Curr Diab Rep (2010) 10:485–497
DOI 10.1007/s11892-010-0154-y

Physiologic Characterization of Type 2 Diabetes–Related Loci

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Published online: 1 October 2010
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Abstract For the past two decades, genetics has been widely explored as a tool for unraveling the pathogenesis of diabetes. Many risk alleles for type 2 diabetes and hyperglycemia have been detected in recent years through massive genome-wide association studies and evidence exists that most of these variants influence pancreatic β-cell function. However, risk alleles in five loci seem to have a primary impact on insulin sensitivity. Investigations of more detailed physiologic phenotypes, such as the insulin response to intravenous glucose or the incretion hormones, are now emerging and give indications of more specific pathologic mechanisms for diabetes-related risk variants. Such studies have shed light on the function of some loci but also underlined the complex nature of disease mechanism. In the future, sequencing-based discovery of low-frequency variants with higher impact on intermediate diabetes-related traits is a likely scenario and identification of new pathways involved in type 2 diabetes predisposition will offer opportunities for the development of novel therapeutic and preventative approaches.

Keywords Type 2 diabetes · Glycemia · β-cell function · Insulin sensitivity · Genetic epidemiology · Physiology

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Introduction

Type 2 diabetes and its complications are major global health problems due to dramatically increasing prevalence in both the Western world and in the developing countries [1]. Type 2 diabetes is characterized by obesity, insulin resistance, and a relative decrease in insulin release from the β-cell. The diminished insulin response is seen as a missing first-phase insulin release after glucose stimulation and also following stimulation with nonglucose secretagogues such as the incretin hormones, glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide. The decreased insulin sensitivity seen in type 2 diabetes patients primarily affects the liver and peripheral tissue leading to increased hepatic glucose output and diminished glucose uptake by skeletal muscle and adipose tissue.

Despite the crucial etiologic role of lifestyle and environmental factors it has for years been recognized that genetic factors are important for the development of type 2 diabetes and related intermediary metabolic traits. The evidence of a genetic component in the pathogenesis of type 2 diabetes and related traits comes from studies of large families, twins and sibpairs, and from adoption studies. Type 2 diabetes clusters in families and offspring have a lifetime risk of developing type 2 diabetes of 35% if one parent has type 2 diabetes and 70% if both parents have type 2 diabetes compared with 10% in the general population [2], translated to a sibling relative risk of 2 to 3 [3]. In studies of monozygotic and dizygotic twins the relative importance of genetic and nongenetic factors can be estimated rather precisely under the assumption that twin pairs share the same prenatal and postnatal environment and that twins resemble singletons according to the phenotype in question [4]. Heritability estimates from twin data have shown variable concordance rates of type 2

diabetes in monozygotic twins from nearly 100% in early studies [5] to more modest rates ranging from 35% to 70% in later reports, as opposed to 20% to 30% in dizygotic twins [6–8]. Similarly, high degrees of heritability of diabetes-related traits have been found. In studies applying intravenous glucose tolerance test (IVGTT), heritability estimates of the acute insulin response to glucose ranged from 35% to 76% [9–11]. Also, the insulin response to nonglucose stimuli is influenced by genetic factors as heritability of GLP-1– and arginine-stimulated insulin release has been estimated to 53% and 80%, respectively [12]. Both basal and insulin-stimulated glucose uptake are also in part genetically determined, with heritability estimates ranging from 40% to 60% [10, 12, 13].

Since 1992, several genetic subtypes of monogenic diabetes have been described in which gene mutations result in diabetes primarily through β-cell dysfunction. This new knowledge means that patients who were previously categorized clinically as having maturity-onset diabetes of the young (MODY), permanent neonatal diabetes mellitus, or transient neonatal diabetes mellitus can now usually be classified by genetic subgroup. Definition of the genetic subgroup can result in appropriate treatment, genetic counseling, and prognostic information. In contrast, progress in identification of the genetic variants influencing predisposition to common forms of type 2 diabetes has, until recently, been slow. The candidate-gene approach has brought some success; however, the overall yield of confirmed disease-susceptibility genes gathered by this approach has been limited. Technologic advances have provided tools for simultaneous genotyping of hundreds of thousands of single nucleotide polymorphisms (SNPs) in individual samples. This has allowed researchers to perform genome-wide association studies (GWAS) that do not rely on prior assumptions regarding biological candidacy, and which are capable of identifying associations within genes that had little or no previous credibility as disease candidates. Even though the unraveling of the molecular pathogenesis of type 2 diabetes is still in its infancy, the last few years have brought some very interesting progress leading to the firm establishment of many genomic loci as contributing etiologic factors in common metabolic traits as type 2 diabetes.

The objective of this review is to summarize the current human physiologic knowledge of all gene variants with a validated impact on type 2 diabetes or traits of glucose homeostasis.

Physiology of Diabetes-Related Loci

Since the discovery of risk variants in *PPARG* in 1998, *KCNJ11* in 2003, and *TCF7L2* in 2006, the emergence of GWAS in 2007 combined with access to large well-powered study populations has quickly expanded the list of validated type 2 diabetes susceptibility alleles [14]. Now 38 loci have shown association with type 2 diabetes at a genome-wide significance level ($P<5\times10^{-8}$). Besides from GWAS of type 2 diabetes in case-control settings, knowledge of the genetic mechanisms in type 2 diabetes has come from GWAS of diabetes-related phenotypes such as fasting and 2-h levels of glucose and insulin during an oral glucose tolerance test (OGTT). These consortium studies have generated a list of 16 loci associated with fasting glucose and five loci associated with 2-h glucose levels [15•, 16], partly overlapping with each other and with the list of type 2 diabetes loci. All loci with a genome-wide significant association with type 2 diabetes or traits of glucose homeostasis are summarized in Table 1.

Given that most risk variants have been detected by the agnostic GWAS approach, the knowledge of the underlying phenotype leading to type 2 diabetes or altered glucose levels is at first very limited for most loci. Several studies have subsequently been performed with the objective to unravel intermediary phenotype of these loci to obtain a more detailed biological knowledge initially seeking to break the diabetes-related phenotype for each locus into the major components of type 2 diabetes pathogenesis being insulin release, insulin sensitivity, and obesity. These studies have taught us that a substantial part of the inherent susceptibility for type 2 diabetes relates to the extent to which pancreatic β-cell function can be maintained (Table 1). This opposes the viewpoint that the majority of type 2 diabetes genes inflict a state of insulin resistance with the β-cell simply failing to respond to the lifelong state of increased insulin demand.

Loci Primarily Associated with Type 2 Diabetes

The pancreatic β-cell has an imperative function to maintain the glucose level and malfunctions in this cell are a requisite for development of type 2 diabetes. The function of the β-cell can be evaluated in humans in different ways. Often the glucose-stimulated release of insulin or C-peptide during an OGTT has been used; however, to map β-cell defects in more detail, a range of more precise measures are warranted (eg, estimation of the acute insulin response after intravenous glucose administration, which provides an estimate of the incretin-independent β-cell function). Also, insulin release during a hyperglycemic clamp in combination with injection of incretins, sulfonylurea, glucagon, or arginine can be used to identify the more specific site of β-cell malfunction. Furthermore, a detailed understanding of genetically caused β-cell dysfunction will often be difficult to reach without also understanding the simultaneous and confounding effect of the mutation in other islet cell types and/or other tissues.

Table 1 Overview of the physiologic impact of loci associated with a diabetes-related trait at genome-wide significance[a]

Nearest gene(s)	Lead SNP[b]	Genome-wide significantly associated trait	Other reported association with physiologic phenotypes	Proposed mechanism
Variants with an effect on β-cell function				
KCNJ11 *ABCC8*	rs5219 (E23K)	T2D	Reduced insulin release during OGTT [20, 31, 34] Increased glucagon levels during hyperglycemic clamp [79]	Impaired β-cell function and impaired glucagon suppression
TCF7L2	rs7901695 rs7903146	T2D, FG, 2 h-G	Impaired conversion of proinsulin to insulin [39, 40••, 80] Reduced insulin release during OGTT [34, 40••, 80, 81] Reduced incretin effect [42, 43•, 44, 45] Reduced glucagon levels [44]	Impaired incretin-stimulated insulin release Impaired expression of prohormone convertases
WFS1	rs10010131	T2D	Reduced insulin release during OGTT [18, 19, 46] Reduced GLP-1 induced insulin release during hyperglycemic clamps [46]	Impaired incretin-stimulated insulin release
HHEX *IDE*	rs1111875 rs5015480	T2D	Reduced insulin release during OGTT [22•], 23•, 24•, 34] Borderline significant reduced insulin release during IV glucose stimulation [22•, 24•, 35–37] Reduced birth weight [67, 82]	β-cell dysfunction
SLC30A8	**rs13266634 (R325W)**	T2D, FG	Impaired conversion of proinsulin to insulin [39, 40••] Reduced insulin release during OGTT [24•, 30, 34, 40••] Reduced insulin release during IVGTT [24•, 25]	Impaired formation of insulin granules impairing insulin release
CDKAL1	rs10946398	T2D	Impaired conversion of proinsulin to insulin [39] Reduced insulin release during OGTT [21, 23•, 34] Reduced insulin release after IV glucose stimulation [35, 83] Reduced birth weight [67, 82, 84]	β-cell dysfunction
CDKN2A *CDKN2B*	rs10811661	T2D	Reduced insulin release during OGTT [22•, [30] Reduced insulin release during IVGTT [22•]	β-cell dysfunction
IGF2BP2	rs4402960	T2D	Reduced insulin release during OGTT [22•, 30, 34] Reduced insulin release during IVGTT [22•, 35] Reduced insulin release after IV tolbutamide stimulation [22•]	β-cell dysfunction
KCNQ1	rs2237895	T2D	Reduced insulin release during OGTT [28, 47] Reduced glucose-stimulated incretin secretion [47]	Decreased incretin secretion
JAZF1	rs864745	T2D	Reduced insulin release derived from OGTT [26]	Possibly β-cell dysfunction

Table 1 (continued)

Nearest gene(s)	Lead SNP[b]	Genome-wide significantly associated trait	Other reported association with physiologic phenotypes	Proposed mechanism
CDC123 CAMK1D	rs12779790	T2D	Reduced insulin release during OGTT [26] Reduced arginine-stimulated and second-phase glucose-stimulated insulin release during hyperglycemic clamp [48•]	Reduced β-cell mass
THADA	rs7578597 (T1187A)	T2D	Reduced GLP-1—and arginine-stimulated insulin release during hyperglycemic clamp [48•]	Reduced β-cell mass due to increased apoptosis
MADD	rs7944584	FG	Higher fasting proinsulin [40••]	Insulin processing defect
ADRA2A	rs10885122	FG	Reduced insulin release during OGTT [66]	β-cell dysfunction
TSPAN8	rs7961581	T2D	Reduced insulin release during OGTT [26]	Possibly β-cell dysfunction
MTNR1B	rs10830963	FG, T2D	Reduced insulin release during OGTT [27••, 29, 34, 40••, 64] Reduced insulin released during IVGTT [29, 34, 48•, 64]	Impaired melatonin-stimulated insulin release
FADS1	rs174550	FG	Reduced insulin release during OGTT [40••, 66]	β-cell dysfunction
GLIS3	rs7034200	FG	Reduced insulin release during OGTT [66]	Possibly β-cell dysfunction
C2CD4B	rs11071657	FG	Reduced insulin release during OGTT [40••, 66] Increased fasting proinsulin [40••]	Impaired insulin processing and release
PROX1	rs340874	FG, T2D	Reduced insulin release during OGTT [40••, 66]	β-cell dysfunction
GCK	**rs1799884 (-30G>A)**	FG, T2D	Increased glucose levels during OGTT [59] Reduced insulin release derived from OGTT [40••]	Increased glucostatic set point and impaired β-cell function
DGKB TMEM195	rs2191349	FG, T2D	Reduced insulin release during OGTT [40••, 66]	β-cell dysfunction
G6PC2	rs560887	FG	Increased insulin release during OGTT [40••, 65] Increased insulin release during IVGTT [65] Increased basal hepatic glucose production [65] Decreased risk of T2D [16, 63]	Unknown
GIPR	rs10423928	2 h-G	Reduced insulin release during OGTT [15•, 40]•• Increased fasting proinsulin [40••] Impaired incretin effect [15•]	Impaired incretin-stimulated insulin release Impaired insulin processing
CENTD2	rs1552224	T2D	Lower HOMA-B [49]	Impaired β-cell function
Variants with an effect on insulin sensitivity				
PPARG	**rs1801282 (P12A)**	T2D	Decreased insulin sensitivity derived from IVGTT and hyperinsulinemic-euglycemic clamp [51]	Whole-body insulin resistance
ADAMTS9	rs4607103	T2D	Reduced insulin-stimulated glucose uptake during hyperinsulinemic-euglycemic clamp [53]	Peripheral insulin resistance

Table 1 (continued)

Nearest gene(s)	Lead SNP[b]	Genome-wide significantly associated trait	Other reported association with physiologic phenotypes	Proposed mechanism
			Increased glucose-stimulated insulin release [48•, 53]	
GCKR	rs780094	FG, T2D, 2 h-G, triglyceride	Increased insulin resistance derived from fasting and OGTT [16, 40••, 55, 57]	Hepatic insulin resistance
	rs1260326 (L446P)		Increased insulin-stimulated hepatic glucose output [56]	
IRS1	rs2943641	T2D	Increased insulin resistance derived from fasting and OGTT [52••]	Whole-body insulin resistance
IGF1	rs35767	FI	Increased HOMA-IR [16] Decreased OGTT-based insulin sensitivity [40••]	Whole-body insulin resistance
Variants with an effect on adiposity				
FTO	rs8050136 rs9939609	BMI, T2D[c]	BMI-dependent insulin resistance [70, 71]	Increased BMI-dependent insulin resistance
Variants with unknown physiology				
HNF1B	rs4430796	T2D		Unknown
ADCY5	rs11708067 rs2877716	FG, 2 h-G, T2D, birth weight	Decreased birth weight [67, 68]	Unknown
NOTCH2	rs10923931	T2D		Unknown
CRY2	rs11605924	FG		Unknown
SLC2A2	rs11920090	FG		Unknown
RBMS1	rs7593730	T2D	Increased HOMA-IR [54]	Unknown
KLF14	rs972283	T2D	Increased fasting insulin and HOMA-IR [49]	Unknown
BCL11A	rs243021	T2D		Unknown
ZBED3	rs4457053	T2D		Unknown
TP53INP1	rs896854	T2D		Unknown
CHCHD9	rs13292136	T2D		Unknown
KCNQ1	rs231362	T2D		Unknown
HMGA2	rs1531343	T2D		Unknown
HNF1A	rs7957197	T2D		Unknown
ZFAND6	rs11634397	T2D		Unknown
PRC1	rs8042680	T2D		Unknown
DUSP9	rs5945326	T2D		Unknown
VPS13C	rs17271305	2 h-G		Unknown

BMI body mass index, *FG* fasting glucose, *FI* fasting insulin, *GLP*-1 glucagon-like peptide-1, *HOMA-B* homeostasis model assessment of β-cell function, *HOMA-IR* homeostasis model assessment of insulin resistance, *IV* intravenous, *IVGTT* intravenous glucose tolerance test, *OGTT* oral glucose tolerance test, *SNP* single nucleotide polymorphism, *T2D* type 2 diabetes, *2 h-G* 2-h glucose

[a] All effects are stated for the allele increasing risk of T2D or increasing fasting/2 h-G or insulin

[b] Functional SNPs in **bold**

[c] *FTO* is only associated with T2D when not adjusting for the effect on BMI

Numerous studies have been performed investigating the glucose-stimulated insulin response in carriers and non-carriers of type 2 diabetes risk alleles and have shown that many of these variants affect this aspect of diabetes pathogenesis. This is the case for risk alleles in or near *KCNJ11*, *TCF7L2*, *WFS1*, *CDKN2A*, *HHEX*, *IGF2BP2*, *CDKAL1*, *SLC30A8*, *JAZF1*, *CDC123*, *TSPAN8*, *MTNR1B*, and *KCNQ1* [17–21, 22•, 23•, 24•, 25, 26, 27••, 28–31], although results for some of the recently identified diabetes risk variants are somewhat conflicting [26, 32–34]. Growing evidence supports the notion that these risk alleles influence different aspects of β-cell function. *CDKAL1*,

CDKN2A, *HHEX*, *IGF2BP2*, *MTNR1B*, and *SLC30A8* have been shown to inflict lower insulin response to intravenous glucose, indicating an incretin-independent pathogenesis [22•, 24•, 25, 27••, 29, 35–37]. For *SLC30A8* a rather detailed knowledge of diabetogenic mechanisms has been disclosed. The *SLC30A8* association signal is limited to an interval containing a single biological candidate, a zinc transporter expressed exclusively in β-cells and implicated in the maintenance of insulin granule function [38]. The strongest association is with a SNP that changes the amino acid structure of the encoded protein (R325W,) which may well be the causal variant. Carriers of the R325W variant are characterized by a reduced serum insulin response following both an intravenous and an oral glucose challenge and with defective proinsulin conversion as indicated by higher fasting and orally glucose-stimulated proinsulin levels [21, 24•, 39, 40••]. It is speculated whether the R325W variant also indirectly has an effect on glucagon secretion from α cells via an altered zinc release from the β-cells [41]. Stimulating the *SLC30A8*-encoded zinc transporter production and/or activity may potentially be a novel approach in the treatment of type 2 diabetes patients, in who zinc depletion is likely to participate in both acute and chronic β-cell dysfunction.

Decreased incretin secretion and/or signaling could also be involved in altered β-cell function. A recent study investigated detailed phenotypes in a small number of *TCF7L2* risk and non-risk allele carriers and observed a 30% lower β-cell sensitivity to incretins in risk-allele carriers [42], which is in line with earlier observations [43•, 44, 45]. The genotype-specific incretin-induced insulin release has also been tested for other diabetes-related risk alleles and, besides *TCF7L2*, risk alleles in or near *KCNJ11*, *KCNQ1*, *WFS1*, and *THADA* influence secretion or action of the incretin hormones as evidenced by studies of hyperglycemic clamp combined with GLP-1 infusion [46, 47, 48•]. Recently, the DIAGRAM (Diabetes Genetics Replication and Meta-analysis) consortium published the results of an updated meta-analysis combining eight GWAS (8130 type 2 diabetes cases and 38,987 controls in the discovery data), additionally identifying 12 novel type 2 diabetes loci including a new independent signal in *KCNQ1* [49]. No detailed physiologic studies have been published on these loci but the discovery paper investigated the basal homeostasis model assessment (HOMA) indices of β-cell function and insulin resistance in about 37,000 individuals. These data suggest that the diabetes-risk allele in *CENTD2* is associated with lower basal β-cell function, whereas the *KLF14* locus may inflict risk of diabetes by increasing insulin resistance [49]. If confirmed, the *KLF14* locus will add to the rather short list of diabetogenic alleles with an effect on this major component of type 2 diabetes pathogenesis.

Years ago, it was recognized that the P12A variant in *PPARG*, an important transcription factor regulating adipocyte differentiation, lipid and glucose homeostasis, and insulin sensitivity, had a type 2 diabetogenic effect caused by lower insulin sensitivity in peripheral insulin target tissues [50, 51]. More recently, a variant located 500 kb upstream of *IRS1* was demonstrated to increase risk of type 2 diabetes and decrease insulin sensitivity through lowering of expression of *IRS1* and diminishing insulin signaling [52••]. The *ADAMTS9* diabetes-associated allele has also been associated with decreased insulin sensitivity, as estimated from the euglycemic-hyperinsulinemic clamp [53]; however, others investigating fasting and OGTT-based insulin sensitivity indices have not been able to detect a significant effect [26, 33]. Future larger studies estimating tissue-specific insulin sensitivity and/or meta-analysis may clarify these inconsistencies. Although it seems evident that *PPARG* primarily exerts its effect in adipose tissue and *IRS1* possibly in skeletal muscle, the putative tissue-specific site of action for *ADAMTS9* has not been determined. One may argue that the lack of effect on basal HOMA of insulin resistance (HOMA-IR) in large samples reflects that a possible insulin-desensitizing effect is not acting in the liver.

In the spring of 2010 a GWAS was published demonstrating the genome-significant association of a SNP near *RBMS1* [54]. Of interest, the lead SNP showed borderline significant association with increased insulin resistance as estimated from HOMA-IR in data provided by the MAGIC (Meta-Analysis of Glucose and Insulin-related Traits Consortium). Despite the huge sample size, the impact on measures of insulin resistance needs validation and refining in further studies.

Finally, variation in *GCKR* has also been shown to influence insulin action. Initially, *GCKR* was found to associate with increased triglyceride levels [55]. Subsequent studies identified the nonsynonymous *GCKR* P446L variant to be the functional variant and demonstrated that the allele reported to be associated with elevated triglyceride levels also associated with decreased plasma glucose levels and vice versa. The genome-wide significant type 2 diabetes risk allele also associated with elevated fasting glucose and elevated levels of insulin during OGTT, demonstrating an insulin resistance phenotype as the probable cause of type 2 diabetes [15•, 16, 56, 57]. The association with OGTT-based insulin sensitivity indices was replicated in 2010 in a large-scale meta-analysis including OGTT data on about 15,000 participants [40••]. *GCKR* associates with basal HOMA-IR, with glucose-stimulated insulin sensitivity, and in a small study sample with increased hepatic glucose output during hyperinsulinemia [56].

Loci Primarily Associated with Quantitative Traits in Glucose Homeostasis

Also, GWAS of quantitative diabetes-related traits have contributed to the genetic understanding of type 2 diabetes and intermediate phenotypes. Mutations in *GCK* cause a mild subtype of MODY and also the common -30G>A polymorphism of the β-cell specific *GCK* promoter is an important player in regulation of fasting glycemia in the general population [58, 59]. Interestingly, despite acting as the glucose sensor of the β-cell and despite conferring a replicated allelic approximately 0.06-mM change in fasting plasma glucose levels, this variant has shown inconsistent association with glucose-stimulated insulin release [40••, 59] and only modest association (OR, 1.05) with type 2 diabetes [16].

By the first wave of GWAS two additional loci, *G6PC2* and *MTNR1B*, regulating fasting glucose levels were identified [27••, 60–63]. Initial and subsequent studies have implicated variants in *MTNR1B* in the risk of type 2 diabetes and decreased insulin release after both oral and intravenous glucose stimulation, thereby seemingly acting independent of the incretin hormones [27••, 64]. Although variants in *MTNR1B* show the expected relationship between effects on fasting glucose, type 2 diabetes, and pancreatic β-cell function, a variant in the *G6PC2* locus surprisingly shows association with elevated fasting glucose, decreased risk of type 2 diabetes [16, 63], increased risk of impaired fasting glycemia, [65] and increased β-cell function after both oral and intravenous glucose loads [40••, 65]. In addition, weak association with increased insulin-stimulated hepatic glucose output has been reported [65]. These complex results leave no clear physiologic footprint because they are not concordant with merely an elevation of the glucostatic set point. However, it has been suggested that results are due to an imbalance between *GCK* and *G6PC2* leading to disturbance of the pulsatile insulin secretion, which is correlated with oscillations in glycolysis [40••]; yet, this hypothesis remains to be tested.

Recent analyses of quantitative glucose traits in huge GWAS meta-analyses performed by MAGIC including more than 45,000 individuals in the discovery stage found a total of 16 loci associated with fasting glucose levels, two loci associated with fasting insulin and HOMA-IR, and five loci associated with 2-h glucose levels during an OGTT [15•, 16]. As might be expected, the list of fasting and 2-h glucose-associated loci is partly overlapping with each other and with the list of type 2 diabetes loci (Table 1).

Of the five known and novel loci associated with 2-h OGTT plasma glucose, the *GIPR* glucose-raising allele showed a strong effect on orally glucose-stimulated insulin release in about 19,500 individuals in the initial discovery paper [15•]. A lack of influence on intravenous glucose-stimulated insulin in 1509 individuals and a diminished incretin effect when comparing insulin response to oral versus intravenous glucose give evidence of an incretin-mediated mechanism [15•]. Paradoxically, this variant only showed nominal significant low-impact association (OR, 1.07) with type 2 diabetes [15•]. Notably, the lead SNP, rs10423928, is in strong linkage disequilibrium (LD) (r^2= 0.93) with the nonsynonymous *GIPR* E354Q substitution, which is likely to be the functional variant. The other novel variants associated with 2-h plasma glucose during an OGTT in the *ADCY5* and *VPS13C* loci show no clear diabetes-related intermediate phenotype [16 ,40••, 66], although *ADCY5* has been associated with decreased birth weight [67, 68]. Another variant in *ADCY5* in high LD was simultaneously associated with both fasting glucose and type 2 diabetes [16], yet does not associate with intermediary phenotypes of glucose metabolism [40••, 66].

In contrary, of the 10 novel fasting glucose-raising alleles, many show association with decreased insulin release during OGTT indicating a general β-cell dysfunction. This seems to be the case for variants in *DGKB*, *C2CD4B*, *GLIS3*, *ADRA2A*, *PROX1*, and *FADS1* [40••, 66]. Interestingly, alleles at *TCF7L2*, *SLC30A8*, *GIPR*, and *C2CD4B* loci showed increased fasting proinsulin levels in a large sample size of more than 17,000 individuals in addition to decreased orally stimulated insulin release [40••], supporting earlier observations in smaller settings for *TCF7L2* and *SLC30A8* risk alleles [39]. This indicates a state of β-cell stress with deficient insulin processing and accumulation of insulin precursor molecules as the intermediate mechanism for β-cell dysfunction, hyperglycemia, and overt type 2 diabetes. For *TCF7L2*, an additional or alternative mechanism for elevated proinsulin levels might be decreased expression of both major genes involved in proinsulin processing (*PCSK1* and *PCSK2*), which contain *TCF7L2* binding sites in their promoters.

One novel locus, *IGF1*, was associated with fasting insulin and HOMA-IR [16] and the effect on insulin resistance has since been replicated by OGTT-based insulin sensitivity indices [40••]. However, the effect is minute (0.5–2% change per allele) and was not statistically significant in 5722 Danish individuals [66] nor in intravenous measurements in 3195 individuals of the former study [40••].

Loci Primarily Associated with Adiposity

Obesity is a major characteristic of type 2 diabetes and one might expect the existence of many common susceptibility alleles for obesity and type 2 diabetes. However, GWAS of

type 2 diabetes and obesity and subsequent association studies have showed that this is not the case. To date, only variation in *FTO* has been convincingly shown to impact type 2 diabetes by an intermediate effect on body mass index (BMI) and adiposity [69]. In addition, variation in *FTO* has revealed association with metabolic phenotypes showing the expected insulin resistance phenotype [70, 71]; however, these effects can be fully explained by the correlation between BMI and traits of insulin resistance [72]. Interestingly, a significant interaction between the *FTO* variant and low physical activity on body fat accumulation and insulin sensitivity has been reported [70].

What Have We Learned?

Genetic Overlap Between Type 2 Diabetes and Diabetes-Defining Glucose Traits

As evidenced by the former sections and by Table 1, many loci showing genome-wide significant association with type 2 diabetes and quantitative traits of glucose homeostasis have been detected. Given the fact that diabetes is defined by glucose levels in the fasting state and at 2 h during an OGTT, one would intuitively expect a considerable overlap between the predisposing genetic factors for these three entities. However, recent discoveries have shown that this is not the case. Some loci (eg, *TCF7L2*, *SLC30A8*, and *GCKR*) show a validated association with both fasting glucose and type 2 diabetes, yet other loci associate only with fasting or 2-h glucose levels (eg, *SLC2A2*) or type 2 diabetes (eg, *HNF1B* and *JAZF1*). At the extremity, the risk allele near *G6PC2* both increases fasting glucose [60, 61] and decreases risk of type 2 diabetes [16, 63].

This apparent paradox raises some interesting issues to be considered. First, quantitative GWAS of glucose traits have all been performed in nondiabetic individuals from the general population, whereas type 2 diabetes loci have been found in studies of disease cases and population controls. Therefore, it is possible that the genetic determinants of glucose levels in the general population are somewhat different from the genetic elements pushing glucose levels to diabetes-defining levels. In this sense diabetes-associated alleles without an impact on glucose levels in the general population would act above a certain glucose threshold. Second, the impact of genetic variants may be age-dependent because quantitative investigations have foremost been performed in middle-aged individuals, whereas ascertained type 2 diabetes cases are generally older. Yet of importance, these coherences between glucose-raising and diabetes-associated alleles may change considerably when causal alleles of the known loci are found. Interestingly, there seems to be an equal fraction of loci with an impact on β-cell function among glucose-raising alleles and diabetes-associated alleles (Table 1).

The Combined Effect of Multiple Risk Alleles

As evidenced above and in Table 1, the majority of diabetes-related SNPs seem to inflict a change in the function of the pancreatic β-cell. Besides SNP-by-SNP studies, this phenomenon has also been investigated in studies combining suspected β-cell gene variants. In 2008, Pascoe et al. [73] found an additive effect of β-cell risk alleles in *CDKAL1*, *HHEX*, and *TCF7L2*, which was also demonstrated in a German study of *TCF7L2*, *CDKAL1*, *HHEX*, and *SLC30A8* [74]. Also, a Swedish prospective study found a relative decline in β-cell function in the quintile of participants carrying the highest number of type 2 diabetes risk alleles [30].

Based on estimates from population-based Inter99 of 5722 nondiabetic individuals, it is evident that the common variants depicted in Fig. 1 and Table 1 individually only have a minor effect on the examined trait. The combined impact of multiple alleles can also be investigated in relation to the extent to which they explain variation in the trait of interest in the general population. The variants depicted in Fig. 1 explain 4% to 6% of the total interindividual variation in insulinogenic index and only 1% to 3% of variation in ISI Matsuda. These figures show that a minor part of the genetic origin of diabetes-related intermediary traits in the general population has been elucidated, telling us that future studies likely will uncover many more risk loci. Also, insights into structural variation, gene-gene and gene-environment interactions, and epigenetic modifications are likely to explain the so-called missing heritability.

Estimation of the Key Diabetes-Related Components: Insulin Release and Insulin Sensitivity

For genetic studies of complex traits such as type 2 diabetes, it has in the last decade been acknowledged that large sample sizes are necessary to estimate the low-impact association with confidence. This development is also reflected in GWAS sample sizes, which now exceeds 120,000 participants including replication sample sets [16]. Also, follow-up studies in cohorts with more detailed diabetes-related phenotypes now frequently include more than 5000 individuals and, recently, the first systematic meta-analysis was performed gathering OGTT data from more than 15,000 participants [40••]. Yet, the sample sizes used to investigate more detailed physiologic traits are severely impeded by low sample size. This is the case for studies applying IVGTT, hyperglycemic-hyperinsulinemic or euglycemic-hyperinsulinemic clamp techniques and

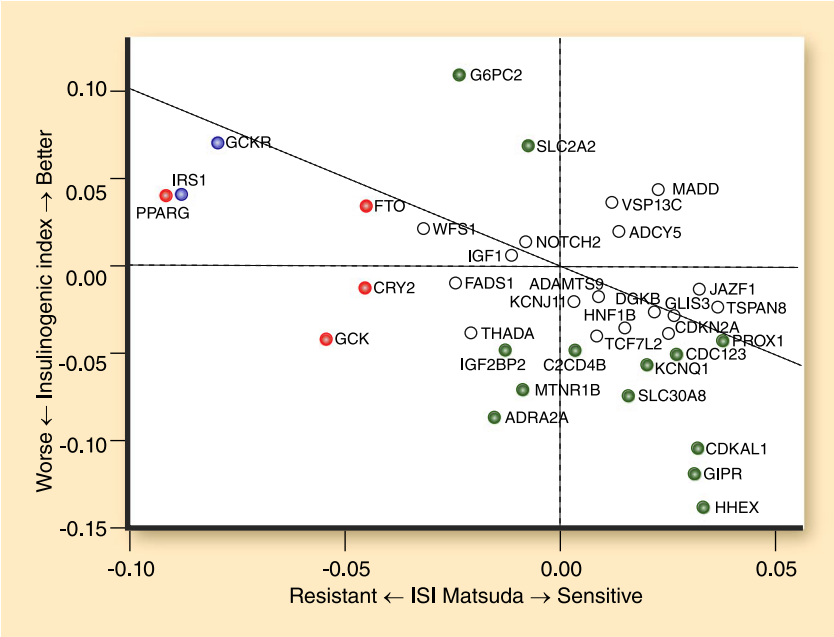

Fig. 1 Graphic representation of association with insulin sensitivity (ISI Matsuda) in relation to association with glucose-stimulated insulin release (insulinogenic index) for 36 single nucleotide polymorphisms (SNPs) with a validated impact on diabetes-related traits in 5722 nondiabetic participants of the population-based Inter99 cohort. The effect sizes are expressed as allelic change in fraction of a standard deviation and have been estimated applying a linear regression model adjusted for sex and age. SNPs represented by *green dots* associate with altered β-cell function using the insulinogenic index ($P<0.05$), SNPs shown in *red dots* associate with altered insulin sensitivity using the Matsuda insulin sensitivity index (ISI Matsuda), and SNPs shown in *blue* associate with altered β-cell function and altered insulin sensitivity. ISI—insulin sensitivity index

entails low statistical power, risk of type 2 errors, and inability to draw robust inferences of no association. Furthermore, many associations seen in such well-characterized samples have only been observed in a single study calling for independent replication.

Several indices of insulin release and insulin sensitivity based on fasting and OGTT data have been validated against more precise measures, the objective being to enable large-scale, low-cost estimation of the primary intermediate components of glucose homeostasis. These estimates have in genetic studies been used to classify diabetes-related risk alleles into broad functional classes such as ones having an impact on insulin release or insulin sensitivity. Yet the question remains whether fasting and OGTT-based indices can also serve to subclassify genetic effects to designate a more precise physiologic mechanism.

What's Next?

Functional Characterization of Diabetes-Related Loci

For most loci summarized in Table 1, the causal gene and mutation leading to the described phenotype has not been determined; instead, these variants may just as well be markers of indirect association. However, for a few loci the molecular effect of the actual causal variant has been discovered opening for much more detailed studies of phenotype and the underlying biology. One such example is *GCKR* for which the initial GWAS SNP marker has been refined by fine-mapping to a nonsynonymous variant (P446L) [56], which was subsequently demonstrated to change regulation of *GCK* in the liver and thereby influence metabolic phenotypes [75]. The functional variant (R325W) in the *SLC30A8* locus has also been established and the first functional studies reported demonstrating a role in insulin granule storage [76]. However, these two examples are special cases because the initial GWAS lead SNP was a nonsynonymous variant or in high LD with a HapMap-genotyped nonsynonymous obvious candidate mutation.

Other loci in which a probable functional variant has been found includes *GIPR*, *GCK*, and *PPARG*. In the *KCNJ11* locus two nonsynonymous variants in two different genes (*KCNJ11* E23K and *ABCC8* S1369A) in high LD (r^2=0.98 [31]) exist. A recent functional study provided evidence for an alteration of the function of the ATP-sensitive potassium channel in the β-cell, encoded by *KCNJ11* and *ABCC8*, when these two variants were coexpressed [77], possibly explaining previous inconclusive reports of investigations of a single mutation. This

example also illustrates the extreme complexity and challenge in finding causal and functional variants in the many recently identified risk loci. For the remaining loci fine-mapping efforts and functional studies are needed to detect and characterize the mutation of interest. Identification of the functional variants will guide the design of more detailed physiologic studies, which will pave the road for a much deeper understanding of the disease mechanisms at the whole body level.

Discovery of Novel Loci

Despite the high number of validated variants with an impact on diabetes-related intermediary traits the explained proportion of variance is rather low, indicating the existence of further genetic susceptibility elements. It seems likely that future studies will discover more common risk variants by GWAS of type 2 diabetes and related traits in even larger samples. However, these variants will probably inflict very modest risk increments and physiologic characterization of such loci will be extremely challenging. Yet, also future decently powered GWAS of specific intermediate phenotypes may discover novel variants, presumably including variants with larger impact on these intermediate traits.

Another avenue in the search for gene variants that impact on the components of type 2 diabetes will probably come from the emerging sequencing studies detecting variation at low frequency in the population. It has been proposed that accumulation of multiple mildly deleterious rare, but not monogenic, gene variants in individual human genomes has a huge impact on the genetic basis for complex diseases contributing with a large relative risk at the individual level [78]. From studies of MODY we know that rare variants can cause Mendelian disease, which affects the β-cell, and it is conceivable that low-frequency variation will have a large impact in certain subsets of the population. The most efficient way to uncover such variation may be to sequence individuals at the ends of distributions of the quantitative trait of interest. The specific physiologic defects could then be investigated in detailed studies of in vivo physiology in individuals recruited by genotype in supplement to association studies at the population level.

Study Designs and Samples for the Study of Quantitative Diabetes-Related Traits

As alluded to previously, the sample size of most studies of detailed human physiology in relation to genetic variants are small, with less than 1000 participants in clamp and IVGTT studies. To close in on the more specific physiology behind crude type 2 diabetes associations and thereby to learn important lessons on biology of type 2 diabetes, more individuals have to be studied with standardized detailed physiologic methods. In the near future these aspects need evaluation and the scientific community and funding agencies will need to consider spending more money on phenotypic characterization of large study samples. Of interest are also large prospective studies that will tell us about the time-dependent and lifestyle-dependent nature of risk allele penetrance.

Conclusions

Many risk alleles for type 2 diabetes and hyperglycemia have been detected in the recent years and evidence exists that most of these variants influence pancreatic β-cell function. Investigations of more detailed physiologic phenotypes, such as of the insulin response to intravenous glucose or the incretion hormones, are now emerging and give indications of more specific pathologic mechanisms for diabetes-related risk variants. Such studies have shed light on the function of some loci but also underlined the complex nature of disease mechanism. In the future the sequencing-based discovery of low-frequency variants with higher impact on intermediate diabetes-related traits is a likely scenario.

Disclosure No potential conflicts of interest relevant to this article were reported.

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• Of importance
•• Of major importance

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