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REVIEW

Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: Where are the insulin resistance genes?

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Abstract Although type 2 diabetes has been traditionally understood as a metabolic disorder initiated by insulin resistance, it has recently become apparent that an impairment in insulin secretion contributes to its manifestation and may play a prominent role in its early pathophysiology. The genetic dissection of Mendelian and, more recently, polygenic types of diabetes confirms the notion that primary defects in insulin synthesis, processing and/or secretion often give rise to the common form of this disorder. This concept, first advanced with the discovery and physiological characterisation of various genetic subtypes of MODY, has been extended to other forms of monogenic diabetes (e.g. neonatal diabetes). It has also led to the identification of common risk variants via candidate gene approaches (e.g. the E23K polymorphism in *KCNJ11* or common variants in the MODY genes), and it has been validated by the description of the robust physiological effects conferred by polymorphisms in the *TCF7L2* gene. More recently, the completion and integration of genome-wide association scans for this disease has uncovered a number of heretofore unsuspected variants, several of which also affect insulin secretion. This review provides an up-to-date account of genetic loci that influence risk of common type 2 diabetes via impairment of beta cell function, outlines their presumed mechanisms of action, and places them in the context of gene–gene and/or gene–environment interactions. Finally, a strategy for the analogous discovery of insulin resistance genes is proposed.

Keywords Genetic associations · Genome-wide association study · Insulin secretion · Pancreatic beta cell · Single nucleotide polymorphisms

Abbreviations

GLP-1	glucagon-like peptide 1
GWAS	genome-wide association scan
HNF	hepatocyte nuclear factor
HOMA-IR	homeostasis model assessment of insulin resistance
siRNA	small interfering RNA
SNP	single nucleotide polymorphism

At the time this paper was undergoing final review, a meta-analysis of three high-density GWAS for type 2 diabetes followed by replication in ~80,000 independent samples was published online [Zeggini E, Scott LJ, Saxena R, Voight BF for the Diabetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet DOI:10.1038/ng.120]. This meta-analysis identified six new loci (*JAZF1*, *CDC123-CAMK1D*, *TSPAN8-LGR5*, *THADA*, *ADAMTS9* and *NOTCH2-ADAM30*) associated with type 2 diabetes at genome-wide statistical significance. Although their effect on beta cell function is not yet known, several of these genes are expressed in the pancreas.

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Introduction

The simple metabolic characterisation of type 1 diabetes as an insulin deficiency syndrome and type 2 diabetes as a primarily insulin-resistant state has been superseded by more refined understanding of the pathophysiology of non-autoimmune forms of diabetes. While hyperinsulinaemia has long been recognised as a primary feature of type 2 diabetes [1, 2], the hyperglycaemia that defines the diagnosis is now viewed as a consequence of a complex interplay between insulin sensitivity and secretion, with a failure of pancreatic beta cells to compensate sufficiently for the increased insulin requirement induced by insulin resistance [3]. Thus, in metabolic studies of beta cell function, insulin secretion should always be interpreted in the context of concomitant insulin sensitivity [4].

Whether insulin resistance or insufficient insulin secretion represents the primary defect in the pathogenesis of type 2 diabetes remains a matter of debate [5–7]. Because glucose tolerance is achieved by the combination of insulin secretion and insulin action, hyperglycaemia (even with values that stay within the normal range) can manifest itself when one component fails and there is no concomitant improvement in the other. While many individuals who are insulin resistant never develop diabetes (indicating the need for a beta cell defect for full-blown hyperglycaemia to manifest itself), secretory deficits can also be demonstrated in normoglycaemic individuals. Thus, it appears that both derangements are necessary, but not sufficient to reach the levels of hyperglycaemia that yield a clinical diagnosis, and that one may occur without the other prior to the onset of overt disease.

This controversy has involved the genetic field. Clearly, a genetic variant that predisposes an individual toward reduced insulin secretion or increased insulin resistance would confer temporal precedence in the pathophysiological cascade to the particular parameter with which it is associated. Epidemiologists who track the current twin explosions of obesity and type 2 diabetes correctly conclude that the environment must play a large role in the epidemic of insulin resistance, as these recent (and rapid) secular trends could not have been driven by genetic changes. Thus, an attractive model has been proposed in which type 2 diabetes emerges when environmentally triggered insulin resistance takes place in the context of genetically programmed beta cell dysfunction [8, 9].

Several predictions could be drawn from this hypothesis: first, genetic mutations that solely affect the beta cell should cause diabetes; second, an unbiased screen for genetic variants associated with common type 2 diabetes should yield a preponderance of beta cell genes; and third, if genetic variants exist that lead to insulin resistance, their mode of action should take place in the context of an interaction with the environment. In the sections that follow, the accumulating evidence supporting each of these three corollaries will be described and an approach to the discovery of insulin resistance genes will be proposed.

Monogenic diabetes

The MODY genes: from Mendelian to common disease The clinical characterisation of MODY established that diabetes could develop on a familial basis without the requirement of insulin resistance [10]. The concerted application of modern genetic linkage and positional cloning techniques led to the successive identification of six MODY genes (*HNF4A*, *GCK*, *TCF1* [also known as *HNF1A*], *PDX1*, *TCF2* [also known as *HNF1B*] and *NEUROD1*), which, overall, are involved in ~85% of MODY cases [11]. All six MODY genes are expressed in the beta cell, with glucokinase (encoded by *GCK*) serving as the glucose sensor that controls the set point for insulin secretion, and the rest acting as transcription factors regulating pancreatic beta cell development and final beta cell mass. These landmark discoveries both established a genetic basis for hyperglycaemia and focused attention on the pancreatic beta cell as a key component in its pathogenesis [12].

In the search for inherited factors that contribute to the more common form of type 2 diabetes, genes implicated in monogenic forms of diabetes—in which a single mutation that has dramatic functional consequences can, by itself, produce the phenotype—have naturally been considered prime biological candidates. Under this paradigm, genetic variants that have a less radical effect on gene expression, transcript processing and/or protein function may induce a metabolic alteration that is sufficient to give rise to a milder form of the disease. Because of the less stringent negative selection pressure on these polymorphisms compared with those linked to a more detrimental phenotype, they would presumably rise to fairly common frequencies in the population. Consequently, common variation in the MODY genes has been studied exhaustively for association with type 2 diabetes.

The MODY gene that has been most extensively examined for association with common type 2 diabetes is *HNF4A*, in part due to its location under a widely replicated linkage peak on chromosome 20 q (reviewed in [13]). Two promoter single nucleotide polymorphisms (SNPs) in *HNF4A* were originally associated with type 2 diabetes in two studies of white populations [14, 15]; this was confirmed in two subsequent large association studies [16, 17], although they yielded more modest ORs. A fifth very large study obtained consistent evidence in a Scandinavian

sample of over 3,000 individuals, but failed to find support in two other white case–control samples totalling over 4,000 individuals [18]. A comprehensive meta-analysis of over 18,000 people has documented a very modest (combined OR ~1.07), but statistically significant (p= 0.003) association for the original SNPs, although coloured by substantial heterogeneity (L. J. Scott, Department of Biostatistics, University of Michigan, Ann Arbor, MI, USA, personal communication). It therefore appears that variants in *HNF4A* contribute a very small proportion of type 2 diabetes risk.

The MODY 3 gene *TCF1* (encoding hepatocyte nuclear factor 1α [HNF1α]) has also received significant attention. In two companion large-scale association studies, meta-analyses of previously published data showed possible associations of two missense SNPs, A98V and I27L, with type 2 diabetes [19, 20]. While inclusion of novel large case–control samples moved both summary ORs towards the null, a current meta-analysis for the A98V polymorphism (J. C. Florez, unpublished results) shows an OR of 1.17 (95% CI 1.03–1.33, p=0.02). Later studies have furnished additional supporting evidence for other SNPs in this gene, including I27L [21–24]. Although a current unpublished meta-analysis of case–control studies for this latter SNP has shown no association (OR 1.02, 95% CI 0.99–1.07, p=0.17), the leucine allele affects the transcriptional activity of HNF1α, is associated with decreased insulin secretion, and appears to raise the risk of diabetes in overweight and/or elderly persons [21–24]. More recently, it has also been shown to predict diabetes onset in a prospective study of a very large Swedish cohort (HR 1.2, 95% CI 1.1–1.3, p=0.0002) [25].

Of particular interest is the intronic SNP rs757210 in *TCF2* (encoding HNF1β). A comprehensive study of several MODY genes noted a modest but robust association of this SNP with type 2 diabetes, which was replicated in additional independent populations: a combined analysis of the >15,000 samples yielded an overall OR of 1.12 and a convincing p value of $<10^{-6}$ [26]. Results consistent with this finding had been obtained in another comprehensive study of MODY genes [21]. Even more intriguing is the association of the protective allele at the same locus with prostate cancer [27], a coincidence that remains unexplained.

Of the other MODY genes, large-scale association studies and meta-analyses have shown an association of the G–30A SNP in the *GCK* promoter with fasting glucose [25, 26, 28]. On the other hand, the previously reported association of the missense A45T SNP in *NEUROD1* with type 2 diabetes has not been substantiated in a comprehensive meta-analysis [29]. Suggestive results for other polymorphisms [21, 26] need to be convincingly replicated to muster persuasive statistical evidence.

With regard to the effect of these associations on beta cell function, a decrease in insulin secretion has been documented for the A98V and I27L SNPs in *TCF1* [22]; nevertheless, the expression patterns and the known impact of functional mutations in these genes on beta cells make it reasonable to presume that other polymorphisms in MODY genes, if truly associated with type 2 diabetes, will likely cause hyperglycaemia via analogous mechanisms of action. Polymorphisms in *GCK* may lead to a resetting of the beta cell glucostat, while other MODY variants may reduce beta cell mass.

The gene encoding wolframin: from syndromic to common diabetes Wolfram syndrome is an autosomal dominant syndromic form of diabetes characterised by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. Affected children develop the clinical manifestations of sensory neuron and beta cell degeneration at approximately 6–8 years of age, and often succumb to the disease. Positional cloning identified the gene *WFS1* on chromosome 4p16 as the cause of the syndrome. Its protein product, wolframin, is expressed in the membranes of neurons and pancreatic beta cells and regulates calcium fluxes in the endoplasmic reticulum [30]. Most mutations that give rise to Wolfram syndrome occur in exon 8 and have major effects on the expressed protein [31].

A team of UK investigators recently evaluated *WFS1* and 83 other candidate genes for association with type 2 diabetes in a set of four white case–control populations [32]. Of 1,536 SNPs genotyped at an initial stage, 18 were promoted to the second stage and two achieved statistical replication. Both SNPs, which accounted for the same association signal, were located in *WFS1*. These and two other correlated SNPs were robustly ($p \sim 10^{-7}$) but modestly (OR ~0.90) associated with type 2 diabetes in a larger set of 9,533 cases and 11,389 controls [32]. This association has been confirmed in several independent cohorts [33, 34], and the implicated polymorphisms appear to increase diabetes risk by compromising beta cell function [34].

Polygenic diabetes

KCNJ11: *from Mendelian to common disease, and back* The gene that encodes the islet ATP-sensitive potassium channel Kir6.2 (*KCNJ11*) spans 1 kb and is located directly downstream of the gene encoding the sulfonylurea receptor SUR1 (*ABCC8*) in the same region of chromosome 11. Both molecules interact physically and functionally to regulate the potassium inward rectifier current and, thereby, beta cell depolarisation, the trigger for insulin release [35].

KCNJ11 was another excellent biological candidate presumed to harbour genetic variants contributing to type 2 diabetes, in part because of known mutations that inactivate the channel and give rise to persistent hyperinsulinaemia of infancy [36], and in part because of the precedent afforded by *PPARG*, the gene that encodes the peroxisome proliferator-activated receptor γ, itself a target for medications that lower insulin resistance: the P12A polymorphism in *PPARG* had been conclusively associated with common type 2 diabetes [37].

A missense variant that changes a glutamic acid residue to lysine at position 23 (E23K) was examined in several early studies that failed to document a significant association [37–40]. As is often the case, larger sample sizes and subsequent meta-analyses confirmed that the E23K polymorphism in *KCNJ11* is also reproducibly associated with type 2 diabetes [41–47], with an OR near 1.15 ($p<10^{-7}$). Functional studies indicate that this polymorphism significantly influences channel gating properties [48–50]. In vivo, human carriers of the lysine risk allele demonstrate impaired insulin secretion [44, 46]. In a research path that retraced the steps of the one travelled by the MODY genes, mutations that have a more potent effect on channel activation have been recently implicated in a subtype of permanent neonatal diabetes [51].

TCF7L2: *the genetic ceiling for type 2 diabetes?* When deCODE (Reykjavik, Iceland) investigators zeroed in on a region of chromosome 10 under a linkage peak and identified polymorphisms in the gene that encodes transcription factor 7-like 2 (*TCF7L2*) as likely contributors to type 2 diabetes, they launched an entirely new direction in diabetes research [52]. In early 2006, Grant et al. reported that a common microsatellite in the *TCF7L2* gene region (DG10S478) was associated with type 2 diabetes in an Icelandic case–control sample (n=2,116), and replicated this result in two additional case–control white cohorts (n=1,658) [53]. The overall estimated allelic relative risk was 1.56 ($p=7.8\times10^{-15}$ after Bonferroni correction for the number of alleles tested). The non-coding SNPs rs12255372 and rs7903146 were in strong linkage disequilibrium with DG10S478, and showed comparably robust associations with type 2 diabetes.

A quick succession of positive replication studies followed. Two meta-analyses of the accumulated evidence (~50,000 individuals) reveal the remarkable consistency of this finding, and yield an overall p value of $<10^{-80}$ [54, 55]. The preponderance of the evidence indicates an additive effect, with a single copy of the risk allele conferring ~40% risk, and two copies (carried by ~10% of the European or African populations) conferring ~80% risk of type 2 diabetes. In addition to rs7903146 [56], a different downstream variant in the same gene seems to increase diabetes risk in Asians [57].

TCF4 (the protein product of *TCF7L2*) belongs to a family of transcription factors that contain high mobility group box DNA-binding domains; it binds β catenin after Wnt activation of its receptor, and this transcriptional complex induces the expression of TCF4 target genes. *Tcf7l2* homozygous null mice lack gut epithelial stem cells, which leads to marked deficits in the enteroinsular system [58]. This specialised gastrointestinal cell compartment is responsible for the manufacture of incretins, hormones secreted by the gut in response to an oral energy load, which regulate gut motility, satiety and energy homeostasis [59]. Pre-eminent among them is glucagon-like peptide 1 (GLP-1), which among other functions stimulates insulin secretion by the pancreatic beta cell; it turns out that TCF4 *trans*-activates the gene that encodes GLP-1 [60].

The link between *TCF7L2* and GLP-1 pointed to the beta cell as the locus in which variants in *TCF7L2* might increase the risk of type 2 diabetes. Consistent with this hypothesis, we [61, 62] and others [63–65] showed that carriers of the risk T allele at rs7903146 have diminished insulin secretion after an oral glucose load. In elegant studies, Lyssenko and colleagues have further shown that the incretin effect is also diminished in risk allele carriers [65]. However the simple model by which gut-secreted GLP-1 mediates the effects of *TCF7L2* has been challenged by newer evidence showing that the defect in insulin secretion can also be seen in risk allele carriers after an intravenous glucose load [66], and that the genotype at *TCF7L2* does not appear to affect basal or glucose-stimulated GLP-1 levels; rather, risk allele carriers demonstrate diminished GLP-1-induced insulin secretion [67]. Interestingly, a recent report shows that the proliferation induced by GLP-1 agonists in INS-1 or mouse primary beta cells requires active Wnt signalling, and that this effect is inhibited by the dominant-negative mutant of *TCF7L2* [68]. Simultaneously, another group has shown that knockdown of *TCF7L2* expression with small interfering RNA (siRNA) leads to increased apoptosis and decreased proliferation of human beta cells [69]. Thus, these various lines of evidence suggest that *TCF7L2* is at work in the beta cell itself, and may modulate beta cell mass.

An alternative hypothesis that is also consistent with the above findings suggests that *TCF7L2* causes a defect in insulin processing [70]. Human islets isolated from T allele carriers exhibit increased *TCF7L2* expression (even after adjusting for diabetes status), and expression of *TCF7L2* is correlated with expression of the gene for insulin (*INS*) [65]. Consistent with this model, siRNA knockdown of *TCF7L2* leads to lower *Ins* gene expression and lower glucose-mediated insulin secretion in mouse insulinoma

cells [71], as well as impaired glucose-stimulated insulin secretion in human beta cells [69]. Despite higher levels of insulin gene expression and higher measurable proinsulin in serum [70, 72], T allele carriers have diminished insulin secretion both in vivo [61–66] and in vitro [65]. Thus, genotype at *TCF7L2* seems to insert a block between *INS* gene expression and post-translational processing or secretion, although this relative increase in proinsulin levels may just be a marker of general beta cell dysfunction.

Multiple genome-wide association scans (GWAS) have shown that *TFC7L2* variants confer the strongest effect on type 2 diabetes risk yet described (see below). Given its ability to predict diabetes incidence in people who are already at high risk of the disease [61, 73], there is great interest in intervening to counteract its biological effects. In support of the hypothesis by which insulin resistance enables the clinical manifestation of genetic beta cell defects, a lifestyle intervention that causes weight loss reduced diabetes incidence to baseline levels even in carriers of the risk variant [61, 73]. Understanding the mechanism by which intronic variants in this gene cause defective insulin secretion is one of the great priorities of ongoing diabetes

Table 1 Genetic variants associated with type 2 diabetes at genome-wide significance known to influence beta cell function

Marker	Chr.	Description	Nearest gene	Gene function/ mechanism of action	Risk allele	Approximate effect size for type 2 diabetes association	*p* value	Insulin secretion associations	Key references
rs5219	11	Missense: E23K	*KCNJ11*	Kir6.2 potassium channel; regulates rectifier current in beta cell under control of ATP	T	1.14	5×10^{-11}	Ins Index	[41–44, 46]
rs7903146	10	Intronic	*TCF7L2*	Transcription factor; *trans*-activates genes encoding proglucagon and insulin	T	1.37	1×10^{-48}	Ins Index, AIR	[53,61, 62, 65]
rs757210	17	Intronic	*TCF2*	Transcription factor involved in pancreatic development	A	1.10	8×10^{-10}		[26, 27]
rs13266634	8	Missense: R325W	*SLC30A8*	Beta cell zinc transporter ZnT-8; insulin storage and secretion	C	1.15	1×10^{-10}	CIR, insulin AUC, DI	[74, 78, 87, 88]
rs1111875	10	7.7 kb downstream	*HHEX*	Transcription factor involved in pancreatic development	C	1.15	7×10^{-17}	I/G_{30}, Ins Index, AIR, insulin AUC	[74, 85–87]
rs10946398	6	Intronic	*CDKAL1*	Homologous to CDK5RAP1, cyclin-dependent kinase 5 inhibitor; islet glucotoxicity sensor	C	1.14	2×10^{-18}	CIR, I/G_{30}, AIR	[75–78, 85, 88]
rs4402960	3	Intronic	*IGF2BP2*	*IGF2* mRNA binding protein; pancreatic development	T	1.14	9×10^{-16}	DI	[75–77, 88]
rs10811661	9	125 kb upstream	*CDKN2A/B*	Cyclin-dependent kinase inhibitor and p15 tumour suppressor; islet regeneration	T	1.20	8×10^{-15}	Ins Index, AIR	[75–77, 86]
rs10010131	4	Intron–exon junction	*WFS1*	Endoplasmic reticulum transmembrane protein	G	1.12	5×10^{-11}	Ins Index	[32–34]

Effect size and *p* values were obtained from Frayling et al. [100], except those for *WFS1*, which were obtained from Franks et al. [33]. Variants that have suggestive evidence of association with type 2 diabetes but have not yet reached genome-wide significance levels ($p\sim5\times10^{-8}$) are not included

AIR, acute insulin response; AUC, area under the curve during a 2 h OGTT; Chr., chromosome; CIR, corrected insulin response; DI, disposition index; Ins Index, insulinogenic index; I/G_{30}, the Δ insulin response between 0 and 30 min divided by the 30 min glucose level during an OGTT

research, which has been spawned by well-designed and adequately powered genetic association studies in humans.

Genome-wide association studies GWAS have both validated known genes and revealed several novel type 2 diabetes loci. The first GWAS reproduced the robust association of *TCF7L2* (OR 1.65, $p<10^{-7}$) while also identifying *SLC30A8* (OR 1.26, $p<10^{-6}$) and *HHEX* (OR 1.21, $p<10^{-5}$) as two new type 2 diabetes-associated genomic regions [74]. Three additional high-density GWAS, which shared results and were published jointly (representing an aggregate of 32,000 samples), confirmed the known *TCF7L2*, *KCNJ11* and *PPARG* associations as well as the recently published *HHEX* and *SCL30A8* associations. They also discovered the novel diabetes loci *CDKAL1* (OR 1.12, $p<10^{-10}$), *IGF2BP2* (OR 1.14, $p<10^{-15}$) and *CDKN2A/B* (OR 1.20, $p<10^{-14}$) [75–77]. Simultaneously, the deCODE investigators and their collaborators corroborated the strong signal of *TCF7L2* and replicated the *HHEX* and *SCL30A8* findings, while independently identifying *CDKAL1* as an additional locus [78]. An initial association of a variant in the *FTO* gene with diabetes was subsequently discovered to be mediated via its impact on obesity [79]. Suggestive variants reported by GWAS conducted at lower density and/or in smaller samples [80–84] await replication in additional cohorts before they reach similar levels of genome-wide statistical significance ($p\sim5\times10^{-8}$).

Interestingly, many of the newly discovered variants appear to influence insulin secretion rather than insulin resistance (Table 1). For example, Steinthorsdottir et al. demonstrated insulin secretion defects in risk allele carriers at *CDKAL1* and *SLC30A8* [78]. Pascoe et al. performed 75 g OGTTs and hyperinsulinaemic–euglycaemic clamps in 1,276 European participants, and showed that variants in *CDKAL1* and *HHEX* are associated with decreased pancreatic beta cell function as measured by the 30 min insulin response, even after correction for insulin resistance; no variant was associated with insulin sensitivity [85]. Grarup et al. simultaneously reported that variants of *HHEX*, *CDKN2A/B* and *IGF2BP2* were associated with type 2 diabetes, and SNPs within the *HHEX* and *CDKN2A/B* loci impaired glucose-induced insulin release in a Danish population sample and in healthy Danes [86]. Staiger et al. found that the major alleles of the *SLC30A8* SNP rs13266634 and the *HHEX* SNP rs7923837 were associated with reduced insulin secretion stimulated by orally administered glucose, but not with insulin resistance [87]. A quantitative trait analysis of GWAS-identified diabetes susceptibility loci was recently completed by Palmer and colleagues in the Insulin Resistance Atherosclerosis (IRAS) Family Study [88]. In this cohort of 1,268 Hispanic and 581 African-American participants, the diabetes risk allele at *CDKAL1* was associated with a diminished acute insulin response during the IVGTT, and risk variants at *SLC30A8* and *IGF2BP2* appeared to be associated with a reduced disposition index. In sum, with few exceptions, the overwhelming majority of newly discovered diabetes genes appear to influence pancreatic beta cell function (see Fig. 1).

Are there insulin resistance genes?

Although rare mutations can cause extreme insulin resistance [89], there seems to be a dearth of common genetic variants in established pathways of insulin action that contribute to type 2 diabetes. Indeed, as outlined above, other than *PPARG* (discovered by candidate gene approaches [37]) and *FTO* (discovered by GWAS [79]), most of the newly identified loci are associated with insulin secretion defects. There are several possible explanation for the relative scarcity of insulin resistance genes discovered via GWAS (see text box: Potential reasons for the relative scarcity of insulin resistance genes found via GWAS approaches).

Potential reasons for the relative scarcity of insulin resistance genes found via GWAS approaches

1. Study design. Deliberate matching of cases and controls for BMI or selecting for lean cases may bias the scan against variants that increase diabetes risk via their effects on fat accumulation
2. Lower heritability of insulin resistance traits indicates a stronger influence of environmental covariates
3. There may be fewer variants that affect insulin resistance
4. Variants that affect insulin resistance may be less frequent in the population (i.e. more difficult to detect with current arrays)
5. Variants that affect insulin resistance may have more modest effect sizes (i.e. more difficult to distinguish from surrounding statistical noise)
6. Ascertainment criteria may impose constraints around insulin resistance measures that restrict the variance in the trait
7. The measures of insulin sensitivity studied in large human studies may be poorly correlated with insulin resistance at the tissue or molecular level

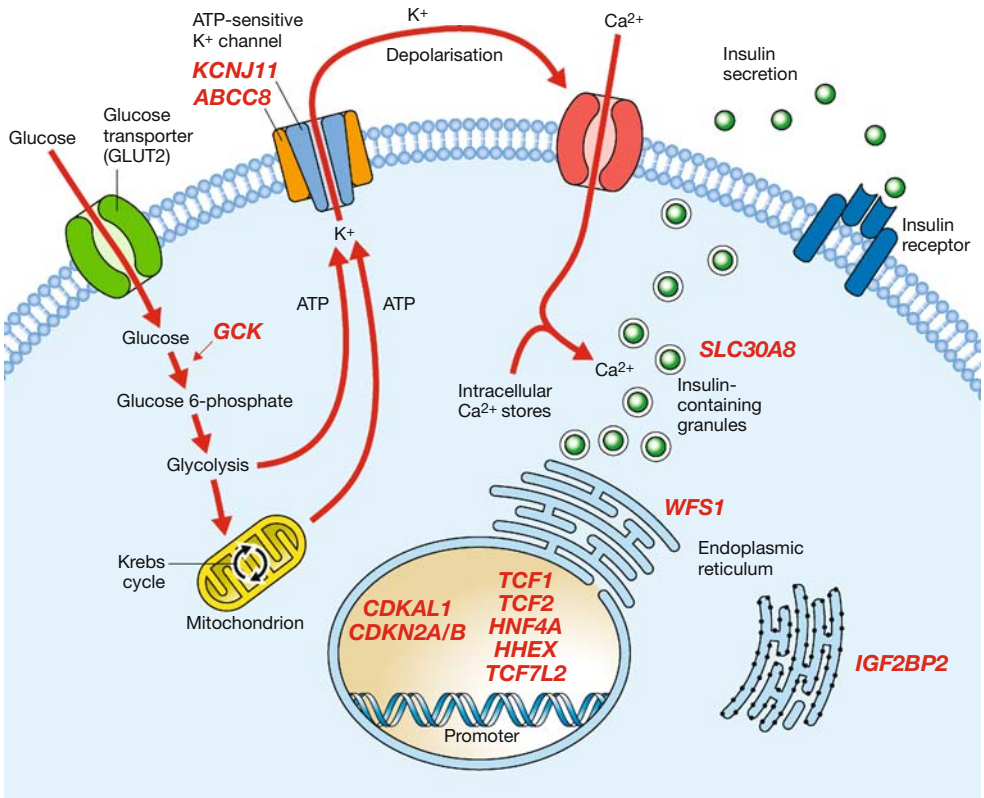

Fig. 1 Schematic diagram of the pancreatic beta cell showing the proposed subcellular localisation of proteins encoded by diabetes-associated genes. *GCK* encodes glucokinase, the glucose sensor of the beta cell. *KCNJ11* encodes the islet ATP-sensitive potassium channel Kir6.2, which interacts with the sulfonylurea receptor (SUR1, encoded by *ABCC8*) to regulate potassium currents across the cell membrane. *HNF4A*, *TCF1* (encoding HNF1α), *TCF2* (encoding HNF1β), *HHEX* and *TCF7L2* encode transcription factors produced in the beta cell and implicated in pancreatic development. *WFS1* encodes wolframin, a protein that regulates calcium transport in the endoplasmic reticulum. *SLC30A8* encodes the ZnT-8 transporter responsible for transporting zinc into insulin secretion granules. *CDKAL1* and *CDKN2A/B* are involved in the cyclin-dependent kinase pathway, and may thus influence beta cell regeneration. *IGF2BP2* encodes a protein that binds *IGF2* mRNA and directs it to specific subcellular locations for protein synthesis. It should be noted that many of these genes are also expressed in several other human tissues

First, study design may be partially responsible. For example, in the GWAS by Sladek et al. [74] diabetic cases were pre-selected below a BMI cut-off value, and in the Diabetes Genetics Initiative GWAS [74] cases and controls were matched for BMI; both deliberate interventions are more likely to bias the scan towards variants that increase diabetes risk without having an effect on BMI. Since BMI and insulin resistance are highly correlated, a design that minimises the contribution of BMI is more likely to identify genetic variants that contribute to hyperglycaemia via defects in beta cell function rather than via peripheral insulin resistance induced by adiposity. In support of this notion, the well-known diabetes-associated polymorphism P12A in *PPARG* (a target for thiazolidinediones) achieved unimpressive *p* values in both of these scans [74, 75], whereas it reached a higher level of statistical significance in scans that did not condition the selection of cases on BMI [76, 77].

Second, OGTT-derived surrogate measures of insulin secretion such as the insulinogenic index are, on average, ~10% more heritable than OGTT-derived measures of insulin resistance such as homeostasis model assessment of insulin resistance (HOMA-IR). This suggests that the environment accounts for a larger proportion of the variance in insulin resistance, and study designs that take into account appropriate covariates (such as obesity traits) are more likely to identify true biological effects.

Third, variants that increase insulin resistance may be fewer in number, less frequent in the population or have more modest effects. Thus, while real, their identification may require deeper explorations down the *p* value distribution and nuanced replication strategies across GWAS.

Finally, in the GWAS for which HOMA-IR values are publicly available (http://www.broad.mit.edu/diabetes, accessed 8 April 2008) [75], the search for HOMA-IR associations was performed in controls whose normoglycaemic status had been ascertained both in the fasting state and after a glucose challenge; while appropriately conservative to exclude participants in whom metabolic derange-

ments may have affected this measure, the establishment of a stringent threshold at the upper bound of normal glucose tolerance must have narrowed the variance around this measure, and thus hampered our ability to demonstrate strong genetic associations with insulin resistance.

Several lines of evidence support the foregoing assertions. While the heritability of insulin resistance traits may be lower than those related to insulin secretion, it is still substantial (in the order of ~0.40 in the Framingham Heart Study [90], for instance). This suggests that insulin resistance genes are there to be found, perhaps further down the *p* value distribution. It should be noted that the various published GWAS did not have full power to detect all of the true positive associations, and the initial analyses have been appropriately conservative. Furthermore, given the correlation between obesity and insulin resistance, the extent and compartmentalisation of adiposity is likely to modulate the effects of genes that influence insulin sensitivity. For example, adjusting for BMI reduced the heritability of HOMA-IR from 0.49 to 0.45 in the Framingham Heart Study [90]. Of the few known genes presumed to induce insulin resistance by their molecular mechanism of action, BMI × genotype interactions have been noted for the *PPARG* P12A polymorphism [91–95] and, more recently, for the missense K121Q polymorphism in the gene encoding ectoenzyme nucleotide pyrophosphate phosphodiesterase (*ENPP1*) [96–98]. This general observation outlines a possible scenario in which a genetic predisposition to insulin resistance may initially have a beneficial effect on BMI, which counterbalances the genetically driven decrease in insulin sensitivity; however, if BMI rises as a result of environmental or other genetic factors, the higher level of insulin resistance caused by both insults (the genetic variant in question plus increased weight) would catapult an individual's glycaemic profile into the diabetic range [99]. Thus, the suggestion that insulin resistance variants may be more detectable within an obesogenic background implies that BMI should be taken into account in the search for such variants. At the same time, whether a particular polymorphism exerts its metabolic effect via BMI (as in the case of *FTO* [79]), or the effect size is simply augmented in the context of increased obesity, must be carefully ascertained in appropriately designed studies.

Conclusions

The discovery and description of monogenic forms of diabetes demonstrated that hyperglycaemia could be caused by primary beta cell defects. The identification of novel common polymorphisms that increase risk of type 2 diabetes, and their recent metabolic characterisation, has extended this fundamental observation. Both sets of findings, however, do not rule out a genetic contribution to insulin resistance. A targeted search for such variants in well-powered samples, in which individual obesity measures are available as covariates and the absence of external constraints ensures a wide range of insulin resistance, followed by an intelligent replication strategy, may indeed reveal the largely missing piece in the genetic foundation of type 2 diabetes.

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