatment success.

A genetic component to T2DM is undeniable given the inheritance seen in families with rare monogenic diabetes (5) as well as the high prevalence for the disease in particular ethnic groups and its modification by genetic admixture (6,7) [for review see (8)] and the difference in concordance rates between monozygotic and dizygotic twins (9,10) and the results of numerous linkage studies [for review see (11)]. The extent to which multiple genes and the environment impact on disease predisposition and progression is an ongoing challenge for researchers but they have several tools at their command. These include genome scans, which use markers spanning the entire genome, which have been used in over 50 family-based linkage studies on a variety of populations. However, until recently [see below (12)] the only T2DM susceptibility gene identified using this technique has been the NIDDM1 gene, calpain 10 (13) and this has been replicated in some but not all populations [for reviews see (14,15)]. Another possibility is to engage in association studies with either of individual genes (candidate gene approach) or of high-density single nucleotide polymorphisms (SNPs) randomly spaced across the entire genome (genome-wide association study). Association studies investigate the relationship between disease status and particular alleles, genotype or haplotype of a genetic marker or a set of markers. This type of study compares the prevalence of a disease marker between affected and unaffected individuals in a case–control manner. A number of genes and polymorphisms have been reproducibly associated with T2DM in a variety of studies (11,16–18).

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CANDIDATE GENES FROM POPULATION STUDIES

Yield from genome scans has been limited, nevertheless many putative localizations have been suggested but not all have been validated by replication across several studies and by several different groups (11). Association studies have had great success in identifying several key genes. The PPARγ gene (PPARG) (19) and KCNJ11 [review (20)] signify the most promising candidate genes for human T2DM susceptibility arising from association studies and are well replicated and supported (Fig. 1). Furthermore, CAPN10 (15,21), HNF4A (22–25) and TCF7L2 (see below) genes identified originally through linkage studies have now been confirmed using the candidate gene association approach [see reviews (11,16,17)]. Indeed, a large prospective study of people in Botnia indicated that variants in CAPN10 and PPARG can predict future T2DM (26).

The recently published work of Grant et al. (12) reported a variant of the transcription factor 7-like 2 gene (TCF7L2), conferring an allele relative risk (RR) of 1.5 per copy carried in T2DM patients from an Icelandic population ($P = 2.1 \times 10^{-5}$). This association was replicated in two further populations, a Danish female cohort ($P = 4.8 \times 10^{-3}$) and a European–American cohort ($P = 3.3 \times 10^{-5}$). The study arose from a previously reported suggested linkage of T2DM to chromosome 10q in the Icelandic population (27) that had also been observed in Mexican Americans (28). To pursue this initial finding, the group used a high density of genotyped microsatellite markers across a 10.5 Mb region corresponding to the locus. A microsatellite, DG10S478, located within intron 3 of TCF7L2 (formerly TCF4) on chromosome 10q25.2 was identified as having association with T2DM. All six alleles of DG10S478 were found to belong to a single monophyletic lineage and were collapsed to form a single composite allele referred to as allele X. Sequencing exon 4 of TCF7L2 in Danish, American and Icelandic cohorts concluded that no other polymorphisms showed stronger association with T2DM than allele X. No significant association was found between body mass and allele X, however, carriers of the at-risk variant, allele X, may have an earlier age of onset than non-carriers ($P = 0.014$). The overall population attributable

![Figure 1. Biological function of T2DM candidate genes arising from animal models and human association studies. Many genes have been associated with T2DM and we include here some of the most promising ([11,16,17] and this review).](https://academic.oup.com/hmg/article-abstract/15/suppl_2/R202/623428/200202623428)
risk is 21% (the fraction by which the disease would be reduced if the risk factor were not present). Transcription factors are associated with maturity-onset diabetes of the young (MODY) as well as in the pathogenesis of T2DM with examples including peroxisome proliferators-activated receptor gamma (PPARγ) (21) and the forkhead gene family (29,30). Although it still remains for the mechanism by which TCF7L2 contributes risk of T2DM to be determined it provides one of the most exciting and significant genetic contributions in recent times. Never before has this gene been linked with T2DM but previous studies suggest evidence of an enteroendocrine role (31–33). Hence, the authors postulate that variants of TCF7L2 could be acting through glucagon-like peptide 1 (GLP-1), which exerts a critical effect on blood glucose homeostasis (34).

INSULIN RESISTANCE, OBESITY AND T2DM

It is believed that polygenic T2DM results from inheritance of a set of susceptibility genes and that each exerts only a partial effect contributing to the development of the disease in full. Only when the effect of these genes is added together in particular combinations and in the presence of certain risk factors, such as obesity, will we see disease (Fig. 1). Up until now specific examples of common genetic variants associated with insulin resistance and obesity have been in short supply but new work has shown that ENPP1 mediates both insulin resistance while concurrently being involved in the development of both obesity and T2DM (35). This discovery strengthens the idea that a similar molecular mechanism underpins both conditions. Meyre et al. (35) provide the first genetic link between these two major metabolic diseases (obesity and T2DM) by investigating family groups with evidence of childhood obesity. In families in which there had been previous evidence of an obesity link to chromosome 6q, it was observed that these children and their parents were more likely to have disrupted glucose metabolism than the other obese subjects. This hypothesis, and data from eight previous genome scans for obesity or T2DM (36–43) allowed the authors to narrow down the region of linkage to a smaller interval on chromosome 6q, which ultimately led to the identification of ENPP1 as a good candidate for further study. This gene was also previously reported to be associated with insulin resistance (44,45). This latest study by Meyre et al. represents the largest undertaken so far investigating ENPP1, in which they sequenced the gene in 48 obese children and 24 non-obese adult controls to find a number of polymorphisms. The strongest association in severe forms of obesity was the K121Q polymorphism and a further two polymorphisms modulated the obesity risk, suggesting that these three polymorphisms together make up a risk haplotype for obesity. Population analysis from 2430 French subjects indicated that this risk haplotype was associated with childhood obesity, morbid obesity in adults as well as less severe forms of adult obesity. The authors then measured ENPP1 levels and found a positive correlation between body mass and serum ENPP1 and also provide evidence for a role of ENPP1 in glucose metabolism by measuring glucose tolerance in two different European populations. Meyre and colleagues propose that genetic variants of ENPP1 influence the amounts of circulating protein and a resulting increase in ENPP1 could impair insulin binding to its receptor in the muscle and brain leading to fat deposition. The association with insulin resistance, earlier onset T2DM and myocardial infarction of the K121Q polymorphism in ENPP1 has also now been supported by three publications in different populations (46–48). However, equally valid are the findings of other groups in which no or weak association was seen (45,46,49–52) and thus further larger studies are required before ENPP1 joins the ranks of susceptibility genes such as PPARγ, KCNJ11 and HNF4A.

But what are the signals that strongly associate weight gain with the onset of insulin resistance? Several promising candidates for future association studies have arisen from biological and physiological studies of models rather than human genetic studies. Yang et al. (53) report that retinol binding protein-4 (RBP4), a fat-derived peptide, can impair insulin sensitivity throughout the whole body by modulating glucose homeostasis. RBP4 is not the first fat-derived hormone to be implicated in controlling body weight, the first was Leptin in 1995 (54). Following the discovery of leptin, a whole family of adipose-derived signaling molecules were identified (examples include, adiponectin, TNF-α and resistin) which indicate changes in adipose tissue mass and energy status and signal this information to organs that control fuel expenditure (55). Expression of the GLUT4 glucose transporter is reduced selectively in adipocytes in insulin-resistant states, including obesity and T2DM. However, skeletal muscle cells and liver cells retain normal GLUT4 levels in rodents and humans that are obese and have insulin resistance (56). This is slightly paradoxical, as skeletal muscle is considered to be the primary site of insulin-stimulated glucose uptake, whereas adipose tissue takes up much less glucose under normal physiological conditions. The first step in uncovering this mystery was to produce transgenic mice with adipose-specific overexpression or reduction of human GLUT4. These mice showed opposite phenotypes; mice overexpressing GLUT4 exhibited enhanced glucose tolerance and insulin sensitivity, whereas mice lacking GLUT4 in their adipose tissue were insulin-resistant in muscle and liver and prone to overt diabetes (57). However, mice lacking GLUT4 in adipose tissue show normal insulin action in muscle in ex vivo experiments; this has led to the search for a circulating factor responsible for communicating between adipose tissue and peripheral tissue resulting in insulin resistance in these mice. Yang et al. undertook a DNA microarray which led to the identification of RBP4, which when elevated causes systemic insulin resistance and when reduced improves insulin action. Yang et al. have provided the first compelling evidence for a link between an adipose-derived factor and modulation of GLUT4, using five independent mouse models of obesity and insulin resistance, as well as obese humans. This is an example of an elegant study, which started its journey using and generating animal models to investigate candidate regulatory factors involved in obesity and insulin resistance, and has related the findings back to human patients with T2DM and obesity. Many questions still remain unanswered, but the authors have also made considerable advances in elucidating possible mechanisms by which RBP4 affects insulin action. RBP4 decreases
the activity of PI-3 kinase and the phosphorylation of insulin receptor substrate-1, both effects clearly suggesting impaired insulin action. RBP4 may act through either retinol-dependent or retinol-independent pathways which could involve a variety of factors, including retinoic acid receptors or other cell surface receptors to which RBP4 binds with high affinity (58,59). Excitingly, studies on human patients show that elevated serum RBP4 is correlated with insulin resistance in subjects with obesity, impaired glucose tolerance or T2DM, and in non-obese, non-diabetic subjects with a strong family history of T2DM. This raises the possibility that RBP4 levels could be used for assessing risk of T2DM and further that RBP4 may play a causal role in insulin resistance and represent a promising therapeutic target (60).

One factor that is also increased with increasing RBP4 is phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in glucose production. Interestingly, PEPCK has also been implicated in other candidate gene models of the disease, including SIRT1 identified by the group of Pere Puigserver (61) and also reported by Moynihan et al. (62). In a paper by Rodgers et al. (61) they report that pyruvate induces SIRT1 in the liver of mice during fasting where it interacts with PGC-1α resulting in induction of PEPCK and hepatic glucose output. SIRT1 was found also to modulate the effects of PGC-1α repression of glycolytic genes implicating SIRT1, formerly known to modulate ageing in several species (63–65), as a modulator of PGC-1α in energy homeostasis and diabetes. Moynihan et al. show that overexpression of Sirt1 improves insulin secretion by the β-cell probably by downregulating UCP2 and thus increasing ATP production efficiency by oxidative phosphorylation. SIRT1 is the mammalian homologue of the NAD+-dependent histone deacetylase Sir2 (silingence information regulator 2) and is known to target MyoD, p53 and forhead transcription factors for deacetylation (66–70). SIRT1 has been investigated in several different model systems, Saccharomyces cerevisiae, Caenorhabditis elegans (64,65,71) and now mice, suggesting that human data is much needed if new therapeutic approaches to diabetes and ageing are to be realized. Forhead transcription factors, for example Foxo1 from the FOXO family and Foxa2 from the FOXA family, are evolutionary conserved downstream targets in the insulin signaling pathway. Both Foxo1 and Foxa2 have been postulated to play vital roles in regulating glucose homeostasis, insulin secretion (Foxo1) and insulin action (Foxo1, Foxa2) (30,72).

**CANDIDATE GENES FROM ANIMAL MODELS**

Candidate genes from animal models are shedding light on just how complex and heterogeneous T2DM is. The Mouse has provided models of insulin resistance, lipodystrophies, β-cell dysfunction and impaired glucose homeostasis and insulin secretion. Animal models of diabetes have become extremely useful, due to the limited availability of human tissue, in providing useful insights into insulin secretory mechanisms and the etiology of human T2DM.

It is now widely recognized that β-cell dysfunction plays a key role in the development of T2DM (73–75). When T2DM is diagnosed patients have severely reduced insulin secretion which continues to diminish throughout the course of the disease, and most likely was reduced prior to disease diagnosis (76). Insulin secretion from the β-cells of the pancreatic islets is critically dependent on cellular ATP levels generated by glucose-stimulated mitochondrial metabolism (77) (Fig. 2). The C57BL/6J mouse strain has impaired glucose tolerance due to loss of first and second phase insulin responses (78–81). In addition, C57BL/6J mice develop obesity, hyperglycemia and insulin resistance when fed a high-fat diet (82). Although the KATP channels in C57BL/6J β-cells have normal ATP sensitivity, glucose fails to close them, or to elevate [Ca2+]i, and stimulate insulin secretion. Genetic mapping identified a loss-of-function mutation in the gene encoding nicotinamide nucleotide transhydrogenase (Nnt) as underlying these defects (81). Transgenic expression of the entire wild-type Nnt gene in C57BL/6J mice rescued their impaired insulin secretion and glucose intolerant phenotype, confirming this idea (83). Nnt is a nuclear-encoded mitochondrial protein that resides in the inner membrane of mitochondria and functions as a redox-driven proton pump, catalyzing the reversible reduction of NADP+ by NADH and conversion of NADH into NAD+ (84). Current studies have revealed that knockdown of Nnt by siRNA in the insulin-secreting cell line MIN6 prevented the rise in [Ca2+]i produced by glucose and led to a dramatic reduction in insulin secretion (85). However, tolbutamide was still effective, suggesting that, as in C57BL/6J β-cells, glucose fails to close KATP channels when Nnt is non-functional. Similarly, studies on islets isolated from mice possessing ENU-induced point mutations in Nnt showed that both homozygous and heterozygous Nnt mutant mice were significantly
Figure 3. Proposed role of Nnt in β-cell metabolism. ROS is generated when there is substantial flux through the respiratory chain at high glucose. ROS accumulation in the mitochondria results in activation of uncoupling protein 2 (UCP2). This leads to enhanced proton leakage across the inner mitochondrial membrane, reducing the electromotive force and thereby ATP production, which in turn promotes activation of \( K_{\text{ATP}} \) channel currents, plasma membrane hyperpolarization and inhibition of insulin secretion. Nnt is postulated to have a role in the TCA cycle as well as in reducing glutathione to detoxify ROS. Abbreviations: reduced nicotinamide nucleotide transhydrogenase (Nnt).

glucose intolerant, and their islets secreted less insulin (85). Furthermore, although basal ATP levels were normal in β-cells from Nnt mutant mice, glucose failed to elevate ATP (85). This suggested that Nnt mutations impair mitochondrial ATP production, possibly through the activation, as opposed to downregulation in the case of SirT1, of UCP2, thus preventing glucose-dependent closure of \( K_{\text{ATP}} \) channels. Consequently, β-cell electrical activity and insulin secretion are impaired (Fig. 3). This is a clear example of a promising candidate gene that has arisen from the study of a mouse model of diabetes. Evidently, it will now be of interest to determine whether polymorphisms in Nnt are associated with human T2DM.

Animal models of well-characterized genes, such as SURI and Kir6.2, are revealing additional roles for these genes outside the β-cell. Pocai et al. (86) have shown that hypothalamic \( K_{\text{ATP}} \) channels are potentially important in insulin resistance and T2DM.

RNA interference has also been a valuable tool in the discovery of the aryl hydrocarbon receptor nuclear translocator (ARNT) (87). This gene was reported to have a 90% reduction in expression in islets of humans with T2DM when compared with unaffected control islets. Following this finding, by real-time PCR, Gunton et al. used siRNA in MIN6 cells to reduce ARNT expression, which resulted in the total loss of glucose-stimulated insulin secretion and downregulation of multiple genes known to have a significant role in β-cell function (87). To determine the role of ARNT in vivo, the group made mice with β-cell specific knockout of ARNT and found that these mice had reduced glucose tolerance and impaired insulin secretion, and reduced mRNA expression of the genes G6PI, aldolase, insulin receptor and Akt2. These gene expression changes reflect those observed in human type-2 diabetic islets. Gene expression alterations reported in this study are the result of short consensus binding sites in the promoters of these genes. In short, this work provides new insights into the pathogenesis of T2DM from new technologies, but much work remains if we are to understand the combined effects of transcription factors and the role they play to as potential targets for disease treatment.

CONCLUSION

Progress in confirming PPAR\(\gamma\), KCNJ11, CAPN10 and HNF4A as important genes conferring risk for T2DM and the notable success in identifying TCF7L2 gives great hope for the future and increasing confidence in the techniques being employed to further our knowledge in the field of human genetics of multifactorial diseases. Other genes, such as ENPP1, that may confer smaller or modifier effects will require further larger studies in order to fully elucidate their role in T2DM.

Studies in animal models are making great progress in identifying genes that are functionally important in the pathophysiology of T2DM. These genes, including RBP4 and NNT, now warrant testing in genetic and functional studies in humans.

How might current findings benefit patients? There is no doubting the growing public health importance of T2DM on a worldwide scale. Hence, the identification of susceptibility genes will allow us to understand better the pathophysiology of the disease but, in terms of direct action for patients, will draw us closer to designing better approaches for diagnosis and treatment. A new class of endogenous regulatory RNAs, microRNAs (miRNAs), may well provide a potential therapeutic target for the future. Poy et al. suggest that miRNAs are involved in exocytosis, the final step in the insulin secretory pathway. The pancreatic islet-specific miRNA miR-375 regulates insulin exocytosis, a key determinant of blood glucose homeostasis, pointing to a possible involvement of miRNAs in T2DM (88).

The cocktail of environmental and genetic factors (26), and the degree to which they interact, cannot afford to be underestimated given the obesity epidemic upon us and thus,
hopefully as we continue to unravel the inherited component to T2DM, we will understand better the role dietary and lifestyle choices play in this complex disease.

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REFERENCES


