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ORIGINAL ARTICLE

Association between insulin secretion, insulin sensitivity and type 2 diabetes susceptibility variants identified in genome-wide association studies

Stephanie-May Ruchat · Cathy E. Elks · Ruth J. F. Loos · Marie-Claude Vohl · S. John Weisnagel · Tuomo Rankinen · Claude Bouchard · Louis Pérusse

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Abstract Several single nucleotide polymorphisms (SNPs) for type 2 diabetes mellitus (T2DM) risk have been identified by genome wide association studies (GWAS). The objective of the present study was to investigate the impact of these SNPs on T2DM intermediate phenotypes in order to clarify the physiological mechanisms through which they exert their effects on disease etiology. We analysed 23 SNPs in 9 T2DM genes (*CDKAL1*, *CDKN2B*, *HHEX/IDE*, *IGF2BP2*, *KCNJ11*, *SLC30A8*, *TCF2*, *TCF7L2* and *WFS1*) in a maximum of 712 men and women from the Quebec Family Study. The participants underwent a 75 g oral glucose tolerance test (OGTT) and were measured for glucose, insulin and C-peptide levels. Indices of insulin sensitivity and insulin secretion were derived from fasting and OGTT measurements. We confirmed the significant associations of variants in *CDKAL1*, *CDKN2B*, *HHEX/IDE*, *KCNJ11* and *TCF7L2* with insulin secretion and also found associations of some of these variants with insulin sensitivity and glucose tolerance. *IGF2BP2* and *SLC30A8* SNPs were not associated with insulin secretion but were with insulin sensitivity and glucose tolerance ($0.002 \leq P \leq 0.02$). To examine the joint effects of these variants and their contribution to T2DM endophenotypes variance, stepwise regression models were used and the model R^2 was computed. The variance in the phenotypes explained by combinations of variants ranged from 2.0 to 8.5%. Diabetes-associated variants in *CDKAL1*, *CDKN2B*, *HHEX/IDE*, *IGF2BP2*, *KCNJ11*, *SLC30A8* and *TCF7L2* are associated with physiological alterations leading to T2DM, such as glucose intolerance, impaired insulin secretion or insulin resistance, supporting their role in the disease aetiology. These variants were found to account for 2.0–8.5% of the variance of T2DM-related traits.

Keywords Glucose tolerance · Insulin secretion · Insulin sensitivity · Diabetes-associated polymorphisms

S.-M. Ruchat · S. J. Weisnagel · L. Pérusse
Department of Preventive Medicine, Laval University, Quebec City, QC, Canada

S.-M. Ruchat · M.-C. Vohl · S. J. Weisnagel · L. Pérusse
Lipid Research Center, CHUL Research Center, Quebec City, QC, Canada

C. E. Elks · R. J. F. Loos
Medical Research Council Epidemiology Unit, Institute of Metabolic Science, Cambridge, UK

T. Rankinen · C. Bouchard
Human Genomics Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, USA

L. Pérusse (✉)
Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Quebec City, QC G1V O6A, Canada
e-mail: louis.perusse@kin.msp.ulaval.ca

Introduction

Type 2 diabetes mellitus (T2DM) currently affects more than 200 million individuals worldwide. T2DM is a multifactorial disease in which environmental factors appear to interact with multiple genetic variants in modulating the predisposition to the disease. Numerous variants within several genes that confer an increased susceptibility to T2DM have been identified by candidate-genes studies but only a small number have been identified as strong candidates for T2DM. These genes include peroxisome proliferator activated receptor-γ (*PPARG*), potassium inwardly-rectifying channel, subfamily J, member 11

(*KCNJ11*), transcription factor 7-like 2 (*TCF7L2*), transcription factor 2, hepatic (*TCF2*) and Wolfram syndrome 1 (*WFS1*) [1].

More recently, genome-wide association studies (GWAS) provided a major increment to our knowledge of the genetics of T2DM. GWAS have identified multiple novel susceptibility variants in Caucasian populations [2–9], increasing the number of confirmed T2DM susceptibility loci to eleven, including CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor 2B (*CDKN2B*), insulin-like growth factor 2 mRNA-binding protein 2 (*IGF2BP2*), haematopoietically expressed homeobox/insulin-degrading enzyme (*HHEX/IDE*), fat mass and obesity-associated (*FTO*) and solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*).

Although results from GWAS based on case-control designs were useful to identify new susceptibility genes associated with increased risk of T2DM, testing the impact of these variants on T2DM intermediate traits remains essential to clarify the physiological mechanisms through which the genes exert their effects. Variants that influence susceptibility to T2DM may be associated with defects in insulin sensitivity, hepatic glucose production and/or insulin secretion. Abnormal insulin action and secretion precede the development of T2DM [10] and represent quantitative traits that can help identify the mechanisms conferring increased risk for the disease. Of interest, most of the loci identified by GWAS appear to be involved in beta-cell function. *TCF7L2* variants were consistently associated with impaired insulin secretion. Florez et al. [11] showed that carriers of the T allele at rs7903146 had significantly lower baseline levels of insulin secretion than did CC homozygotes, while Saxena et al. [12] found that TT homozygous individuals have a ~50% reduction in insulinogenic index and insulin disposition index. The T-allele of rs7903146 also strongly predicted future T2DM in two independent cohorts followed for up to 22 years [13]. Other studies have produced similar results with measures of insulin secretion and/or insulin sensitivity [14–23] or with impaired beta-cell proinsulin processing [24–26].

CDKAL1 has also been associated in several studies with insulin resistance and/or defects in insulin secretion, derived either from fasting measures, oral glucose tolerance tests (OGTT), intravenous glucose-tolerance tests (IVGTT) or euglycemic hyperinsulinemic clamps [24, 27–31]. Steinthorsdottir et al. [6] reported that the insulin response in rare homozygotes for rs7756992 was approximately 20% lower than in heterozygotes or noncarriers of the variant. *CDKN2B* [28, 32], as well as *IGF2BP2* [28, 29, 32], *HHEX/IDE* [24, 30, 32–34] and *SLC30A8* [6, 24, 27, 33, 35] were also reported to be associated with T2DM-related traits. However, only a small number of the above mentioned studies have used method such as the OGTT that allows assessment of insulin secretion and insulin sensitivity in dynamic conditions.

The objective of our study was to assess the relationship between diabetes susceptibility variants, recently identified by GWAS, and phenotypes of insulin secretion and insulin sensitivity derived from fasting and OGTT measures, in the well characterized Quebec Family Study (QFS).

Methods

Study design

The design of QFS has been described in detail elsewhere [36]. Briefly, QFS is composed of French-Canadian families living in and around the Quebec City area. The QFS sample is composed of a mixture of randomly ascertained families (phase 1) and families ascertained through an obese [body mass index (BMI) $\geq$30 kg/m^2] proband. The total QFS sample includes 951 individuals from 223 families. The present study includes 712 nondiabetic adults (NGT) who underwent an OGTT. Following the OGTT, 25 individuals were newly diagnosed as diabetic (2-h glycemia $\geq$11.1 mmol/l, T2D) and 134 individuals had impaired glucose tolerance (7.8 mmol/l $\leq$ 2-h glycemia $<$ 11.1 mmol/l, IGT). Since the individuals with type 2 diabetes were diagnosed at the screening visit, we decided to keep them in the analyses. These individuals were not aware of their diabetes and thus had not changed their lifestyle or taken medication. They therefore represent the extreme end of a population-based sample. The Medical Ethics Committee of Laval University approved the protocol and all participants gave their written consent to participate in the study.

Measurements of T2D-related phenotypes

Several indicators of insulin sensitivity and insulin secretion were considered. Fasting blood samples were collected after an overnight fast and plasma glucose, insulin and C-peptide levels were assayed as previously described [37].

In the fasting state, insulin sensitivity was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) and insulin secretion using the homeostasis model assessment for insulin secretion (HOMA-B) [38]. The participants underwent a 75 g OGTT, after an overnight fast. Blood glucose, insulin and C-peptide levels were measured at $-$15, 0, 15, 30, 45, 60, 120, 150 and 180 min after the glucose load. OGTT areas under the curve (AUC) were calculated using the trapezoid method.

In the present study, we also used different other indices of insulin sensitivity and beta-cell function derived from

plasma levels of glucose, insulin and C-peptide obtained during the OGTT. The Cederholm index [39] is a numerical index of the curve relating glucose uptake (*M*) to log insulin. *M* was considered as the difference between the glucose load and the increase in the amount of glucose in the glucose space during the OGTT. Insulin sensitivity is then expressed as the ratio of the metabolic clearance rate (*M*/mean blood glucose) to log mean serum insulin. The Cederholm index is calculated using the following equation [75,000 + (glucose 0 min − glucose 120 min) × 1.15 × 180 × 0.19 × weight]/[120 × log (insulin mean) × glucose mean] and high values represent high insulin sensitivity. The C-peptide/glucose ratio (P30/G30: C-peptide 30 min − C-peptide 0 min/glucose 30 min − glucose 0 min) [40] quantifies the pancreatic C-peptide response to glucose during the first 30 minutes of the OGTT, and the insulogenic index (I30/G30: insulin 30 min − insulin 0 min/glucose 30 min − glucose 0 min) [41] the pancreatic insulin response to glucose during the first 30 min of the test. Both ratios are indicators of early beta-cell response following glucose ingestion. Finally, the disposition index (P30/G30 × Cederholm index) is a measure of insulin secretion corrected for insulin sensitivity [42].

These indices have been validated against more direct measures of insulin sensitivity and insulin secretion [43, 44].

Genotyping

A total of 23 SNPs in 9 genes were retained for the present study (see Table 2). The variants were selected from recent GWAS published in 2007 [2–7, 9] and included 2 SNPs in *TCF7L2*, 7 SNPs in *CDKN2B*, 4 SNPs in *KCNJ11*, 3 SNPs in *HHEX/IDE*, 2 SNPs in *CDKAL1* and one SNP for each of *IGF2BP2, SLC30A8, TCF2* and *WFS1*. One SNP (rs1828390) was not located in a known gene.

All variants except rs4430796 in *TCF2* and rs10010131 in *WFS1* were genotyped using the Illumina Golden Gate assay on the Illumina Bead Station platform (Illumina Inc., San Diego, CA, USA). Variants in *TCF2* and *WFS1* were genotyped using TaqMan methodology of Applied Biosystem Company [45].

Statistical analysis

Deviation from Hardy–Weinberg equilibrium (HWE) and the linkage disequilibrium (LD) among polymorphisms were tested in unrelated individuals using the ALLELE procedure implemented in SAS (SAS Institute, Cary, NC, version 9.1.3). The pairwise LD among the SNPs was assessed by r^2 and D'[46].

Association with T2DM endophenotypes was tested using a regression model implemented in the MIXED procedure of SAS (SAS Institute, Cary, NC, version 9.13). A sandwich estimator was used to take into account the nonindependence of the data resulting from the familial relationships by correcting the standard errors of estimates for the dependencies of individuals within families. We tested for association assuming a dominant model when the frequency of the rare homozygotes was below 2% (i.e. for rs495490 and rs1828390). All phenotypes, except C-peptide AUC, Cederholm index and disposition index, were natural log transformed, whereas fasting insulin was square root transformed. The analyses were done on data adjusted for age and sex, while insulin secretion phenotypes were further adjusted for insulin sensitivity in order to assess the effect of genetic variants on beta-cell function independently of insulin sensitivity. Thus, fasting C-peptide and HOMA-B were adjusted for HOMA-IR and insulin AUC, C-peptide AUC, I30/G30 and P30/G30 were adjusted for the Cederholm index. We also verified whether the associations were independent of BMI by further adjustment for BMI. Finally, to examine the joint effect of the variants and their contribution to the variance of the T2DM endophenotypes, stepwise regression analyses were performed on adjusted data (as described above) using the 23 SNPs genotypes as independent variables.

Results

The characteristics of the 712 participants are presented in Table 1 and the genotypic and allelic frequencies are shown in Table 2. All polymorphisms were in Hardy–Weinberg equilibrium. Results of association analyses are presented in Table 3 for SNPs showing significant ($P < 0.05$) evidence of association.

Glucose tolerance

The strongest evidence for association with glucose tolerance was with the *IGF2BP2* rs4402960 (fasting glucose, $P = 0.010$, glucose AUC, $P = 0.002$ and 2-h glucose, $P = 0.016$), and with two variants in *CDKN2B* (rs3731201: fasting glucose, $P = 0.031$ and 2-h glucose, $P = 0.018$; rs10811661: glucose AUC, $P = 0.013$ and 2-h glucose, $P = 0.024$). These associations remained significant after adjustment for BMI (Table 3). Other variants in *CDKN2B*, *HHEX/IDE* and *TCF7L2* were also associated with phenotypes of glucose tolerance ($0.03 \leq P \leq 0.04$), but these associations were no longer significant after adjustment for BMI. Variants in *CDKAL1*, *KCNJ11* and *SLC30A8* were not associated with glucose tolerance. Using stepwise regression analyses, we showed that *IGF2BP2* rs4402960 accounted for a significant fraction of

Table 1 Characteristics of the participants

Phenotypes	*N*	Men ($n = 310$)	Women ($n = 402$)
Age (years)	712	40.99 ± 15.16	40.02 ± 14.55
BMI (kg/m^2)	703	27.61 ± 6.69	27.74 ± 7.99
Fasting glucose (mmol/l)	712	5.41 ± 0.82	5.12 ± 0.58§
Fasting insulin (pmol/l)	706	72.88 ± 55.76	72.99 ± 60.52
Fasting C-peptide (pmol/l)	700	799.83 ± 498.19	797.45 ± 407.56
HOMA-IR	706	3.08 ± 2.85	2.92 ± 3.08
HOMA-B	706	126.57 ± 80.54	175.56 ± 583.74
Glucose AUC (mmol/l × min)	706	1260.20 ± 339.78	1198.00 ± 282.29#
Insulin AUC (nmol/l × min)	698	77.13 ± 57.34	79.75 ± 53.68
C-peptide AUC (nmol/l × min)	684	496.11 ± 257.81	541.00 ± 233.13*
2-h glucose (mmol/l)	712	6.41 ± 2.37	6.47 ± 1.99
I30/G30	677	122.40 ± 522.25	179.18 ± 684.50
P30/G30	667	574.13 ± 1895.10	823.47 ± 3117.80
Cederholm	697	15.89 ± 5.27	16.18 ± 4.55
Disposition index	681	40.942 ± 20.612	44.234 ± 21.894**

BMI body mass index; *AUC* area under the curve; I30/G30, insulin 30 min − insulin 0 min/glucose 30 min − glucose 0 min (insulinogenic index); P30/G30, C-peptide 30 min − C-peptide 0 min/glucose 30 min − glucose 0 min

Data presented are means ± SD

§ ≤ 0.0001, # 0.01, * 0.02 and ** $P = 0.05$ for differences between men and women

the variance in fasting glucose (1.72%), glucose AUC (2.00%) and 2-h glucose (1.64%) (Table 4).

Insulin sensitivity

HOMA-IR was strongly modulated by variants in *CDKN2B* (rs3731201, $P = 0.004$) and *HHEX/IDE* (rs7923837, $P = 0.002$), whereas the Cederholm index was associated with variants in *SLC30A8* (rs13266634, $P = 0.005$) and *IGF2BP2* (rs4402960, $P = 0.007$) (Table 3). These associations remained significant after adjustment for BMI. Variants in *KCNJ11* and *TCF7L2* were not associated with insulin sensitivity. The larger part of the variance in HOMA-IR and in the Cederholm index was explained by *HHEX/IDE* rs7923837 (1.89%) and *IGF2BP2* rs4402960 (2.25%), respectively (Table 4).

Insulin secretion

The strongest evidence for association with insulin secretion was between HOMA-B and *KCNJ11* (rs2285676, $P = 0.003$ and rs11024273, $P = 0.01$). Variants in *CDKAL1*, *CDKN2B*, *HHEX/IDE* and *TCF7L2* showed also evidence of association with insulin secretion indices derived from the OGTT ($P = 0.01$–0.02). On the other hand, variants in *IGF2BP2* (rs4402960) and *SLC30A8* (rs13266634) were not associated with insulin secretion ($P > 0.05$). The stepwise regression analyses showed that *CDKN2B* variants accounted for most of the variance in insulin secretion-related phenotypes, with a R^2 up to 3.14% (Table 4).

No evidence of association were found with rs7756992 in *CDKAL1*, rs3217992 in *CDKN2B*, rs7911264 in *HHEX/IDE*, rs5215 and rs11024273 in *KCNJ11*, rs7901695 in *TCF7L2*, rs4430796 in *TCF2*, rs10010131 in *WFS1* and rs1828390, located in an unknown gene.

Discussion

Recent GWAS have identified novel T2DM susceptibility loci within the genes encoding *CDKAL1*, *CDKN2B*, *HHEX/IDE*, *IGF2BP2*, *SLC30A8* and *TCF2* and have confirmed those in *PPARG*, *KCNJ11*, *TCF7L2* and *WFS1* [2–7, 9]. The purpose of this study was to examine the relationship between these susceptibility genes and T2DM intermediate phenotypes derived from measures of glucose, insulin and C-peptide in a fasting state and following an OGTT.

The strongest evidence of association was observed with variants in the *IGF2BP2*, *CDKN2B*, *TCF7L2*, and *HHEX/IDE* genes. These loci have shown clear associations with increased T2DM risk in GWAS, with odds ratios (ORs) per allele ranging from 1.14 to 1.37 [47]. Our results reveal that the *IGF2BP2* variant (rs4402960) was associated with insulin sensitivity (the Cederholm index) and with all the glucose tolerance phenotypes (fasting glucose, glucose AUC and 2-h glucose). Similarly, we observed that rs3731201 in *CDKN2B* was associated with fasting glucose, 2-h glucose and HOMA-IR. As glucose tolerance is the result of the interaction between insulin sensitivity and insulin secretion, the latter observations suggest that these variants affect glucose tolerance through their effect on insulin sensitivity, effect that is not compensate by insulin secretion. *IBF2BP2* was previously associated with the disposition index in Hispanic Americans but not in African Americans [29], with HOMA-B in nondiabetic Japanese individuals [28] and with lower acute insulin release and

Table 2 Genotypic and allelic distribution at each SNP

Genes	Polymorphisms	Genotypes	*F* (%)	Alleles	*F* (%)
CDKAL1	rs7756992	AA	55.8	A	75.6
		AG	39.6	G	24.4
		GG	4.6		
	rs10946403	AA	69.3	A	83.9
		AG	29.1	G	16.1
		GG	1.5		
CDKN2B	rs3731211	TT	50.0	T	71.2
		TA	42.5	A	28.8
		AA	7.5		
	rs3731201	AA	68.0	A	82.8
		AG	29.5	G	17.2
		GG	2.5		
	rs3217992	GG	32.8	G	58.0
		GA	50.3	A	42.0
		AA	16.9		
	rs495490	AA	86.2	A	92.9
		AG	13.5	G	7.1
		GG	0.3		
	rs523096	AA	35.9	A	59.4
		AG	46.9	G	40.6
		GG	17.2		
	rs564398	AA	40.5	A	62.7
		AG	44.5	G	37.3
		GG	15.0		
	rs10811661	AA	62.3	A	77.8
		AG	31.0	G	22.2
		GG	6.7		
HHEX/IDE	rs7911264	GG	25.5	G	50.3
		GA	49.7	A	49.7
		AA	24.8		
	rs1111875	GG	33.1	G	58.9
		GA	51.5	A	39.3
		AA	15.3		
	rs7923837	GG	35.9	G	60.7
		GA	49.7	A	39.3
		AA	14.4		
IGF2BP2	rs4402960	CC	52.1	C	71.8
		CA	39.3	A	28.2
		AA	8.6		
KCNJ11	rs1002227	CC	49.7	C	70.6
		CA	41.7	A	29.5
		AA	8.6		
	rs2285676	AA	40.8	A	63.5
		AG	45.4	G	36.5
		GG	13.8		
	rs5215	AA	35.0	A	58.7
		AG	47.5	G	41.3
		GG	17.5		
	rs11024273	CC	48.8	C	69.6
		CA	41.7	A	30.4
		AA	9.5		
SLC30A8	rs13266634	GG	48.2	G	69.3
		GA	42.3	A	30.7
		AA	9.5		
TCF7L2	rs7903146	GG	44.8	G	67.8
		GA	46.0	A	32.2
		AA	9.2		
	rs7901695	TT	40.9	T	65.0
		TC	48.2	C	35.0
		CC	10.9		
TCF2	rs4430796	GG	27.2	G	52.8
		GA	51.1	A	47.2
		AA	21.7		
WFS1	rs10010131	GG	35.9	G	61.3
		GA	50.8	A	38.7
		AA	13.3		
Unknown gene	rs1828390	AA	81.3	A	89.9
		AG	17.2	G	10.1
		GG	1.5		

lower insulin release in response to tolbutamide injection in young healthy Caucasian individuals [32]. Interestingly, the latter authors did not replicate their initial findings on insulin secretion in a large cohort of NGT, IGT and T2D subjects but found an association with 2-h glucose levels. As for *CDKN2B,* it was hypothesized that the increased T2DM susceptibility conferred by the gene could be mediated through a decrease in beta-cell mass and subsequent low insulin release under high insulin demand conditions (i.e. after OGTT or IVGTT) [32]. In accordance with this hypothesis, we observed that *CDKN2B* rs10811661 variant influenced glucose tolerance (glucose AUC and 2-h glucose), potentially through its effect on insulin secretion (the disposition index) as the variant did not affect insulin sensitivity. Furthermore, we reported weaker associations between other variants in *CDKN2B* (rs523096, rs495490 and rs564398) and insulin release during the OGTT (I30/G30, P30/G30 and C-peptide AUC).

TCF7L2 was the most extensively studied gene in relation to T2DM risk and number of studies have supported its role in insulin secretion [11–17, 19, 22–25, 48]. We observed an influence of rs790146 variant on insulin secretion during the OGTT (insulin AUC) that, in the absence of a compensatory response in insulin sensitivity, results in an effect on 2-h glucose levels. However, the effect of this gene on 2-h glucose levels was abolished after adjustment for BMI, suggesting that this gene might have a pleiotropic effect.

Finally, we reported a strong association between rs7923837 variant in *HHEX/IDE* and fasting insulin secretion (fasting C-peptide) and insulin sensitivity (HOMA-IR), what resulted in an effect on glucose

Table 3 Associations between T2DM susceptibility genes and measures of glucose tolerance, insulin sensitivity and insulin secretion

	CDKAL1		*CDKN2B*[a]					*HHEX/IDE*[b]		*IGF2BP2*	*KCNJ11*[c]		*SLC30A8*	*TCF7L2*
	rs10946403 **A** > G	rs523096 A > **G**	rs10811661 **A** > G	rs3731211 T > **A**	rs3731201 A > **G**	rs495490# **A** > G	rs564398 **A** > G	rs7923837 G > **A**	rs1111875 G > **A**	rs4402960 C > **A**	rs2285676 **A** > G	rs11024273 **C** > A	rs1326663 **G** > A	rS7903146 G > **A**
Glucose tolerance														
Fasting glucose				0.041	0.031			0.031*		0.010				
Glucose AUC		0.021*	0.013							0.002				
2-h glucose			0.024		0.018					0.016				0.025*
Insulin sensitivity														
Fasting insulin														
HOMA-IR	0.040			0.019	0.004			0.002	0.019*					
Cederholm index										0.007			0.005	
Insulin secretion														
Fasting C-peptide								0.014*						
HOMA-B											0.003	0.010		
Insulin AUC														0.019
C-peptide AUC	0.010													
I30/G30		0.042					0.037							
P30/G30						0.044								
Disposition index			0.014											

Allele in bold represent the at-risk allele

Analyses were performed on data adjusted for age, sex and insulin sensitivity for insulin secretion-related phenotypes. The *P* values reported in the table are under additive model, except for # indicating dominant model (homozygote for the common allele versus carriers of the rare allele)

* Nonsignificant after further adjustment for BMI

[a] LD between *CDKN2B* SNPs: rs3731211 − rs3731201: $D' = 1$, $r^2 = 0.5$; rs3731201 − rs523096: $D' = 1$, $r^2 = 0.14$; rs3731201 − rs564398: $D' = 1$, $r^2 = 0.12$; rs495490 − rs523096: $D' = 1$, $r^2 = 0.11$; rs523096 − rs564398: $D' = 1$, $r^2 = 0.87$

[b] LD between *HHEX/IDE* SNPs: rs7911264 − rs1111875: $D' = 0.99$, $r^2 = 0.68$

[c] LD between *KCNJ11* SNPs: rs2285676 − rs11024273: $D' = 1$, $r^2 = 0.76$

Table 4 Contribution of the variants to the prediction of T2DM-related phenotypes

Genes	Variants	R^2 (%)	*P* value
Glucose tolerance			
Fasting glucose			
CDKN2B	**rs3731211, rs10811661**	1.86	
IGF2BP2	**rs4402960**	1.72	
HHEX/IDE	**rs7923837**	1.17	
KCNJ11	**rs5215**	0.65	
Unknown gene	**rs1828390**	0.65	
Model		6.05	<0.0001
Glucose AUC			
IGF2BP2	rs4402960	2.00	
KCNJ11	**rs5215**	1.26	
HHEX/IDE	**rs7923837**	1.04	
CDKN2B	**rs10811661**	0.79	
TCF7L2	rs7903146	0.35	
Model		**5.43**	<0.0001
2-h Glucose			
IGF2BP2	**rs4402960**	1.64	
KCNJ11	**rs5215**	0.96	
HHEX/IDE	**rs7923837**	0.88	
TCF7L2	**rs7903146**	0.73	
CDKAL1	rs10946403	0.32	
Model		**4.53**	<0.0001
Insulin sensitivity			
Fasting insulin			
HHEX/IDE	**rs7923837, rs1111875**	1.26	
TCF7L2	**rs7903146**	1.14	
CDKN2B	rs10811661	0.46	
Model		**2.86**	0.0007
HOMA-IR			
HHEX/IDE	**rs7923837**	1.89	
IGF2BP2	**rs4402960**	1.02	
CDKN2B	**rs523096**, rs495490	0.97	
KCNJ11	rs1002227	0.44	
Model		**4.32**	<0.0001
Cederholm index			
IGF2BP2	**rs4402960**	2.25	
CDKN2B	rs10811661, rs523096, rs3217992, rs495490	1.52	
HHEX/IDE	**rs7923837**	1.15	
KCNJ11	**rs5215**	0.98	
SLC30A8	rs13266634	0.37	
Model		**6.27**	<0.0001
Insulin secretion			
Fasting C-peptide			
CDKN2B	**rs495490, rs3217992,** rs3731201	3.14	
CDKAL1	**rs10946403**, rs7756992	2.03	
HHEX/IDE	**rs7923837,** rs1111875	1.25	
WFS1	**rs10010131**	0.75	
KCNJ11	rs1002227, rs11024273	0.71	
SLC30A8	rs13266634	0.49	
Model		**8.37**	<.0001
HOMA-B			
CDKN2B	**rs10811661**	1.49	
Unknown gene	**rs1828390**	0.86	
IGF2BP2	**rs4402960**	0.68	
TCF7L2	**rs7903146**	0.57	
Model		**3.60**	<0.0001
Insulin AUC			
TCF7L2	**rs7903146,** rs7901695	2.12	
CDKN2B	**rs495490**, rs3731211, rs564398	1.69	
WFS1	rs10010131	0.41	
Model		**4.22**	<0.0001
C-peptide AUC			
CDKN2B	**rs495490,** rs3217992	2.69	
CDKAL1	**rs10946403,** rs7756992	1.29	
WFS1	**rs10010131**	0.98	
SLC30A8	**rs13266634**	0.82	
HHEX/IDE	**rs7923837**	0.67	
Model		**6.45**	<0.0001
I30/G30			
CDKN2B	**rs3217992, rs495490**	1.42	
HHEX/IDE	rs7923837	0.51	
Model		**1.93**	0.005
P30/G30			
CDKN2B	**rs3217992, rs495490**	1.57	
HHEX/IDE	**rs7911264**	0.67	
Model		**2.24**	0.002
Disposition index			
CDKN2B	**rs10811661**	1.42	
CDKAL1	**rs10946403,** rs7756992	1.29	
KCNJ11	rs1002227	0.41	
TCF7L2	rs11196205	0.31	
Model		**3.43**	0.0004

In bold, the variants which contribute significantly ($P < 0.05$) to the model

tolerance (fasting glucose). Several authors reported associations between *HHEX/IDE* variants (rs7923837 and rs1111875) and insulin secretion response following a glucose load [24, 30, 32–34], suggesting that this gene might influence T2DM risk primarily through an effect on beta cell function.

Our results suggest a significant implication of these T2DM variants in beta-cell dysfunction and, to a lesser extent, in insulin sensitivity. Despite evidence that these variants are associated with T2DM intermediate phenotypes, their contributions to the variance of these phenotypes remain small. Considering the polygenic nature of these phenotypes which imply the contribution of many loci with small effects on the phenotype, it is not a surprise to find that genes identified through GWAS explain only a small portion of the variance of complex traits such as those investigated in the present study. Indeed, Palmer et al. [29] reported that diabetes variants accounted for no more than 0.01 and 0.004% in the phenotypic variance in insulin secretion and insulin sensitivity indices, respectively. In the current study, using linear regression models that included the 23 variants, we reported that specific combination of these variants may account for up to 8.37% of the phenotypic variance in insulin secretion (fasting C-peptide), 6.27% of the phenotypic variance in insulin sensitivity (the Cederholm index) and 6.05% in that of glucose tolerance (fasting glucose).

These findings suggest that the variants which have been shown to be associated with an increased risk of T2DM appear to have a stronger influence on insulin secretion than on insulin sensitivity phenotypes, in line with the hypothesis that beta cell dysfunction is genetically programmed [49]. Furthermore, these observations also suggest that combining genetic information from several susceptibility genes allows to better explain inter-individual differences in T2DM-related phenotypes.

In the present study we reported 27 significant associations at a P value <0.05, of which nine were significant a P value ≤ 0.01. The Bonferroni method which is traditionally used to adjust P values for multiple testing assumes that the tests are independent. In genetic association studies, linkage disequilibrium (LD) between SNPs and the occurrence of multiple genes implicated in various metabolic pathways give rise to correlations among the SNPs. In addition to correlations among SNPs, we have correlations among phenotypes. The objective of performing adjustment for multiple testing is to reduce the likelihood of obtaining false positive results (type I errors). However, in the context of a replication study like the present one, one can argue that it is not the number of tests being performed that is important, but the a priori probability of finding associations. In this respect, it has been showed that two studies reporting association with P value <0.01 constitute a strong predictor of future replication [50]. For these reasons we believed that adjustment for multiple testing would be overly conservative in the context of the present study and we have chosen to report only the nominal P values.

Following to the identification of novel T2DM susceptibility genes by GWAS and the confirmation of the implication of well-known variants in the disease risk, it was important to investigate the relationship between these genes and T2DM intermediate traits in order to determine whether these variants influence the risk of disease primarily through effects on beta-cell function, insulin action or glucose tolerance. Using T2DM endophenotypes derived from an OGTT, we were able to confirm the significant association of diabetes susceptibility genes with beta-cell dysfunction. The presence of associations with insulin sensitivity and glucose tolerance also suggests that diabetes susceptibility genes may influence the risk of disease through different biological pathways. However, despite the high population attributable risk of these T2DM variants, their contribution to the variance of glucose and insulin metabolism related phenotypes range from about 2 to 9%, suggesting that there are probably many other genes influencing the risk of T2DM.

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