

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

J. C. Florez

Received: 16 February 2008 / Accepted: 4 April 2008 / Published online: 27 May 2008
© Springer-Verlag 2008

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, pro-

cessing 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.

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.

J. C. Florez (✉)
Diabetes Unit and Center for Human Genetic Research,
Massachusetts General Hospital,
Simches Research Building—CPZN 5.250, 185 Cambridge Street,
Boston, MA 02114, USA
e-mail: jcflorez@partners.org

J. C. Florez
Program in Medical and Population Genetics,
Broad Institute of Harvard and MIT,
Cambridge, MA, USA

J. C. Florez
Department of Medicine, Harvard Medical School,
Boston, MA, USA

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

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 \sim 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 1 α [HNF1 α]) 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 HNF1 α , 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 HNF1 β). 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 \sim 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 ($p=7.8 \times 10^{-15}$ 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 *TCF7L2* 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	<i>p</i> value	Insulin secretion associations	Key references
rs5219	11	Missense: E23K	<i>KCNJ11</i>	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	<i>TCF7L2</i>	Transcription factor; <i>trans</i> -activates genes encoding proglucagon and insulin	T	1.37	1×10^{-48}	Ins Index, AIR	[53, 61, 62, 65]
rs757210	17	Intronic	<i>TCF2</i>	Transcription factor involved in pancreatic development	A	1.10	8×10^{-10}		[26, 27]
rs13266634	8	Missense: R325W	<i>SLC30A8</i>	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	<i>HHEX</i>	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	<i>CDKAL1</i>	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	<i>IGF2BP2</i>	<i>IGF2</i> mRNA binding protein; pancreatic development	T	1.14	9×10^{-16}	DI	[75–77, 88]
rs10811661	9	125 kb upstream	<i>CDKN2A/B</i>	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	<i>WFS1</i>	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 ($p \sim 5 \times 10^{-8}$) 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/G₃₀, 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 *SLC30A8* 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 *SLC30A8* 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 ($p \sim 5 \times 10^{-8}$).

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

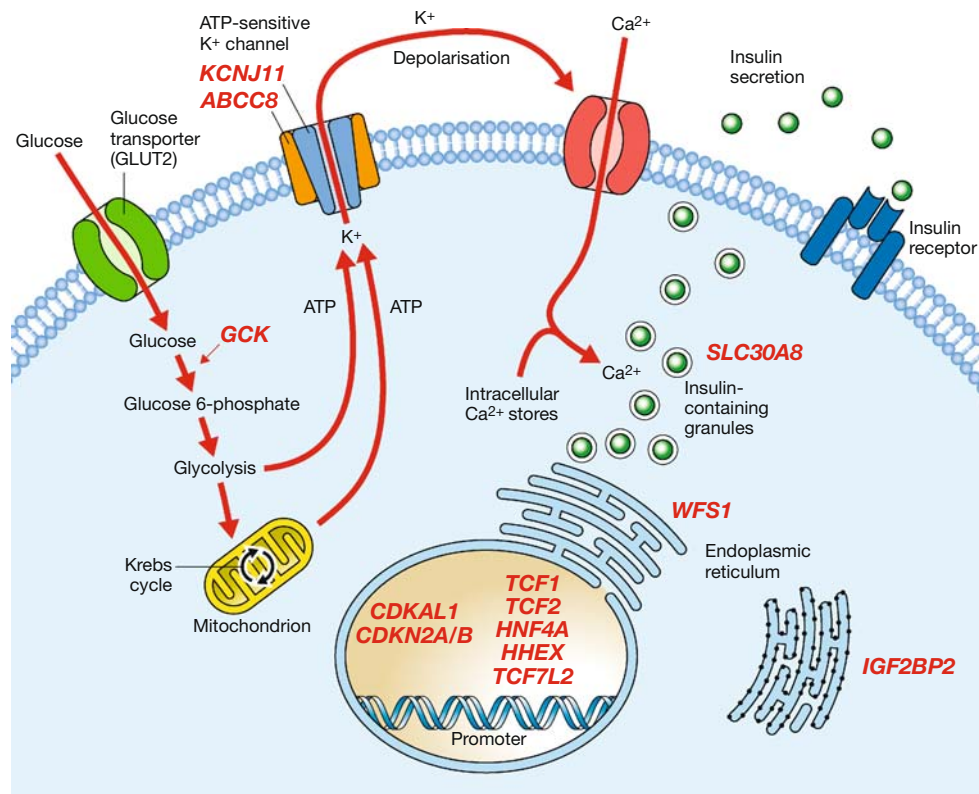


Fig. 1 Schematic diagram of the pancreatic beta cell showing the proposed subcellular localisation of proteins encoded by diabetes-associated genes. *GCK* encodes glucokinase, the glucose sensor of the beta cell. *KCNJ11* encodes the islet ATP-sensitive potassium channel Kir6.2, which interacts with the sulfonylurea receptor (SUR1, encoded by *ABCC8*) to regulate potassium currents across the cell membrane. *HNF4A*, *TCF1* (encoding HNF1 α), *TCF2* (encoding HNF1 β), *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 \times 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.

Acknowledgements I thank W. Winckler for assistance with the current *TCF1* meta-analyses. Supported by National Institutes of Health Research Career Development Award 1 K23 DK65978-04.

Duality of interest J. C. Florez has received a consulting honorarium from Publicis Healthcare Communications Group, a global advertising agency engaged by Amylin Pharmaceuticals.

References

1. Yalow RS, Berson SA (1960) Immunoassay of endogenous plasma insulin in man. *J Clin Invest* 39:1157–1175
2. Reaven GM, Bernstein R, Davis B, Olefsky JM (1976) Non-ketotic diabetes mellitus: insulin deficiency or insulin resistance? *Am J Med* 60:80–88
3. Ferrannini E (1998) Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. *Endocr Rev* 19:477–490
4. Kahn SE (2003) The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia* 46:3–19
5. Lillioja S, Mott DM, Spraul M et al (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 329:1988–1992
6. Haffner SM, Miettinen H, Gaskill SP, Stern MP (1995) Decreased insulin secretion and increased insulin resistance are independently related to the 7-year risk of NIDDM in Mexican-Americans. *Diabetes* 44:1386–1391
7. Gerich JE (2000) Insulin resistance is not necessarily an essential component of type 2 diabetes. *J Clin Endocrinol Metab* 85:2113–2115
8. Pimenta W, Korytkowski M, Mitrakou A et al (1995) Pancreatic beta-cell dysfunction as the primary genetic lesion in NIDDM. Evidence from studies in normal glucose-tolerant individuals with a first-degree NIDDM relative. *JAMA* 273:1855–1861
9. Polonsky KS, Sturis J, Bell GI (1996) Non-insulin-dependent diabetes mellitus—a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 334:777–783
10. Fajans SS, Bell GI, Polonsky KS (2001) Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:971–980
11. Vaxillaire M, Froguel P (2006) Genetic basis of maturity-onset diabetes of the young. *Endocrinol Metab Clin N Am* 35:371–384
12. Bell G, Polonsky KS (2001) Diabetes mellitus and genetically programmed defects in beta-cell function. *Nature* 414:788–791
13. Florez JC, Hirschhorn JN, Altshuler D (2003) The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. *Annu Rev Genom Hum Genet* 4:257–291

14. Love-Gregory LD, Wasson J, Ma J et al (2004) A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 α gene on chromosome 20 q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashkenazi Jewish population. *Diabetes* 53:1134–1140
15. Silander K, Mohlke KL, Scott LJ et al (2004) Genetic variation near the hepatocyte nuclear factor-4 α gene predicts susceptibility to type 2 diabetes. *Diabetes* 53:1141–1149
16. Weedon MN, Owen KR, Shields B et al (2004) Common variants of the hepatocyte nuclear factor-4 α P2 promoter are associated with type 2 diabetes in the U.K. population. *Diabetes* 53:3002–3006
17. Hansen SK, Rose CS, Glumer C et al (2005) Variation near the hepatocyte nuclear factor (HNF)-4 α gene associates with type 2 diabetes in the Danish population. *Diabetologia* 48:452–458
18. Winckler W, Graham RR, de Bakker PIW et al (2005) Association testing of variants in the hepatocyte nuclear factor 4 α gene with risk of type 2 diabetes in 7,883 people. *Diabetes* 54:886–892
19. Winckler W, Burt NP, Holmkvist J et al (2005) Association of common variation in the HNF1 α gene region with risk of type 2 diabetes. *Diabetes* 54:2336–2342
20. Weedon MN, Owen KR, Shields B et al (2005) A large-scale association analysis of common variation of the HNF1 α gene with type 2 diabetes in the U.K. Caucasian population. *Diabetes* 54:2487–2491
21. Bonnycastle LL, Willer CJ, Conneely KN et al (2006) Common variants in Maturity-Onset Diabetes of the Young genes contribute to risk of type 2 diabetes in Finns. *Diabetes* 55:2534–2540
22. Holmkvist J, Cervin C, Lyssenko V et al (2006) Common variants in HNF-1 α and risk of type 2 diabetes. *Diabetologia* 49:2882–2891
23. Willer CJ, Bonnycastle LL, Conneely KN et al (2007) Screening of 134 single nucleotide polymorphisms (SNPs) previously associated with type 2 diabetes replicates association with 12 SNPs in nine genes. *Diabetes* 56:256–264
24. McAteer J, Jablonski KA, Pollin TI et al (2007) Discovery of rare variants in MODY genes and impact on progression to diabetes in the Diabetes Prevention Program (DPP). ADA Annual Scientific Sessions Abstracts 392-OR. http://professional.diabetes.org/Abstracts_Display.aspx?TYP=1&CID=55551, accessed April 2008
25. Holmkvist J, Almgren P, Lyssenko V et al (2008) Common variants in MODY genes and future risk of type 2 diabetes. *Diabetes* (in press). DOI 10.2337/db06-1464
26. Winckler W, Weedon MN, Graham RR et al (2007) Evaluation of common variants in the six known Maturity-Onset Diabetes of the Young (MODY) genes for association with type 2 diabetes. *Diabetes* 56:685–693
27. Gudmundsson J, Sulem P, Steinthorsdottir V et al (2007) Two variants on chromosome 17 confer prostate cancer risk, and the one in *TCF2* protects against type 2 diabetes. *Nat Genet* 39:977–983
28. Weedon MN, Clark VJ, Qian Y et al (2006) A common haplotype of the glucokinase gene alters fasting glucose and birth weight: association in six studies and population-genetics analyses. *Am J Hum Genet* 79:991–1001
29. Kavvoura FK, Ioannidis JP (2005) Ala45Thr polymorphism of the *NEUROD1* gene and diabetes susceptibility: a meta-analysis. *Hum Genet* 116:192–199
30. Inoue H, Tanizawa Y, Wasson J et al (1998) A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nat Genet* 20:143–148
31. Hardy C, Khanim F, Torres R et al (1999) Clinical and molecular genetic analysis of 19 Wolfram syndrome kindreds demonstrating a wide spectrum of mutations in *WFS1*. *Am J Hum Genet* 65:1279–1290
32. Sandhu MS, Weedon MN, Fawcett KA et al (2007) Common variants in *WFS1* confer risk of type 2 diabetes. *Nat Genet* 39:951–953
33. Franks PW, Rolandsson O, Debenham SL et al (2008) Replication of the association between variants in *WFS1* and risk of type 2 diabetes in European populations. *Diabetologia* 51:458–463
34. Florez JC, Jablonski KA, McAteer J et al (2008) Testing of diabetes-associated *WFS1* polymorphisms in the Diabetes Prevention Program. *Diabetologia* 51:451–457
35. Aguilar-Bryan L, Bryan J (1999) Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 20:101–135
36. Ashcroft FM, Gribble FM (1999) ATP-sensitive K⁺ channels and insulin secretion: their role in health and disease. *Diabetologia* 42:903–919
37. Altshuler D, Hirschhorn JN, Klannemark M et al (2000) The common PPAR γ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76–80
38. Sakura H, Wat N, Horton V, Millns H, Turner RC, Ashcroft FM (1996) Sequence variations in the human Kir6.2 gene, a subunit of the beta-cell ATP-sensitive K-channel: no association with NIDDM in white Caucasian subjects or evidence of abnormal function when expressed *in vitro*. *Diabetologia* 39:1233–1236
39. Inoue H, Ferrer J, Warren-Perry M et al (1997) Sequence variants in the pancreatic islet beta-cell inwardly rectifying K⁺ channel Kir6.2 (Bir) gene: identification and lack of role in Caucasian patients with NIDDM. *Diabetes* 46:502–507
40. Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen JO, Pedersen O (1997) Amino acid polymorphisms in the ATP-regulatable inward rectifier Kir6.2 and their relationships to glucose- and tolbutamide-induced insulin secretion, the insulin sensitivity index, and NIDDM. *Diabetes* 46:508–512
41. Hani EH, Boutin P, Durand E et al (1998) Missense mutations in the pancreatic islet beta cell inwardly rectifying K⁺ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. *Diabetologia* 41:1511–1515
42. Gloyn AL, Hashim Y, Ashcroft SJ, Ashfield R, Wiltshire S, Turner RC (2001) Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with type 2 diabetes mellitus (UKPDS 53). *Diabet Med* 18:206–212
43. Gloyn AL, Weedon MN, Owen KR et al (2003) Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) confirm that the *KCNJ11* E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572
44. Nielsen E-MD, Hansen L, Carstensen B et al (2003) The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52:573–577
45. Love-Gregory L, Wasson J, Lin J, Skolnick G, Suarez B, Permutt MA (2003) E23K single nucleotide polymorphism in the islet ATP-sensitive potassium channel gene (Kir6.2) contributes as much to the risk of type II diabetes in Caucasians as the PPAR γ Pro12Ala variant. *Diabetologia* 46:136–137
46. Florez JC, Burt N, de Bakker PIW et al (2004) Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. *Diabetes* 53:1360–1368
47. van Dam RM, Hoebee B, Seidell JC, Schaap MM, de Bruin TWA, Feskens EJM (2005) Common variants in the ATP-

- sensitive K⁺ channel genes *KCNJ11* (Kir6.2) and *ABCC8* (SUR1) in relation to glucose intolerance: population-based studies and meta-analyses. *Diabet Med* 22:590–598
48. Schwanstecher C, Meyer U, Schwanstecher M (2002) KIR6.2 polymorphism predisposes to type 2 diabetes by inducing overactivity of pancreatic β -cell ATP-sensitive K⁺ channels. *Diabetes* 51:875–879
 49. Riedel MJ, Boora P, Steckley D, de Vries G, Light PE (2003) Kir6.2 polymorphisms sensitize β -cell ATP-sensitive potassium channels to activation by acyl CoAs: A possible cellular mechanism for increased susceptibility to type 2 diabetes? *Diabetes* 52:2630–2635
 50. Riedel MJ, Light PE (2005) Saturated and cis/trans unsaturated acyl CoA esters differentially regulate wild-type and polymorphic β -cell ATP-sensitive K⁺ channels. *Diabetes* 54:2070–2079
 51. Gloyn AL, Pearson ER, Antcliff JF et al (2004) Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 350:1838–1849
 52. Zeggini E, McCarthy MI (2007) *TCF7L2*: the biggest story in diabetes genetics since HLA? *Diabetologia* 50:1–4
 53. Grant SFA, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat Genet* 38:320–323
 54. Cauchi S, El Achhab Y, Choquet H et al (2007) *TCF7L2* is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med* 85:777–782
 55. Florez JC (2007) The new type 2 diabetes gene *TCF7L2*. *Curr Opin Clin Nutr Metab Care* 10:391–396
 56. Miyake K, Horikawa Y, Hara K et al (2008) Association of *TCF7L2* polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects. *J Hum Genet* 53:174–180
 57. Chang YC, Chang TJ, Jiang YD et al (2007) Association study of the genetic polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene and type 2 diabetes in the Chinese population. *Diabetes* 56:2631–2637
 58. Korinek V, Barker N, Moerer P et al (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet* 19:379–383
 59. Drucker DJ (2007) The role of gut hormones in glucose homeostasis. *J Clin Invest* 117:24–32
 60. Yi F, Brubaker PL, Jin T (2005) TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β -catenin and glycogen synthase kinase-3 β . *J Biol Chem* 280:1457–1464
 61. Florez JC, Jablonski KA, Bayley N et al (2006) *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241–250
 62. Saxena R, Gianniny L, Burt NP et al (2006) Common single nucleotide polymorphisms in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55:2890–2895
 63. Damcott CM, Pollin TI, Reinhart LJ et al (2006) Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish: Replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55:2654–2659
 64. Munoz J, Lok KH, Gower BA et al (2006) Polymorphism in the transcription factor 7-like 2 (*TCF7L2*) gene is associated with reduced insulin secretion in nondiabetic women. *Diabetes* 55:3630–3634
 65. Lyssenko V, Lupi R, Marchetti P et al (2007) Mechanisms by which common variants in the *TCF7L2* gene increase risk of type 2 diabetes. *J Clin Invest* 117:2155–2163
 66. Palmer ND, Lehtinen AB, Langefeld CD et al (2008) Association of *TCF7L2* gene polymorphisms with reduced acute insulin response in Hispanic Americans. *J Clin Endocrinol Metab* 93:304–309
 67. Schafer SA, Tschritter O, Machicao F et al (2007) Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (*TCF7L2*) gene polymorphisms. *Diabetologia* 50:2443–2450
 68. Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of *TCF7L2*-dependent Wnt signaling enhances pancreatic beta cell proliferation. *J Biol Chem* 283:8723–8735
 69. Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K (2008) Transcription factor 7-like 2 regulates β -cell survival and function in human pancreatic islets. *Diabetes* 57:645–653
 70. Loos RJF, Franks PW, Francis RW et al (2007) *TCF7L2* polymorphisms modulate proinsulin levels and β -cell function in a British European population. *Diabetes* 56:1943–1947
 71. Da Silva Xavier G, Rutter GA (2007) *Tcf7l2* regulates glucose-stimulated insulin secretion and insulin gene expression in Min6 β -cells. ADA Annual Scientific Sessions Abstracts 1725-P. http://professional.diabetes.org/Abstracts_Display.aspx?TYP=1&CID=55224, accessed April 2008
 72. Kirchoff K, Machicao F, Haupt A et al (2008) Polymorphisms in the *TCF7L2*, *CDKAL1* and *SLC30A8* genes are associated with impaired proinsulin conversion. *Diabetologia* 51:597–601
 73. Wang J, Kuusisto J, Vanttinen M et al (2007) Variants of transcription factor 7-like 2 (*TCF7L2*) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. *Diabetologia* 50:1192–1200
 74. Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:828–830
 75. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University and Novartis Institutes for BioMedical Research (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336
 76. Zeggini E, Weedon MN, Lindgren CM et al (2007) Replication of genome-wide association signals in U.K. samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341
 77. Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345
 78. Steinthorsdottir V, Thorleifsson G, Reynisdottir I et al (2007) A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770–775
 79. Frayling TM, Timpson NJ, Weedon MN et al (2007) A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889–894
 80. Salonen JT, Uimari P, Aalto JM et al (2007) Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am J Hum Genet* 81:338–345
 81. Hayes MG, Pluzhnikov A, Miyake K et al (2007) Identification of type 2 diabetes genes in Mexican Americans through genome-wide association studies. *Diabetes* 56:3033–3044
 82. Hanson RL, Bogardus C, Duggan D et al (2007) A search for variants associated with young-onset type 2 diabetes in American Indians in a 100K genotyping array. *Diabetes* 56:3045–3052
 83. Rampersaud E, Damcott CM, Fu M et al (2007) Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: Evidence for replication from diabetes-related quantitative traits and from independent populations. *Diabetes* 56:3053–3062
 84. Florez JC, Manning AK, Dupuis J et al (2007) A 100 K genome-wide association scan for diabetes and related traits in the Framingham Heart Study: replication and integration with other genome-wide datasets. *Diabetes* 56:3063–3074
 85. Pascoe L, Tura A, Patel SK et al (2007) Common variants of the novel type 2 diabetes genes, *CDKAL1* and *HHEX/IDE*, are

- associated with decreased pancreatic β -cell function. *Diabetes* 56:3101–3104
86. Grarup N, Rose CS, Andersson EA et al (2007) Studies of association of variants near the *HHEX*, *CDKN2A/B* and *IGF2BP2* genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes* 56:3105–3111
 87. Staiger H, Machicao F, Stefan N et al (2007) Polymorphisms within novel risk loci for type 2 diabetes determine beta-cell function. *PLoS ONE* 2:e832
 88. Palmer ND, Goodarzi MO, Langefeld CD et al (2008) Quantitative trait analysis of T2D susceptibility loci identified from whole genome association studies in the IRAS Family Study. *Diabetes* 57:1093–1100
 89. Taylor SI (1992) Lilly Lecture: molecular mechanisms of insulin resistance. Lessons from patients with mutations in the insulin-receptor gene. *Diabetes* 41:1473–1490
 90. Panhuysen CIM, Cupples LA, Wilson PWF, Herbert AG, Myers RH, Meigs JB (2003) A genome scan for loci linked to quantitative insulin traits in persons without diabetes: the Framingham Offspring Study. *Diabetologia* 46:579–587
 91. Lyssenko V, Almgren P, Anevski D et al (2005) Genetic prediction of future type 2 diabetes. *PLoS Med* 2:e345
 92. Vanttinen M, Nuutila P, Pihlajamaki J et al (2005) The effect of the Ala12 allele of the peroxisome proliferator-activated receptor- γ 2 gene on skeletal muscle glucose uptake depends on obesity: a positron emission tomography study. *J Clin Endocrinol Metab* 90:4249–4254
 93. Tonjes A, Scholz M, Loeffler M, Stumvoll M (2006) Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor γ with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 29: 2489–2497
 94. Florez JC, Jablonski KA, Sun MW et al (2007) Effects of the type 2 diabetes-associated *PPARG* P12A polymorphism on progression to diabetes and response to troglitazone. *J Clin Endocrinol Metab* 92:1502–1509
 95. Ludovico O, Pellegrini F, Di Paola R et al (2007) Heterogeneous effect of peroxisome proliferator-activated receptor γ 2 Ala12 variant on type 2 diabetes risk. *Obesity* 15:1076–1081
 96. Bochenski J, Placha G, Wanic K et al (2006) New polymorphism of *ENPP1* (*PC-1*) is associated with increased risk of type 2 diabetes among obese individuals. *Diabetes* 55:2626–2630
 97. Chandalia M, Grundy S, Adams-Huet B, Abate N (2007) Ethnic differences in the frequency of *ENPP1/PC1* 121Q genetic variant in the Dallas Heart Study cohort. *J Diabetes Complicat* 21:143–148
 98. McAteer JB, Prudente S, Bacci S et al (2007) The *ENPP1* K121Q polymorphism is associated with type 2 diabetes in European populations: Evidence from an updated meta-analysis in 42,042 subjects. *Diabetes* 57:1125–1130
 99. Bacci S, De Cosmo S, Prudente S, Trischitta V (2007) *ENPP1* gene, insulin resistance and related clinical outcomes. *Curr Opin Clin Nutr Metab Care* 10:403–409
 100. Frayling TM (2007) Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 8:657–662