Diabetologia (2009) 52:253–261
DOI 10.1007/s00125-008-1196-4

ARTICLE

Type 2 diabetes-associated genetic variants discovered in the recent genome-wide association studies are related to gestational diabetes mellitus in the Korean population

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Received: 21 July 2008 / Accepted: 10 October 2008 / Published online: 11 November 2008

Abstract
Aims/hypothesis New genetic variants associated with susceptibility to type 2 diabetes mellitus have been discovered in recent genome-wide association (GWA) studies. The aim of the present study was to examine the association between these diabetogenic variants and gestational diabetes mellitus (GDM).
Methods The study included 869 Korean women with GDM and 345 female and 287 male Korean non-diabetic controls. We genotyped the single nucleotide polymorphisms (SNPs) rs7756992 and rs7754840 in *CDKAL1*; rs564398, rs1333040, rs10757278 and rs10811661 in the *CDKN2A−CDKN2B* region; rs8050136 in *FTO*; rs1111875, rs5015480 and rs7923837 in *HHEX*; rs4402960 in *IGF2BP2*; and rs13266634 in *SLC30A8*. In addition, rs7903146 and rs12255372 in *TCF7L2*; rs5215 and rs5219 in *KCNJ11*; and rs3856806 and rs1801282 in *PPARG* were genotyped. The genotype frequencies in the GDM patients were compared with those in the non-diabetic controls.
Results Compared with controls (men and women combined), GDM was associated with rs7756992 and rs7754840 (OR 1.55, 95% CI 1.34–1.79, $p=4.17\times10^{-9}$) in *CDKAL1*; rs10811661 (OR 1.49, 95% CI 1.29–1.72, $p=1.05\times10^{-7}$) in the *CDKN2A−CDKN2B* region; rs1111875 (OR 1.27, 95% CI 1.09–1.49, $p=0.003$), rs5015480, and rs7923837 in *HHEX*; rs4402960 (OR 1.18, 95% CI 1.01–1.38, $p=0.03$) in *IGF2BP2*; rs13266634 (OR 1.24, 95% CI 1.07–1.43, $p=0.005$) in *SLC30A8*; and rs7903146 (OR 1.58, 95% CI 1.03–2.43, $p=0.038$) in *TCF7L2*. The risk alleles of the SNPs rs7756992 and rs7754840 in *CDKAL1*; rs10811661 in the *CDKN2A–CDKN2B* region; and rs1111875, rs5015480 and rs7923837 in *HHEX* were associated with significant decreases in the insulin AUC during a 100 g OGTT performed at the time of diagnosis of GDM.
Conclusions/interpretation Some of the type 2 diabetes-associated genetic variants that were discovered in the recent GWA studies are also associated with GDM in Koreans.

Keywords Genetic association · Gestational diabetes mellitus · Type 2 diabetes

Abbreviations

CDK5	cyclin-dependent kinase 5
GDM	gestational diabetes mellitus
GWA	genome-wide association
HOMA-IR	homeostasis model assessment of insulin resistance

Electronic supplementary material The online version of this article (doi:10.1007/s00125-008-1196-4) contains supplementary material, which is available to authorised users.

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KCNJ11	potassium inwardly rectifying channel, subfamily J, member 11
SNP	single nucleotide polymorphism
TCF7L2	transcription factor 7-like 2

Introduction

Gestational diabetes mellitus (GDM)—glucose intolerance of varying degrees of severity that is first recognised during pregnancy [1]—affects 2–14% of all pregnancies [2–4]. GDM may have the same genetic background as type 2 diabetes mellitus as there is evidence for the clustering of type 2 diabetes and impaired glucose tolerance in families with women with GDM [5] and for a higher prevalence of type 2 diabetes in mothers of women with GDM [6]. Furthermore, it is well known that women with GDM are at a greater risk of developing type 2 diabetes later in life [7–9]. However, knowledge regarding the genetics of GDM is limited [7, 10]. Several studies have examined candidate genes in women with and without GDM. Positive associations were shown for genes encoding glucokinase [11], calpain-10 [12], sulfonylurea receptor 1 [13], potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*) [14], β_3 adrenergic receptor [15], plasminogen activator inhibitor 1 [16] and transcription factor 7-like 2 (*TCF7L2*) [17, 18]. Except for the effects of *TCF7L2* in Scandinavian women [17], no robust associations of genetic variants with GDM have been demonstrated.

In addition to those in *KCNJ11*, *PPARG* and *TCF7L2*, the recent genome-wide association (GWA) studies have identified new genetic variants with reproducible associations with susceptibility to type 2 diabetes [19–23], the majority of which were found in genes that were not even considered candidates [24]. Furthermore, robust signals ($p<5\times10^{-7}$) were identified in certain gene regions (i.e. the *HHEX–IDE* and *CDKN2A–CDKN2B* regions) [19–23]. We recently found that the diabetogenic genetic variants reported by the large-scale GWA studies in Europids [19–23] were also associated with the risk of type 2 diabetes in Asian populations, including Koreans [25].

If GDM and type 2 diabetes share a common genetic background, the genetic variants determining the risk of type 2 diabetes may also be associated with GDM. In the present study, we compared the genotype frequencies of the single nucleotide polymorphisms (SNPs) in the diabetogenic genes in GDM patients with those in non-diabetic controls.

Methods

Patients with GDM This study included 869 Korean women diagnosed with GDM at the Samsung Cheil Hospital (Seoul, Korea) between January 1996 and February 2003. During the study period, 39,190 consecutive women underwent screening for GDM. We followed a previously described protocol for the screening and diagnosis of GDM [3, 26]. In brief, all pregnant women without a previous diagnosis of glucose intolerance were screened for GDM between 24 and 28 weeks of gestation by using the 50 g, 1 h glucose challenge test as recommended by the Third International Workshop-Conference on GDM [1]. A plasma glucose concentration of 7.2 mmol/l or more was considered positive for GDM and was followed by a 100 g OGTT. GDM was diagnosed according to the criteria of the Third International Workshop-Conference on GDM [1]. The threshold glucose values were as follows: fasting ≥5.8 mmol/l, 1 h ≥10.5 mmol/l, 2 h ≥9.2 mmol/l and 3 h ≥8.0 mmol/l.

Gestational age at the time of the screening test was 27.9±2.9 weeks (mean±SD). Plasma glucose concentration was measured by the glucose oxidase method using an YSI 2300 STAT analyser (Yellow Springs Instrument Company, Yellow Springs, OH, USA). Serum insulin concentration was measured using insulin-specific radioimmunoassay kits (Linco Research, St Louis, MO, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula developed by Matthews et al. [27]. GAD antibodies were measured using a radioimmunoassay method (RSR, Cardiff, UK).

The clinical characteristics of women with GDM at 6 weeks postpartum are shown in Table 1. Height and body weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, with the patient standing barefoot and in light clothing. BMI was calculated as body weight in kilograms divided by the square of height in metres. Blood pressure was measured after the participant had remained seated for 10 min. Measurements were taken twice, 5 min apart. The frequency of a positive family history of type 2 diabetes in women in the GDM group was 41.3%. Women who tested positive for GAD antibodies were excluded from the study.

Non-diabetic controls We recruited 632 non-diabetic controls (345 women, 287 men) who visited Seoul National University Hospital for a routine health check-up. The following selection criteria were used: age ≥60 years, no history of type 2 diabetes, no first-degree relatives with type 2 diabetes, fasting plasma glucose level <6.1 mmol/l and HbA_{1c} level <5.8%. Therefore, controls were expected to be at a very low risk of type 2 diabetes. Table 1 shows the clinical characteristics of the controls in detail. It was not confirmed whether the non-diabetic female participants had experienced pregnancy without GDM. However, the ascertainment bias should be minimal given that the prevalence of GDM is estimated to be very low in Korea, at a rate of 2.2 cases per 100 pregnant women [3].

Table 1 Clinical characteristics of the study participants

	GDM[a] (n=869)	Non-diabetic controls		
		All (n=632)	Men (n=287)	Women (n=345)
Age (years)	32.0±3.9	64.7±3.6	64.9±3.8	64.4±3.3
BMI (kg/m^2)	23.1±3.6	23.3±3.0	22.9±2.7	23.9±3.3
Systolic BP (mmHg)	118±13	128±20	128±19	129±20
Diastolic BP (mmHg)	73±9	80±10	81±11	79±11
Fasting plasma glucose (mmol/l)	5.5±1.7	4.9±8.7	5.0±0.5	4.9±0.5
HbA_{1c} (%)	NA	5.3±0.3	5.2±0.3	5.3±0.3
Total cholesterol (mmol/l)	5.22±0.85	4.98±0.90	4.83±0.90	5.10±0.88
Triacylglycerol (mmol/l)	3.89±2.68	3.30±1.66	3.22±1.63	3.37±1.68
HDL-cholesterol (mmol/l)	1.37±0.33	1.20±0.33	1.21±0.35	1.20±0.31
LDL-cholesterol (mmol/l)	3.09±0.70	3.12±0.89	2.98±0.91	3.23±0.84
Fasting plasma insulin (pmol/l)	64±32	47±29	43±20	51±35
HOMA-IR	2.6±1.5	1.7±1.1	1.6±0.8	1.9±1.3

Data are presented as mean±SD
[a] All values for the GDM group were measured at 6 weeks postpartum
NA, not available

Ethical considerations The Institutional Review Board of the Clinical Research Institute of Seoul National University Hospital and the Research and Ethics Committee of the Samsung Cheil Hospital approved the study protocol, and informed consent was obtained from each study participant. The study was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Gene and SNP selection SNPs from six novel genetic loci, which were identified through the recent GWA studies [19–23] and showed reproducible associations with type 2 diabetes in Europeans, were selected for this study. Specifically, these were rs7756992 and rs7754840 in *CDKAL1*; rs564398 and rs10811661 in *CDKN2A–CDKN2B*; rs8050136 in *FTO*; rs1111875, rs5015480 and rs7923837 in *HHEX*; rs4402960 in *IGF2BP2*; and rs13266634 in *SLC30A8*. Two representative SNPs (rs1333040 and rs10757278) close to *CDKN2A–CDKN2B* that were associated with coronary heart disease and myocardial infarction were also selected [28–30]. In addition, rs7903146 and rs12255372 in *TCF7L2* [31]; rs5215 and rs5219 in *KCNJ11* [32]; and rs3856806 and rs1801282 in *PPARG* [33] were also genotyped.

Genotyping We genotyped 18 SNPs in nine genes from genomic DNA. An allelic discrimination assay was performed in 5 μl of 1× TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) containing 20 ng of genomic DNA and 0.125 μl of 40× Assays-on-Demand SNP genotyping product (Applied Biosystems), according to the manufacturer's instructions. (A list of the assay ID numbers and public ID numbers for the Assays-on-Demand SNP genotyping products used in this study is presented in Electronic supplementary material [ESM] Table 1.) Next, the plate was placed in a thermal cycler (PE 9700; Applied Biosystems) and heated for 2 min at 50°C and 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. The TaqMan assay plate was then transferred to a Prism 7900HT instrument (Applied Biosystems) and the fluorescence intensity of each well was read. The fluorescence data files for each plate were analysed using Sequence Detection System version 2.1 (Applied Biosystems). Forty-four blind duplicates and eight blank samples were used as positive and negative controls, respectively. The overall genotyping success rate was 99.4% (range 97.4–99.8%; ESM Table 2) and the concordance rate based on blind duplicate comparisons was 99.6%.

Statistical analyses All continuous variables are expressed as mean±SD. Statistical analyses were conducted using SPSS for Windows, version 11.0 (SPSS, Chicago, IL, USA). To determine whether individual polymorphisms were in Hardy–Weinberg equilibrium, χ^2 tests were used. The result of the test for Hardy–Weinberg equilibrium and the statistical power for each variant are shown in ESM Table 2. Since there were no differences in the genotype frequencies between male and female non-diabetic controls (ESM Table 3), we initially compared the genotype frequencies of the 869 GDM patients with those of the 632 controls (i.e. both men and women), which was considered to be a non-diabetic gene pool. In addition, we compared the genotype frequencies of the 869 GDM patients with those of the 345 female controls. Logistic regression analyses were used for calculating the ORs, 95% CIs and the corresponding p values with regard to the number of risk alleles using an additive model. Genotypes were given codes of 0, 1 and 2, and the OR was expressed

Table 2 Comparison of genotype frequencies between GDM patients and non-diabetic controls

Gene	Locus (rs number)	Allele (major/minor)	Genotype	GDM *n* (%)	Control *n* (%)	OR (95% CI)	*p* value
CDKAL1	rs7756992	G/A	AA	145 (17.1)	137 (21.7)	1.39 (1.20–1.61)	9.14×10^{-9}
			GA	374 (44.0)	325 (51.4)		
			GG	331 (38.9)	170 (26.9)		
	rs7754840	C/G	GG	171 (19.8)	178 (28.3)	1.55 (1.34–1.79)	4.17×10^{-9}
			CG	389 (45.1)	319 (50.6)		
			CC	303 (35.1)	133 (21.1)		
CDKN2A/2B	rs564398	T/C	TT	653 (75.8)	485 (76.7)	1.03 (0.84–1.27)	0.78
			CT	189 (22.0)	132 (20.9)		
			CC	19 (2.2)	15 (2.4)		
	rs1333040	T/C	TT	378 (43.8)	295 (46.7)	1.11 (0.95–1.30)	0.20
			CT	386 (44.7)	275 (43.5)		
			CC	99 (11.5)	62 (9.8)		
	rs10757278	A/G	AA	238 (28.4)	183 (29.3)	1.05 (0.91–1.22)	0.50
			AG	419 (50.1)	318 (50.9)		
			GG	180 (21.5)	124 (19.8)		
	rs10811661	T/C	CC	137 (15.8)	152 (24.1)	1.49 (1.29–1.72)	1.05×10^{-7}
			CT	399 (46.1)	313 (49.5)		
			TT	330 (38.1)	167 (26.4)		
FTO	rs8050136	C/A	CC	643 (74.4)	486 (77.3)	1.12 (0.90–1.40)	0.30
			AC	208 (24.1)	132 (21.0)		
			AA	13 (1.5)	11 (1.7)		
HHEX	rs1111875	T/C	TT	352 (40.7)	299 (47.5)	1.27 (1.09–1.49)	0.003
			CT	413 (47.6)	278 (44.1)		
			CC	102 (11.8)	53 (8.4)		
	rs5015480	T/C	TT	525 (60.9)	416 (65.8)	1.22 (1.01–1.47)	0.035
			CT	300 (34.8)	197 (31.2)		
			CC	37 (4.3)	19 (3.0)		
	rs7923837	A/G	AA	481 (55.9)	388 (61.7)	1.26 (1.06–1.50)	0.011
			AG	329 (38.3)	218 (34.7)		
			GG	50 (5.8)	23 (3.7)		
IGF2BP2	rs4402960	G/T	GG	389 (45.4)	313 (49.9)	1.18 (1.01–1.38)	0.034
			GT	365 (42.6)	257 (41.0)		
			TT	103 (12.0)	57 (9.1)		
SLC30A8	rs13266634	C/T	TT	126 (14.6)	107 (17.1)	1.24 (1.07–1.43)	0.005
			CT	372 (43.2)	306 (48.8)		
			CC	363 (42.2)	214 (34.1)		
TCF7L2	rs7903146	C/T	CC	803 (95.2)	596 (95.1)	1.58 (1.03–2.43)	0.038
			CT	63 (7.3)	31 (4.9)		
			TT	2 (0.2)	0 (0)		
	rs12255372	G/T	GG	860 (99.2)	628 (99.7)	2.56 (0.53–12.34)	0.24
			GT	7 (0.8)	2 (0.3)		
			TT	0 (0)	0 (0)		
KCNJ11	rs5215	A/G	AA	308 (35.6)	251 (41.0)	1.11 (0.96–1.28)	0.17
			AG	416 (48.1)	260 (42.5)		
			GG	140 (16.2)	101 (16.5)		
	rs5219	G/A	GG	298 (35.2)	254 (40.4)	1.12 (0.97–1.30)	0.13
			AG	407 (48.1)	273 (43.4)		
			AA	141 (16.7)	102 (16.2)		
PPARG	rs3856806	C/T	TT	28 (3.2)	22 (3.5)	1.09 (0.90–1.32)	0.37
			CT	228 (26.3)	178 (28.3)		
			CC	612 (70.5)	430 (68.3)		
	rs1801282	C/G	GG	1 (0.1)	2 (0.3)	1.27 (0.90–1.79)	0.17
			CG	71 (8.2)	63 (10.0)		
			CC	793 (91.7)	567 (89.7)		

p values were not corrected for multiple comparisons

Table 3 Comparison of significant genetic variants associated with type 2 diabetes and GDM in European and Korean populations

Gene	Locus (rs number)	Risk allele	Type 2 diabetes, European[a]			Type 2 diabetes, Korean			Korean GDM			*p* value for heterogeneity of OR	
			Control Risk AF	OR (95% CI)	*p* value	Control Risk AF	OR (95% CI)	*p* value	Control Risk AF	OR (95% CI)	*p* value	*p* value[b]	*p* value[c]
Total *n*			55,826			1,393			1,501				
Cases/controls			21,733/34,093			761/632			869/632				
CDKAL1	rs7756992	G	0.26	1.20 (1.13–1.27)	7.7×10^{-9}	0.53	1.26 (1.09–1.47)	0.002	0.53	1.39 (1.20–1.61)	9.1×10^{-9}	0.17	0.52
	rs7754840	C	0.31	1.12 (1.08–1.16)	4.1×10^{-11}	0.46	1.24 (1.07–1.44)	0.005	0.46	1.55 (1.34–1.79)	4.2×10^{-9}	0.002	0.14
CDKN2A/2B	rs10811661	T	0.83	1.20 (1.14–1.25)	7.8×10^{-15}	0.51	1.55 (1.33–1.81)	2.0×10^{-8}	0.51	1.49 (1.29–1.72)	1.1×10^{-7}	0.046	0.80
FTO	rs8050136	A	0.39	1.17 (1.12–1.22)	1.3×10^{-12}	0.12	1.15 (0.92–1.44)	0.23	0.12	1.12 (0.90–1.40)	0.295	0.78	0.91
HHEX	rs1111875	C	0.53	1.13 (1.08–1.17)	5.7×10^{-10}	0.31	1.21 (1.03–1.43)	0.019	0.31	1.27 (1.09–1.49)	0.003	0.30	0.76
	rs7923837	G	0.62	1.22 (1.01–1.43)	2.2×10^{-5}	0.21	1.33 (1.12–1.59)	0.002	0.21	1.26 (1.06–1.50)	0.011	0.80	0.76
IGF2BP2	rs4402960	T	0.29	1.14 (1.11–1.18)	8.9×10^{-16}	0.30	1.18 (1.00–1.39)	0.049	0.30	1.18 (1.01–1.38)	0.034	0.76	1.00
SLC30A8	rs13266634	C	0.65	1.12 (1.07–1.16)	5.3×10^{-8}	0.59	1.18 (1.02–1.38)	0.029	0.59	1.24 (1.07–1.43)	0.005	0.35	0.75
TCF7L2	rs7903146	T	0.26	1.37 (1.31–1.43)	1.0×10^{-48}	0.03	1.53 (0.98–2.39)	0.06	0.03	1.58 (1.03–2.43)	0.038	0.62	0.94

The SNP included from the European ancestry combined study was rs7756992 [47]. The SNPs included from DGI, WTCCC/UKT2D and FUSION, and Korean studies were rs7754840, rs10811661, rs8050136, rs111187, rs4402960, rs13266634, and rs7903146 [20, 23, 25]. The SNP included from Icelandic study was rs7923837 [22]

p values were not corrected for multiple comparisons

[a] Meta-analysis in Europeans was performed by fixed effects Cochran-Mantel-Haenszel test based on available SNPs reported in GWA studies of European populations

[b] Comparison of European type 2 diabetic and Korean GDM patients

[c] Comparison of Korean type 2 diabetic and Korean GDM patients

AF, allele frequency

per difference in the number of risk alleles. For the comparison of representative SNPs for type 2 diabetes and GDM in European and Korean populations, the heterogeneity of ORs among studies or populations was assessed by Cochran's Q statistic, as shown elsewhere [25]. Insulin resistance was evaluated using the HOMA-IR, and insulin secretory function was assessed by determination of the insulin AUC during a 100 g OGTT performed at the time of diagnosis of GDM. Multivariate linear regressions, adjusted for age and BMI, were used for comparing insulin resistance and insulin secretory function in GDM patients according to genotype. A p value of less than 0.05 was considered statistically significant.

Results

We compared the genotype frequencies in the 869 GDM patients with those in the 632 non-diabetic controls (345 women, 287 men) who were expected to be at very low risk of type 2 diabetes. All SNPs were in Hardy–Weinberg equilibrium (ESM Table 2). Compared with the 632 controls, we found that GDM was significantly associated with rs7756992 ($p=9.14\times10^{-9}$) and rs7754840 ($p=4.17\times10^{-9}$) in *CDKAL1*; rs10811661 ($p=1.05\times10^{-7}$) in *CDKN2A–CDKN2B*; rs1111875 ($p=0.003$), rs5015480 ($p=0.035$), and rs7923837 ($p=0.011$) in *HHEX*; rs4402960 ($p=0.034$) in *IGF2BP2*; rs13266634 ($p=0.005$) in *SLC30A8*; and rs7903146 ($p=0.038$) in *TCF7L2* (Table 2). In this analysis, three SNPs (rs7756992 and rs7754840 in *CDKAL1* and rs10811661 in *CDKN2A–CDKN2B*) met the stringent criterion for robust association ($p<5\times10^{-7}$) [19], and their ORs ranged from 1.39 to 1.55. When we compared the genotype frequencies in the GDM patients with those in the 345 female controls, rs7756992 ($p=0.001$) and rs7754840 ($p=1.36\times10^{-5}$) in *CDKAL1*; rs10811661 ($p=1.42\times10^{-7}$) in *CDKN2A–CDKN2B*; and rs13266634 ($p=0.044$) in *SLC30A8* showed significant associations with GDM (ESM Table 4).

Table 3 compares the ORs for the associations between the variants and GDM in our study with those previously reported for the associations of these variants with type 2 diabetes in European and Korean populations [20, 22, 23, 25, 47]. There were no significant differences in ORs between type 2 diabetes and GDM in Koreans. Although the effects on the risk of diabetes were in the same direction, the effect sizes of rs7754840 and rs10811661 were slightly greater for GDM in Koreans than for type 2 diabetes in Europeans.

Next, we examined the associations between the risk alleles and insulin resistance (i.e. HOMA-IR) and insulin secretory capacity (i.e. AUC of insulin during a 100 g OGTT; Table 4). No SNPs were significantly associated with HOMA-IR, with the exception of rs7754840 in *CDKAL1* and rs1111875 in *HHEX*, which showed modest associations (adjusted $p=0.049$ and 0.026, respectively). The risk alleles of the SNPs rs7756992 and rs7754840 in *CDKAL1* and rs10811661 in *CDKN2A–CDKN2B*, which, as mentioned above, showed robust associations with GDM, were associated with a marked decrease in AUC of insulin during a 100 g OGTT. Interestingly, the risk alleles of rs1111875, rs5015480 and rs7923837 in *HHEX* were also significantly associated with a reduced insulin AUC (adjusted $p=0.0000002$, 0.0002, and 0.006, respectively). In contrast, the risk allele of rs8050136 in the *FTO* gene was associated with an increased AUC of insulin (adjusted $p=0.006$) but was not associated with HOMA-IR.

Discussion

We found that some of the SNPs recently identified as genetic determinants of type 2 diabetes by GWA studies [19–23] were also associated with GDM in Koreans. Three SNPs in particular (rs7756992 and rs7754840 in *CDKAL1* and rs10811661 in *CDKN2A–CDKN2B*) were very strongly associated with GDM. These same SNPs were significantly associated with insulin secretory capacity as assessed by the insulin AUC during a 100 g OGTT performed at the time of the diagnosis of GDM. However, they did not show any robust associations with insulin resistance. Their association with pancreatic beta cell function is consistent with the results obtained in a study on type 2 diabetes in the Japanese population, which showed that risk alleles at *CDKAL1* (rs7756992) and *CDKN2A–CDKN2B* (rs10811661) were associated with impaired beta cell function [34].

It is well known that normal pregnancy is accompanied by a marked increase in insulin resistance, which may be the result of both increased maternal adiposity and the insulin-antagonising effects of several placental hormones [7]. Therefore, maternal pancreatic beta cell compensation is crucial for overcoming the insulin resistance provoked by pregnancy and for maintaining the metabolic balance during pregnancy. It has recently been shown that prolactin represses islet menin levels and stimulates beta cell proliferation during pregnancy in mice. It was found that the transgenic expression of the gene encoding menin in maternal beta cells inhibited islet expansion and led to the development of GDM phenotypes [35]. In human studies, inadequate compensatory insulin secretion in the face of increased insulin resistance has consistently been observed in patients with GDM [36–38]. In this regard, the risk alleles of rs7756992 and rs7754840 in *CDKAL1* and rs10811661 in *CDKN2A–CDKN2B* may play crucial roles in the pathogenesis of GDM through impaired compensatory insulin secretion by pancreatic beta cells.

Table 4 Associations between risk alleles and insulin resistance and insulin secretory function

Parameter	Gene	rs number	Allele (major/minor)	Homozygote of protective allele	Heterozygote	Homozygote of risk allele	*p* value (unadjusted)	*p* value (adjusted for age and BMI)
HOMA-IR	*CDKAL1*	rs7756992	G/A	3.18±2.17	2.91±1.73	2.81±1.62	0.048	0.07
		rs7754840	C/G	3.12±2.08	2.94±1.75	2.75±1.58	0.029	0.049
	CDKN2A/2B	rs564398	T/C	2.96±1.82	2.85±1.65	2.30±1.11	0.15	0.79
		rs1333040	T/C	2.95±1.51	2.95±2.07	2.66±1.38	0.29	0.98
		rs10757278	A/G	2.95±2.00	2.83±1.75	3.10±1.53	0.51	0.96
		rs10811661	T/C	3.14±2.58	2.87±1.64	2.88±1.49	0.26	0.15
	FTO	rs8050136	C/A	2.90±1.69	2.98±1.97	3.06±2.44	0.53	0.73
	HHEX	rs1111875	T/C	3.10±1.75	2.81±1.86	2.72±1.41	0.017	0.026
		rs5015480	T/C	2.98±1.90	2.80±1.55	2.96±1.63	0.29	0.12
		rs7923837	A/G	2.93±1.75	2.88±1.83	2.92±1.46	0.80	0.42
	IGF2BP2	rs4402960	G/T	2.94±1.64	2.97±1.93	2.60±1.51	0.23	0.35
	SLC30A8	rs13266634	C/T	2.95±1.46	3.02±2.05	2.78±1.53	0.17	0.05
	TCF7L2	rs7903146	C/T	2.93±1.77	2.74±1.75	3.57±3.17	0.56	0.63
		rs12255372	G/T	2.92±1.77	2.62±1.36	NA	0.66	0.44
	KCNJ11	rs5215	A/G	2.94±1.56	3.00±2.04	2.65±1.25	0.25	0.59
		rs5219	G/A	2.93±1.57	2.96±2.02	2.65±1.24	0.23	0.53
	PPARG	rs3856806	C/T	3.27±2.33	2.91±1.74	2.90±1.75	0.50	0.84
		rs1801282	C/G	2.52	2.76±1.36	2.93±1.81	0.41	0.37
AUC of insulin during 100 g OGTT at the time of diagnosis of GDM (pmol l^{-1} ×h)	*CDKAL1*	rs7756992	G/A	1,573±956	1,350±767	1,287±756	0.0012	0.0002
		rs7754840	C/G	1,555±926	1,362±774	1,248±743	0.0001	0.00004
	CDKN2A/2B	rs564398	T/C	1,355±800	1,406±828	1,222±585	0.83	0.28
		rs1333040	T/C	1,391±797	1,342±822	1,333±738	0.40	0.77
		rs10757278	A/G	1,380±736	1,344±801	1,378±924	0.93	0.33
		rs10811661	T/C	1,530±991	1,380±782	1,275±727	0.002	0.020
	FTO	rs8050136	C/A	1,317±772	1,452±788	2,000±1,745	0.002	0.006
	HHEX	rs1111875	T/C	1,530±897	1,286±732	1,106±600	0.0000001	0.0000002
		rs5015480	T/C	1,458±846	1,230±718	1,158±631	0.00005	0.0002
		rs7923837	A/G	1,423±833	1,289±771	1,234±652	0.013	0.006
	IGF2BP2	rs4402960	G/T	1,378±826	1,346±771	1,373±819	0.78	0.34
	SLC30A8	rs13266634	C/T	1,419±758	1,360±883	1,337±728	0.36	0.33
	TCF7L2	rs7903146	C/T	1,372±811	1,255±684	984±496	0.21	0.08
		rs12255372	G/T	1,361±798	1,361±798	NA	0.39	0.69
	KCNJ11	rs5215	A/G	1,378±786	1,354±815	1,347±806	0.67	0.60
		rs5219	G/A	1,383±795	1,351±814	1,350±799	0.64	0.55
	PPARG	rs3856806	C/T	1,270±671	1,369±845	1,364±791	0.78	0.81
		rs1801282	C/G	1,701	1,291±626	1,368±816	0.52	0.89

Data are presented as mean±SD
p values were not corrected for multiple comparisons
NA, not available—no participants homozygous for the risk allele

CDKAL1 is expressed in human pancreatic islets and shows considerable homology with *CDK5RAP1*, a well-known inhibitor of cyclin-dependent kinase 5 (CDK5) activation [23]. CDK5 has been suggested to downregulate insulin expression through the formation of p35/CDK5 complexes [39, 40]. In addition, CDK5 transduces glucose toxicity signals in pancreatic beta cells [39].

The 5′ sequence upstream of rs10811661 contains *CDKN2B* and *CDKN2A* (encoding $p15^{INK4b}$ and $p16^{INK4a}$, respectively) [23]. $p16^{INK4a}$ is known to inhibit CDK4, a powerful regulator of pancreatic beta cell replication [41–44]. *CDKN2B* overexpression was found to be related to islet hypoplasia and diabetes mellitus in murine models [45]. Furthermore, both *CDKN2A* and *CDKN2B* are expressed at high levels in pancreatic islets [23]. It was reported that the SNPs in this region showed a stronger signal as a haplotype [23], but we could not find such a trend in our study (ESM Tables 5 and 6)

In our study, the association of *TCF7L2* with GDM was not as strong as that reported by the Scandinavian study [17]. This discrepancy may be explained by the difference in the frequency of the rs7903146 risk allele between the

two studies (risk allele frequency in the control group was 0.238 vs 0.025, respectively), even though the effect size was similar (OR 1.49, 95% CI 1.28–1.75, $p=4.9\times10^{-7}$ vs OR 1.58, 95% CI 1.03–2.43, $p=0.038$, respectively).

Although *HHEX* was not robustly associated with GDM, it was strongly associated with insulin secretory capacity. Interestingly, the protective allele of rs1111875 in *HHEX* was very modestly associated with increased insulin resistance (adjusted $p=0.026$) but very strongly associated with increased insulin secretory capacity (adjusted $p=0.0000002$). This finding suggests that rs1111875 may be more likely to be one of the genetic factors involved in beta cell compensatory insulin secretion in the face of insulin resistance induced by pregnancy. It is known that *HHEX* is expressed at high levels in the fetal and adult pancreas [23] and is crucial for the development of the ventral pancreas [46]. In Europids [20–23], rs1111875 is found within an extended region of linkage disequilibrium that contains not only *HHEX* but also *KIF11* and *IDE*. Therefore, the influence of *KIF11* and/or *IDE* cannot be ignored.

Although *FTO* did not show any significant association with GDM or insulin resistance, the risk allele of rs8050136 in the *FTO* gene was found to be associated with an increased insulin AUC (age- and BMI-adjusted $p=0.006$). In addition, the pre-pregnancy BMI did not differ according to the rs8050136 genotype (data not shown). We suggest that rs8050136 does not increase the risk of GDM but may afford protective by increasing insulin secretory capacity, at least in the Korean population.

We found that the risk allele frequencies of the SNPs in patients with GDM did not differ from those in patients with type 2 diabetes in the Korean population (Table 3). In this regard, some genetic factors (especially, rs7756992 and rs7754840 in *CDKAL1* and rs10811661 in *CDKN2A–CDKN2B*) are associated with GDM in particular, as well as type 2 diabetes in general.

We recently confirmed that these SNPs were associated with the risk of type 2 diabetes in Koreans, showing similar effect sizes to those in Europeans, although the risk allele frequencies of most of these SNPs were different between populations [25]. The effect sizes of rs7754840 and rs10811661 were slightly greater in Korean women with GDM than in Europeans with type 2 diabetes, although the effects were in the same direction.

This study is subject to certain limitations. We compared the genotype frequencies in GDM patients with those in non-pregnant, non-diabetic controls. The results of the current study may therefore be regarded as a comparison of genotype frequency among gene pools of patients with GDM and individuals with a very low risk of type 2 diabetes. A control group consisting of age- and BMI-matched pregnant women without GDM may be more suitable for identification of the GDM susceptibility genes. It was not confirmed whether the non-diabetic female controls who were enrolled in this study had experienced pregnancy without GDM. However, the ascertainment bias should be minimal because the prevalence of GDM is estimated to be very low (2.2 cases per 100 pregnant women in Korea) [3]. Our study was underpowered to detect associations of some of the SNPs with GDM (see ESM Table 2), probably because of their low frequencies, which may have resulted in some associations being overlooked.

In conclusion, some of the type 2 diabetes-associated genetic variants discovered in the recent GWA studies are also associated with GDM in Koreans. Further studies need to be conducted to examine whether these risk variants predict the development of type 2 diabetes later in life.

Acknowledgements This study was supported by a grant from the Korea Health 21 R&D Project (Ministry of Health, Welfare and Family Affairs, Republic of Korea, no. 00-PJ3-PG6-GN07-001) and a Clinical Research Award from the Korean Diabetes Association.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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