Genome-wide association studies of Type 2 diabetes: are these ready to make an impact in the clinic?

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Practice Points

- More than 40 genetic susceptibility variants for Type 2 diabetes (T2D) have been identified so far, largely through genome-wide association studies.
- The majority of genome-wide association studies findings have highlighted genetic variants primarily affecting β-cell function rather than insulin resistance.
- Together, the identified genetic loci explain a small fraction of the overall heritable risk for T2D (~10%).
- Potential areas of clinical translation include risk prediction, prevention, pharmacogenetics and development of novel therapeutics.
- Ongoing research studies are focused towards identifying rare genetic variants conferring a larger effect on risk of T2D, which could facilitate clinical translation.
- Future research includes detailed physiological and functional studies to identify the underlying molecular defects associated with each genetic susceptibility variant.
- Clinicians should be aware that T2D genetic research is currently experiencing a dramatic revolution and remain optimistic that these landmark studies will translate into improved care for diabetic patients.

SUMMARY

Genome-wide association studies have facilitated a substantial and rapid rise in the number of confirmed susceptibility variants for Type 2 diabetes. This has inevitably led to widespread hope that these findings will translate into improved clinical care for the growing numbers of patients with diabetes. This article summarizes recent discoveries in the field of Type 2 diabetes genetics and will discuss their importance and the current obstacles to clinical translation.

The importance of genetic factors in the etiology of Type 2 diabetes (T2D) is well established from family and twin studies [1,2]. However, it has been challenging to identify the specific genetic variants associated with increased diabetes risk. A significant breakthrough in understanding the genetic basis of complex diseases including T2D has been facilitated by the arrival of genome-wide association studies (GWAS). Since 2007, GWAS have led to a rapid rise in the number of...
confirmed susceptibility variants for T2D [3–7]. Understandably these landmark studies have fuelled widespread expectation that genetic information will provide useful insights into the underlying pathophysiology of T2D and, ultimately, translate into improved care and novel therapeutics for patients with diabetes. This article summarizes recent advances in the field and discusses their clinical implications and the current obstacles to clinical translation.

T2D genetics before GWAS

Early research techniques to establish a link between genotype and phenotype included linkage analysis and candidate gene association studies. Linkage analysis detects genetic loci co-segregating with diabetes within families and was successful in uncovering the molecular genetic basis of monogenic β-cell dysfunction (maturation-onset diabetes of the young [MODY]) [8]. Candidate gene studies examine specific genes postulated to have a role in the pathogenesis of T2D. Despite considerable efforts, these methods initially yielded only two confirmed T2D susceptibility variants; the P12A change in the peroxisome proliferator activated receptor-γ (PPARG) gene [9] and the E23K variant in the ATP-sensitive potassium channel (K\(_{ATP}\)) gene, KCNJ11 [10]. More recent large-scale association studies have reported that common genetic variation in the WSF1 and HNF1B genes also confer susceptibility to T2D [11,12]. In 2006 the deCODE investigators identified a susceptibility variant in TCF7L2 (encoding the transcription factor 7-like 2 protein) through regional exploration of a previously demonstrated linkage signal on chromosome 10q [13]. This susceptibility variant has been subsequently replicated in multiple European and non-European groups [14] (although not in Pima Indians [15]) and has the largest effect on T2D risk described to date with a per-allele odds ratio (OR) of approximately 1.4.

GWAS era of T2D genetics

Genome-wide association studies are a powerful biology-agnostic method to detect genetic variation predisposing to disease by screening the entire genome of individuals with and without the disorder of interest for a large number of common single nucleotide polymorphisms (SNPs). These studies have been facilitated by several recent developments including completion of the Human Genome Project, availability of affordable high-throughput genotyping technologies, development of statistical and computational software to analyze the huge datasets and international collections of many thousands of individuals with well-characterized phenotypes. A higher frequency of a particular allele at a given SNP in the cases versus the controls suggests that it is associated with the disease, with a p value of 5 × 10\(^{-8}\) being required to satisfy genome-wide significance [16]. Each GWAS typically involves hundreds of thousands of simultaneous tests of association, therefore, this stringent threshold reflects the usual p value of 0.05 with a Bonferroni correction for 1 million statistical tests, and reduces the number of false-positive SNPs identified. Even with such strict statistical thresholds, positive findings are routinely replicated in independent datasets to verify or refute the association of a SNP with the phenotype of interest. The data from several case–control collections can be merged and summarized via meta-analysis, which has enabled identification of SNPs of smaller effect size by increasing the overall sample size. To date GWAS have identified over 40 susceptibility loci for T2D in European and Asian populations (Figure 1) [3,17–24].

Obstacles to clinical translation

One obstacle to clinical translation is that, although many new and interesting susceptibility loci have been identified, the majority are associated with small effect sizes (OR ~1.1–1.3). Typically each copy of a susceptibility allele at each locus is associated with only a 15–20% increase in the lifetime risk of T2D [25]. Together, the current confirmed susceptibility variants account for approximately only 10% of the known T2D heritability [26]. An additional obstacle to translation is that a SNP identified as associated with disease by GWAS is usually annotated by the gene in closest proximity. In fact, in the majority of cases the causal variants and molecular mechanisms for diabetes risk are unknown. Furthermore, most genetic risk variants are found in the intronic or noncoding regions of genes and most likely to affect the regulation of transcription rather than gene function per se. One of the biggest current challenges is characterizing the downstream consequences of these variants, which requires both physiological and functional studies to demonstrate a causal relationship [27]. Current rudimentary understanding of the underlying mechanism associated with each susceptibility variant limits translation to the clinical setting.
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Pathophysiological insights into T2D arising from GWAS

Genome-wide association studies allow an unbiased global search of the entire human genome and can, therefore, identify novel and unsuspected pathways involved in the pathogenesis of T2D. Most of the identified T2D susceptibility variants are not close to obvious candidate genes, suggesting that there is much left to be elucidated regarding the pathophysiology of this disease. There are some common emergent themes; the genes implicated are largely involved in β-cell function and insulin secretion rather than insulin resistance [19,28]. This may partly reflect design of some of the GWAS, which matched for BMI and therefore removed adiposity and insulin resistance from the equation. The FTO gene, predisposing to obesity, was only identified in the UK GWAS, which did not match for BMI [29]. However, these results do emphasize the critical role of the β-cell in all types of diabetes.

A further significant observation from the GWAS is the potential role of cell cycle regulation abnormalities in the pathogenesis of T2D, following the identification of several risk loci mapping close to genes involved in this process (such as CDKN2A/B) [4–6]. Additional evidence for the role of cell cycle regulation in β-cell mass includes islet hypoplasia and development of a T2D phenotype in rodents with overexpression of Cdken2a [30]. Furthermore, GWAS have highlighted that T2D susceptibility loci share associations with other common diseases including certain cancers [31]. Interestingly, CDKN2A/B encodes cyclin-dependent kinase inhibitors that are known tumor suppressors [32] and germ line loss-of-function mutations in CDKN2A cause a familial melanoma syndrome [32,33]. Ongoing research in this area may clarify epidemiological data linking diabetes and cancer [34].

Another epidemiological observation supported by recent genetic studies is the link between low birth weight and T2D risk [35]. The T2D susceptibility variants within or near ADCY5, CDKAL1 and HHEX-IDE genes have all been robustly associated with low birth weight [36,37] and provide further support for the fetal insulin hypothesis. This hypothesis proposes that genetic variants predisposing to reduced insulin secretion or action can restrict intra-uterine growth and, therefore, lower birth weight as well as the development of T2D in later life [38].

Potential clinical applications of the GWAS findings in diabetes

Risk prediction & prevention

The development of personalized susceptibility profiles based on genetic information in order to aid prediction, early detection and prevention of T2D is one potential clinical application of the recent GWAS. In fact genome-wide genetic profiling is already commercially available for prediction of T2D. This clinical application has been investigated in several prospective cohort studies including the Framingham Offspring study [39], Malmo Preventative Project (MPP) and Botnia study [40], Genetics of Diabetes Audit and Research Tayside (GoDARTS) study [26] and the Whitehall II prospective cohort study [41]. In these studies 16–20 susceptibility loci were genotyped, and a genetic score based on the number of risk alleles was calculated in those who developed diabetes during the follow-up period and those who remained disease free. This genetic score was compared with established risk prediction models (such as the Cambridge T2D risk score and Framingham offspring study T2D risk score), which incorporate various clinical and biochemical factors including BMI, parental history of diabetes and lipid profile [26,39–41]. The power of the phenotype-based risk models was significantly better than the genotype-based risk model to predict the individuals who developed T2D in this population [26,39–41]. Importantly the addition of the genetic score...
did not improve the discriminative accuracy of the phenotypic models. These studies have all concluded that traditional clinical risk factors most reliably define future risk of developing T2D, and that there is only marginal improvement with the addition of currently available genotypic information. This may reflect that phenotypic information already incorporates the genotypic information from the available risk alleles, the current number of susceptibility variants may be too low to accurately predict overall predisposition to T2D, or environmental factors such as obesity and diet might have a stronger affect on T2D risk. Interestingly, subset analyses of the study cohorts suggested that genetic testing may be beneficial in younger patients prior to the clinical manifestation of phenotypic characteristics associated with T2D. This was recently examined by de Miguel-Yanes and colleagues by re-calculating the genotype score using the updated list of 40 T2D susceptibility variants in the Framingham Offspring Study [42]. This study demonstrated that the genetic score marginally improved the ability to predict future diabetes in subjects younger than 50 years compared with phenotypic features. In fact this age group may be the most likely to benefit from lifestyle and medical intervention, prior to the development of adverse clinical features, although this would need to be confirmed in prospective studies.

Robust evidence that the use of genetic information can produce meaningful changes in human behavior or allow therapeutic intervention should precede widespread use of genetic testing for prediction. Genetic investigation of the Diabetes Prevention Program (DPP) suggested that homozygous carriers of the TCF7L2 risk allele randomized to the lifestyle intervention arm did not have an increased risk of T2D despite carrying the two copies of the risk allele (it usually confers 80% increased risk of developing diabetes) [43]. One study reported that a ‘high-risk’ result from genetic testing would inspire the 71% of 152 healthy subjects interviewed to adopt healthy lifestyle changes [44]. A second study found no evidence that direct-to-consumer genetic testing had any effect on short-term lifestyle behavior (diet and exercise) or psychological health [45]. This emphasizes that prospective clinical trials would be required to demonstrate that the anticipated enthusiasm would translate into measurable patient outcomes.

**Pharmacogenetics**

Pharmacogenetics is the effect of genetic variation on the therapeutic response and side-effect profile of oral hypoglycemic agents and may reflect differences in drug pharmacokinetics or metabolism at a molecular level. Genetic profiling may allow true ‘personalization’ of medicine by optimizing an individual’s treatment choices to maximize clinical efficacy and minimize toxicity. Experience with monogenic diabetes has already demonstrated that genetic information can guide clinical practice and management decisions; for example individuals with MODY caused by heterozygous HNF1A mutations are exquisitely sensitive to sulfonylureas [46] and children with permanent neonatal diabetes caused by activating mutations in the KCNJ11 gene can safely transfer from insulin to sulfonylureas [47]. Application of pharmacogenetics to T2D is more challenging. Initial studies were underpowered and rarely replicated, which led to inconclusive and conflicting results. However, some recent studies have demonstrated robust association between genetic variation and therapeutic response to commonly used oral hypoglycemic agents.

Metformin is the first-line treatment for T2D. It is not metabolized and is excreted unchanged via the kidneys and the biliary tract and so studies have focused on specific drug transporters. The multidrug and toxin extrusion 1 transporter, encoded by the SLC47A1 gene, is responsible for the final step of metformin clearance through bile and urine. Two independent studies reported that a variant in SLC47A1 was associated with reduced HbA1c in metformin-treated T2D patients [48,49]. However, the most compelling evidence to date though comes from a recent GWAS, which demonstrated that common variants near the ataxia telangiectasia mutated gene (ATM) are associated with an effective treatment response to metformin (defined as achieving HbA1c ≤7%) in over 1000 individuals with T2D from Scotland, which was subsequently replicated in two independent datasets from the UK [50]. Homozygous loss-of-function ATM mutations result in ataxia telangiectasia, a severe neurodegenerative disorder and, interestingly, these individuals have an increased risk of diabetes [51]. Furthermore, inhibition of ATM influences activation of AMP-activated protein kinase, which is widely considered to be a molecular target of metformin [52].
Pharmacogenetics has also been studied in patients treated with sulfonylureas. In 2007 Pearson and co-workers demonstrated that patients homozygous for the diabetes risk allele (G) of the TCF7L2 variant were twice as likely to fail sulfonylurea therapy compared with those homozygous for the T allele [53]. Another study showed that homozygous carriers of the ABCC8 A1369 risk allele (which is in complete linkage disequilibrium with the E23K KCNJ11 variant) had a greater therapeutic response to sulfonylureas in a prospective trial of over 1000 lean, Chinese T2D patients treated with gliclazide for 8 weeks [54,55]. This observation is supported by recent functional studies that have demonstrated that variant channels have an enhanced response to class A sulfonylureas such as gliclazide compared with wild-type channels [56]. Other groups have investigated the effect of variants in CYP2C9, which encodes the cytochrome p450 responsible for metabolizing sulfonylureas in the liver [49]. Zhou and colleagues demonstrated that homozygous carriers of the loss-of-function CYP2C9 alleles were 3.4-times more likely to achieve HbA1c targets and had lower risk of failure of sulfonylurea therapy than wild-type carriers [57]. These studies indicate that genetic variation can influence response to oral hypoglycemic agents. Genetic background alone is insufficient to predict response at an individual level but pharmacogenetics is a developing field with potential to advance the goal of personalized medicine. Future progress will require study of larger cohorts in which drug response is well characterized and eventually, prospective clinical trials to assess whether genotypic information can guide therapeutic choices effectively.

**Novel therapeutic targets**

As well as providing useful insights into the pathophysiology of T2D, it is likely that the susceptibility loci identified in the GWAS could highlight novel biological pathways or molecular targets that may be amenable to therapeutic intervention. The two oldest T2D susceptibility variants lie within the genes PPARG and KCNJ11/ABCC8, which encode targets of established oral hypoglycemic agents, thiazolidinediones and sulfonylureas, respectively. This highlights that genetic variants of modest effect may illuminate pathways that could be targeted for drug development. For example, the first T2D GWAS identified a susceptibility variant in the SLC30A8 gene (which encodes the β-cell zinc transporter ZnT-8) [3]. This protein transports zinc into the β-cell for insulin storage and secretion [58]. A therapeutic agent that enhances the intracellular function of this transporter could hypothetically increase insulin secretion and lower blood glucose levels. Perhaps not surprisingly given the relatively short time since their discovery, no medical therapies have directly stemmed from GWAS findings so far, but this remains a distinct future possibility.

**Biomarkers to aid diabetes diagnostics**

One potential clinical application that has arisen directly from GWAS is based on the observation that patients with MODY caused by HNF1A mutations have significantly lower blood levels of high-sensitivity C-reactive protein compared with other types of diabetes [59]. This could form the basis of a useful diagnostic test in order to select subjects with common types of diabetes who would benefit from diagnostic testing for underlying MODY mutations. This finding stemmed from two separate GWAS showing that C-reactive protein levels are influenced by common variation near the HNF1A gene in healthy adults [60,61]. Individuals with HNF1A-MODY are commonly misdiagnosed despite important clinical implications [62] and, therefore, a widely-available, cost-effective biomarker could improve diagnosis rate of monogenic diabetes. Analogous to this example in monogenic diabetes, it is possible that the presence of specific underlying genetic variants could allow sub-classification according to the underlying molecular pathway in T2D. This could facilitate a more personalized pathway of management and treatment in T2D as well.

**Future perspective**

The heritability of T2D remains largely unexplained by the growing list of T2D susceptibility variants. One hypothesis to account for the ‘missing heritability’ is the role of low-frequency (LF) risk variants (Figure 2) [63]. GWAS have focused on finding common variants associated with disease and, therefore, the role of variants with a minor allele frequency (MAF) of <5% is unknown. LF variants are predicted to confer a larger effect on T2D risk to the individual with an OR of approximately 2–3. Nejentsev and colleagues resequenced coding regions of ten candidate genes to find causative variants...
for Type 1 diabetes, and identified four rare variants within the IFIH1 (MAF ≤ 1.1%) that lowered Type 1 diabetes risk (OR: 0.5–0.7), independently of each other [64]. Although LF variants have been implicated in the pathogenesis of other complex diseases such as hypertriglyceridemia [65,66], none have yet been identified that are associated with T2D. The identification of large effect susceptibility variants will enhance potential for clinical translation. The search for LF variants will be facilitated via the 1000 Genomes Project [67]. This international collaborative initiative is using next generation whole-genome sequencing technology to systematically catalog all variants with a minor allele frequency of greater than 1% of at least 1000 genomes. Early pilot analyses have identified more than several million new polymorphisms including insertions, deletions and large structural variants [68]. The discrimination of pathogenic mutations from incidental genetic variation will be challenging and will require robust statistical, functional and physiological studies. Additional research resources are being directed towards other types of genetic variations including structural variations (e.g., copy number variants) [69] and epigenetic modification (e.g., DNA methylation and histone acetylation) [70,71]. Furthermore, the majority of GWAS have been conducted in European populations and so a priority is to examine non-European populations. This may highlight different metabolic pathways or identify shared variants that exist at higher frequencies in non-European populations and are, therefore, more easily detected.

It is clear that full understanding of GWAS results will require identification of the causal gene associated with a susceptibility variant. This requires ‘fine-mapping’, functional and physiological investigation, which are labor intensive and time consuming. For example, despite several functional studies, the precise mechanism through which TCF7L2 modulates pancreatic function remains incompletely understood. It is a component of the Wnt signaling pathway and may have a role in the preservation of β cell mass [72]. Physiological studies of nondiabetic TCF7L2 risk allele homozygote carriers have shown reduced insulinogenic index and insulin disposition index during oral glucose tolerance tests [73] and impaired incretin effect [74]. A recent study has shed light on the potential molecular mechanism of how TCF7L2 could influence gene function. It was demonstrated in human pancreatic islets that the TCF7L2 risk variant alters chromatin state and that carriers of the risk variant have more open chromatin at this locus [75]. Since open chromatin state correlates with increased transcriptional activity, this provides one possible mechanism linking an intronic variant with a disease phenotype. Rigorous functional assessment of the susceptibility variants should facilitate advances in clinical translation of risk alleles identified through GWAS.

Finally, any proposed clinical application based on new genetic technologies should be evaluated in well-conducted prospective trials prior to widespread use.

Conclusion
The exciting results generated by GWAS have led to intense speculation regarding their clinical utility. The lack of clinical impact to date is not surprising as this branch of genetic research is very new. Importantly, experience within monogenic diabetes has proven that genetic studies can affect treatment and diagnosis. It is challenging to translate the GWAS findings into improved care for diabetic patients. Current obstacles to clinical translation are the focus of ongoing research efforts; these include detailed functional characterization of the identified T2D susceptibility variants as well as searching for ‘missing heritability’. Clinicians should maintain interest in the current revolution in T2D genetic research and acknowledge these studies will
undoubtedly facilitate broadened understanding of the pathogenesis of this complex disease. Potential clinical repercussions of the current and future discoveries are likely to occur in a gradual manner. At present, the era of ‘personalized medicine’ – specifically individualized disease prediction, prevention and therapy is a distant prospect. Benefits are more likely to stem from unsuspected insights into the pathogenesis of T2D and identification of molecular targets for the development of novel therapeutic agents.

**Bibliography**

Papers of special note have been highlighted as:

- of interest
- of considerable interest


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**MANAGEMENT PERSPECTIVE**

**Financial & competing interests disclosure**

Katharine R Owen is an NIHR-funded Clinician Scientist. Anna L Gloyn is a Medical Research Council New Investigator (81696). This work is also supported by the Oxford NIHR Biomedical Research Centre. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.
Genome-wide association studies identified variant to be associated with response to metformin, which highlights an unexpected link between glucose homeostasis and DNA repair mechanisms.

First robustly replicated common genetic variant to be associated with response to metformin, which highlights an unexpected link between glucose homeostasis and DNA repair mechanisms.

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**Potential example of clinical translation arising from a genome-wide association studies finding.**


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