

A Genome-Wide Association Study of Gestational Diabetes Mellitus in Korean Women

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Knowledge regarding the genetic risk loci for gestational diabetes mellitus (GDM) is still limited. In this study, we performed a two-stage genome-wide association analysis in Korean women. In the stage 1 genome scan, 468 women with GDM and 1,242 nondiabetic control women were compared using 2.19 million genotyped or imputed markers. We selected 11 loci for further genotyping in stage 2 samples of 931 case and 783 control subjects. The joint effect of stage 1 plus stage 2 studies was analyzed by meta-analysis. We also investigated the effect of known type 2 diabetes variants in GDM. Two loci known to be associated with type 2 diabetes had a genome-wide significant association with GDM in the joint analysis. rs7754840, a variant in *CDKAL1*, had the strongest association with GDM (odds ratio 1.518; $P = 6.65 \times 10^{-16}$). A variant near *MTNR1B*, rs10830962, was also significantly associated with the risk of GDM (1.454; $P = 2.49 \times 10^{-13}$). We found that there is an excess of association between known type 2 diabetes variants and GDM above what is expected under the null hypothesis. In conclusion, we have confirmed that genetic variants in *CDKAL1* and near *MTNR1B* are strongly associated with GDM in Korean women. There seems to be a shared genetic basis between GDM and type 2 diabetes. *Diabetes* 61:531–541, 2012

There has been a marked increase in our understanding of the genetic predisposition to type 2 diabetes as a result of the technical advances in array-based genotyping and the knowledge derived from the Human Genome Project (1). Recent genome-wide association (GWA) studies and meta-analyses, including the Diabetes Genetics Replication and Meta-analysis+ (DIAGRAM+) Study (2), the Meta-analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) Study (3), and the recent GWA studies of type 2 diabetes in Asians (4,5), enlisted up to 41 genetic risk loci for type 2 diabetes or glycemic traits (2,3,6–10). However, these loci

explain only a limited part of the expected heritability of type 2 diabetes (11). A complementary approach to improve our insight into the genetics of diabetes might involve the identification of genetic risk loci in a different subtype of diabetes, such as gestational diabetes mellitus (GDM). This approach could enable us to compare the genetic risk factors between type 2 diabetes and GDM and relate the similarities and dissimilarities to the pathophysiology of the two closely related diseases.

GDM is defined as abnormal glucose tolerance first recognized during pregnancy (12). The estimated prevalence of GDM varies according to ethnicity (13), and in Korean women, 2–5 of 100 pregnancies are complicated by GDM (14). During pregnancy, women are faced with increased adiposity and increased insulin resistance. The insulin resistance that develops during pregnancy is explained in part by the increased production of human placental lactogen, estrogen, and prolactin (15–17). Those who have limited β -cell capacity for the compensation of insulin resistance are likely to develop GDM (18). Women with GDM are assumed to have decreased β -cell insulin secretory function similar to type 2 diabetes (18). After parturition, nearly one-half of these women progress to type 2 diabetes within 5 years (19–21). Therefore, GDM is often regarded as a herald of type 2 diabetes in later life.

Based on these findings, it can be hypothesized that GDM and type 2 diabetes share a common genetic background. We have previously reported that some of the genetic variants that are strongly associated with type 2 diabetes are similarly associated with GDM risk (22). However, genetic knowledge in the context of GDM is still limited, and systematic approaches such as GWA studies have not been applied to GDM. Thus, it is not known whether there are genetic risk loci specific to GDM. In this study, we performed a GWA study to investigate genetic risk factors for GDM in Korean women and we also compared the genetic basis of GDM and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Stage 1 genome scan study subjects. A total of 468 women with GDM and 1,242 nondiabetic control women were recruited for the stage 1 genome scan. The GDM group was selected from a hospital-based cohort enrolled between January 1996 and February 2003 from Cheil General Hospital. A 50-g 1-h glucose challenge test was performed during 24–28 weeks' gestation in order to screen for GDM, and a glucose level of ≥ 7.2 mmol/L was considered positive and warranted a diagnostic 100-g oral glucose tolerance test. Glucose and insulin concentrations were measured at 0, 1, 2, and 3 h of the glucose challenge. GDM was diagnosed according to the criteria of the Third International Workshop Conference on GDM (12). The thresholds for the diagnosis of GDM were as follows: fasting ≥ 5.8 mmol/L, 1 h ≥ 10.6 mmol/L, 2 h ≥ 9.2 mmol/L, and 3 h ≥ 8.1 mmol/L. The clinical characteristics of stage 1 subjects are summarized in Table 1. The mean gestational age at GDM diagnosis was 27.9 ± 2.9 weeks.

Nondiabetic control subjects were selected from two population-based cohort studies, the rural Ansung and the urban Ansan cohorts. The two cohorts comprised the Korean Genome Epidemiology Study and included 5,018 and

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TABLE 1
Clinical characteristics of the study participants in the GDM GWA analysis

	Stage 1 genome scan		Stage 2 follow-up	
	GDM	Nondiabetic control subjects	GDM	Nondiabetic control subjects
<i>n</i>	468	1,242	931	783
Age (years)	31.5 ± 4.0	59.1 ± 5.6	32.5 ± 4.0	66.1 ± 7.5
BMI (kg/m ²)	23.3 ± 3.2	24.6 ± 3.2	25.0 ± 4.7	23.9 ± 3.2
Systolic blood pressure (mmHg)	116 ± 13	122 ± 19	114 ± 13	132 ± 19
Diastolic blood pressure (mmHg)	70 ± 9	77 ± 11	69 ± 9	82 ± 11
Fasting plasma glucose (mmol/L)	5.4 ± 1.1	4.4 ± 0.4	5.2 ± 1.0	4.9 ± 0.4
HbA _{1c} (%)	NA	5.5 ± 0.3	NA	5.5 ± 0.3

Data are means ± SD. Data for women with GDM were measured during the diagnostic 100-g oral glucose tolerance test. NA, not available.

5,020 subjects, respectively (23). Only women were eligible for enrollment. From the Korean Genome Epidemiology Study subjects, we included 1,242 nondiabetic women according to the following criteria: age ≥50 years, no previous history of type 2 diabetes, no first-degree relatives with type 2 diabetes, fasting plasma glucose level <5.6 mmol/L, and HbA_{1c} <6.0%. Information on parity and glucose tolerance status during pregnancy was not available for the control group.

Stage 2 follow-up study subjects. A total of 931 women with GDM and 783 nondiabetic control women were included in the stage 2 follow-up study (Table 1). The GDM group consisted of two subgroups: 1) 426 women with GDM recruited from the Cheil General Hospital between January 1996 and February 2003 and 2) 505 women with GDM recruited as an independent cohort from the same hospital between March 2003 and February 2008. The diagnostic criteria of GDM in stage 2 were the same as those in stage 1. As control subjects, we enrolled nondiabetic women from four different institutes: 1) Seoul National University Hospital (*n* = 162), 2) Seoul National University Bundang Hospital (*n* = 96), 3) Kyungpook National University Hospital (*n* = 201), and 4) Korea National Institute of Health (from the Health T2D Study) (*n* = 324). The criteria for selecting control subjects were as follows: age ≥60 years, no previous history of type 2 diabetes, no first-degree relatives with type 2 diabetes, fasting plasma glucose level <5.6 mmol/L, and HbA_{1c} <6.0%.

The institutional review board of the Clinical Research Institute at Seoul National University Hospital approved the study protocol (institutional review board no. H-0412-138-017), and written informed consent was obtained from each subject. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Biochemical measurements. The anthropometric and metabolic phenotypes of the women with GDM were measured at the time of the 100-g oral glucose tolerance test. Plasma glucose concentration was determined by YSI 2300 STAT (YSI, Yellow Springs, OH) using the glucose oxidase method. The insulin concentration was measured using a human-specific radioimmunoassay kit (Linco Research, St. Charles, MO). The homeostasis model assessment (HOMA) was used to estimate insulin resistance (HOMA-IR) and β-cell function (HOMA-B) with paired fasting glucose and insulin concentrations (24).

Genotyping. We extracted genomic DNA from peripheral leukocytes. Genotyping for the stage 1 genome scan was performed using the Affymetrix Genome-Wide Human Single Nucleotide Polymorphism (SNP) Array 5.0, and genotypes were called using the Birdseed version 2.0 algorithm. Extensive quality-control protocols were applied. Markers with significant deviation from Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-6}$), genotype call rate < 97%, and minor allele frequency < 0.01 were excluded. The genotyping for the case and control subjects was conducted in the same laboratory but at a different time. To exclude the potential batch effect, we also excluded markers with significant differences ($P < 1.0 \times 10^{-3}$) in missingness between case and control subjects. Finally, 321,654 markers remained for analysis.

The genotype concordance rate in 10 replicated samples was >99.7% in the stage 1 control group. We excluded one sample with sex inconsistency and five samples with cryptic first-degree relatedness assessed by identity-by-descent analysis, resulting in 468 case and 1,242 control subjects. The overall genomic inflation factor (λ) of the stage 1 genome scan was 1.042, which suggests a low level of population stratification. A quantile-quantile (QQ) plot indicated that the observed distribution of the *P* values of the logistic regression analysis were similar to that expected under the null hypothesis, except for the deviation in the extremely low *P* values (Fig. 1A). These findings indicate that our study has good-quality data and that statistically significant signals are enriched.

Genotype cluster plots were manually inspected for suggestive loci in the stage 1 genome scan. For stage 2 follow-up genotyping, the TaqMan assay (Applied Biosystems, Carlsbad, CA) was used, as previously described (25), except for the 324 subjects from the Health T2D Study, where genotyping was done using the

Affymetrix Genome-Wide Human SNP Array 6.0 (26). Overall, the TaqMan genotyping success rate was 99.3%, and the concordance rate based on 15 blind duplicate comparisons was 100% in the stage 2 study. For the SNP genotyping in Health T2D Study, standard quality-control measures were applied and imputation was conducted using IMPUTE version 1.0 (<http://mathgen.stats.ox.ac.uk/impute/impute.html>) (27).

Statistical analyses. Most of the association testing was performed using PLINK version 1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) (28), and genotype imputation was conducted using MACH software (<http://www.sph.umich.edu/csg/abecasis/mach/>) (29). The genotype data that passed our quality-control criteria and had minor allele frequency >5% were used as input data for genotype imputation. The HapMap-phased genotype information of Japanese in Tokyo, Japan, and Han Chinese in Beijing, China (build 36 release 21) was used as a reference. After postimputation quality control using $r^2 \geq 0.3$ (squared correlation between imputed and true genotypes), a total of 2,188,613 SNPs were available for analysis. The association between genotypes and GDM risk was assessed by logistic regression analysis in an additive model. For imputed markers, an association test was performed with dosage data using mach2dat software (<http://www.sph.umich.edu/csg/abecasis/mach/>) (29).

Markers with suggestive genome-wide significance ($P < 2.0 \times 10^{-5}$) or markers near known type 2 diabetes risk loci with moderate significance ($P < 1.0 \times 10^{-3}$) in the stage 1 genome scan were further genotyped in stage 2 subjects. The significance threshold for the stage 2 follow-up study was $P < 0.05$. A joint analysis of stage 1 plus stage 2 results was performed with METAL (<http://www.sph.umich.edu/csg/abecasis/metal/>), using an inverse-variance method assuming fixed effects (30). An additional association analysis was performed using the EMMAX (<http://genetics.cs.ucla.edu/emmax/>), a variance component association method, to account for possible hidden relatedness and population stratification in the stage 1 genome scan (31). The association between genetic variants and fasting glucose, fasting insulin concentration, HOMA-IR, and HOMA-B were analyzed using linear regression in case and control subjects separately, assuming an additive genetic model. None of the women with GDM or the control subjects was using antidiabetes medications, including insulin, at the time of glucose and insulin measurement. The fasting insulin concentration, HOMA-IR, and HOMA-B was log₁₀ transformed before analysis. The linear regression analysis was controlled for age in women with GDM and for age and BMI in control women.

The statistical power of the joint stage 1 plus stage 2 analysis was calculated using the CaTS power calculator (<http://www.sph.umich.edu/csg/abecasis/cats/>) (32). The QQ plot, which shows the distribution of the observed *P* values of the logistic regression analysis against the expected distribution under the null hypothesis, was generated using the R statistical package (<http://www.r-project.org>). The Manhattan plot, which depicts the negative log₁₀ of *P* values derived from the logistic regression analysis, was plotted against the chromosomal position using the R statistical package. The dense regional association results of stage 1, stage 2, and the joint analysis of stage 1 plus stage 2 were plotted using LocusZoom software (<http://csg.sph.umich.edu/locuszoom/>) (33). To compare the effect size of known type 2 diabetes variants in GDM and type 2 diabetes, the β-coefficient of the logistic regression analysis derived from our stage 1 genome scan and from the stage 1 meta-GWA analysis of the Asian Genetic Epidemiology Network (AGEN) type 2 diabetes report (26) was plotted.

RESULTS

Stage 1 genome scan. In the stage 1 genome scan, logistic regression analysis using an additive genetic model was used to test for the association between the genotypes and GDM. The negative log₁₀ of the *P* values from the

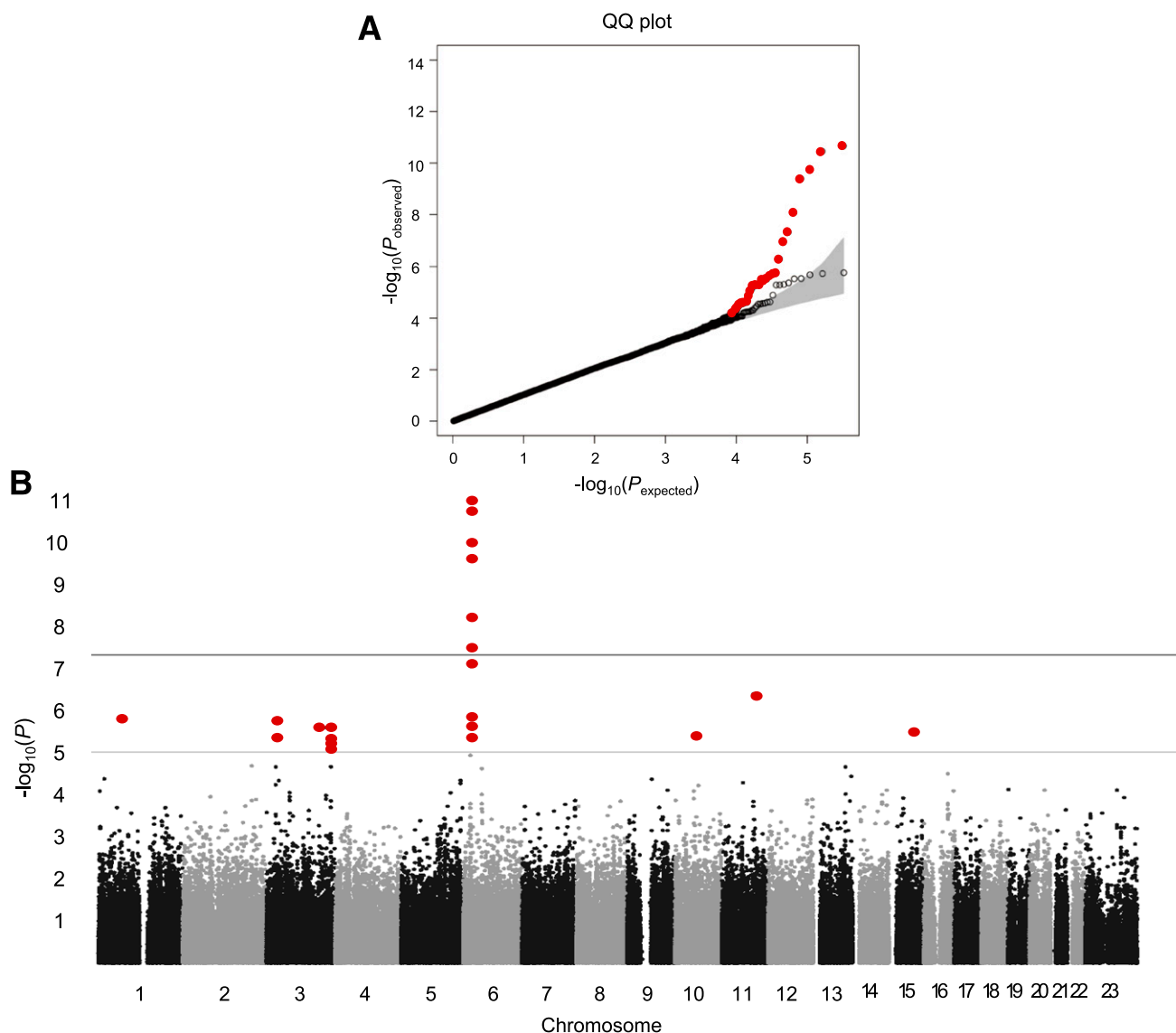


FIG. 1. Stage 1 genome scan results. **A:** QQ plot showing the distribution of the observed P values from the logistic regression analysis for the stage 1 genome scan against the expected distribution under the null hypothesis. The gray zone indicates the 95% CI. Colored circle, distribution of excess association signals driven by the known type 2 diabetes variants (listed in Table 4) and markers in LD ($r^2 > 0.8$) with them. \circ , distribution after excluding the known type 2 diabetes variants. **B:** Manhattan plot depicting the significance of all the association results of the stage 1 genome scan. SNP locations are plotted on the x-axis according to their chromosomal position. The negative \log_{10} of P values derived from the logistic regression analysis under the additive model are plotted on the y-axis. (A high-quality color representation of this figure is available in the online issue.)

association test were plotted against their genomic position in Fig. 1B. The association test results for SNPs with $P < 0.0001$ in the stage 1 genome scan are listed in Supplementary Table 1. A total of nine independent (pairwise linkage disequilibrium [LD] $r^2 < 0.5$) SNPs were suggestive of an association according to our predefined arbitrary threshold of $P < 2.0 \times 10^{-5}$. Among these, variants in *CDKAL1* (rs7754840) and near *MTNR1B* (rs10830962) showed the strongest association with GDM risk. We added two additional variants (rs10757261 near *CDKN2A/2B* and rs10882066 in *IDE*) from the type 2 diabetes risk loci, which had suggestive P values in our stage 1 results. Among the 11 SNPs, 4 were substituted to imputed SNPs (rs6499500, rs12715106, rs9395950, and rs187230), which had more significant P values and had strong LD ($r^2 > 0.8$) with the original SNPs. To eliminate hidden population stratification and cryptic relatedness, the EMMAX, a variance component approach accounting for hidden sample structure,

was used to test the association between genetic variants and GDM. The P values of the EMMAX are listed in Supplementary Table 1. The results of the EMMAX were similar to those of the original logistic regression analysis. The Pearson correlation coefficient for the \log_{10} of the P values of both analyses was 0.921 ($P < 1.51 \times 10^{-288}$). This implies that population stratification was minimal in our study samples.

Stage 2 follow-up and joint analysis. In stage 2 follow-up, we genotyped 11 SNPs in 931 case and 783 control subjects. The results of the stage 2 association test are shown in Table 2. Among these, rs7754840 in *CDKAL1* (odds ratio [OR] 1.396 [95% CI 1.222–1.594]; $P = 2.90 \times 10^{-7}$), rs10830962 near *MTNR1B* (1.442 [1.259–1.651]; $P = 6.95 \times 10^{-8}$), rs1470579 in *IGF2BP2* (1.236 [1.068–1.430]; $P = 0.0042$), and rs10882066 in *IDE* (1.203 [1.013–1.428]; $P = 0.035$) were significantly associated with GDM in the stage 2 samples. None of the other SNPs showed evidence

TABLE 2
Association between SNPs and GDM in stage 1, stage 2, and joint stage 1 plus stage 2 analysis

SNP	CHR	Risk allele	Nearest gene	Stage 1 genome scan				Stage 2 follow-up				Stage 1 plus stage 2			
				RAF (GDM)	RAF (control)	OR (95% CI)	P	RAF (GDM)	RAF (control)	OR (95% CI)	P	n (GDM subjects/control subjects) = 931/783	OR (95% CI)	P	n (GDM subjects/control subjects) = 1,399/2,025
				0.575	0.445	1.707 (1.459–1.997)	2.15×10^{-11}	0.556	0.467	1.396 (1.222–1.594)	2.90×10^{-7}	1.518 (1.372–1.680)	6.65×10^{-16}		
rs7754840	6	C	<i>CDKALI</i>	0.575	0.445	1.707 (1.459–1.997)	2.15×10^{-11}	0.556	0.467	1.396 (1.222–1.594)	2.90×10^{-7}	1.518 (1.372–1.680)	6.65×10^{-16}	709	
rs10830962	11	G	<i>MTNR1B</i>	0.529	0.430	1.469 (1.266–1.705)	5.02×10^{-7}	0.537	0.444	1.442 (1.259–1.651)	6.95×10^{-8}	1.454 (1.315–1.608)	2.49×10^{-13}	836	
rs1470579	3	C	<i>IGF2BP2</i>	0.377	0.293	1.465 (1.242–1.707)	3.03×10^{-6}	0.337	0.291	1.236 (1.068–1.430)	0.0042	1.332 (1.197–1.484)	1.67×10^{-7}	1,707	
rs6499500*	16	C	<i>FTSJDI/CALB2</i>	0.387	0.304	1.473 (1.254–1.730)	2.68×10^{-6}	0.328	0.305	1.110 (0.962–1.281)	0.148	1.258 (1.131–1.400)	2.57×10^{-5}	2,603	
rs12898654	15	G	<i>LBXCOR1</i>	0.210	0.145	1.592 (1.306–1.941)	4.37×10^{-6}	0.170	0.153	1.127 (0.937–1.355)	0.205	1.323 (1.156–1.515)	4.74×10^{-5}	3,036	
rs9395950*	6	A	<i>TINAG</i>	0.770	0.693	1.485 (1.245–1.771)	1.03×10^{-5}	0.723	0.703	1.099 (0.950–1.272)	0.195	1.242 (1.110–1.390)	1.59×10^{-4}	2,853	
rs10882066	10	G	<i>IDE</i>	0.215	0.167	1.376 (1.136–1.667)	0.0011	0.210	0.181	1.203 (1.013–1.428)	0.035	1.277 (1.124–1.451)	1.81×10^{-4}	3,527	
rs10757261	9	G	<i>CDKN2A/2B</i>	0.732	0.671	1.330 (1.128–1.568)	0.0007	0.699	0.680	1.095 (0.946–1.267)	0.221	1.193 (1.070–1.331)	0.0015	4,157	
rs7513574	1	G	<i>CACHD</i>	0.352	0.274	1.482 (1.262–1.740)	1.75×10^{-5}	0.277	0.283	0.973 (0.838–1.129)	0.711	1.182 (1.060–1.319)	0.0027	5,214	
rs12715106*	3	G	<i>LRRC3B</i>	0.764	0.683	1.536 (1.285–1.836)	2.57×10^{-6}	0.724	0.726	0.988 (0.847–1.152)	0.876	1.192 (1.061–1.340)	0.0031	4,277	
rs187230*	3	C	<i>PLDI</i>	0.731	0.655	1.439 (1.216–1.703)	2.33×10^{-5}	0.669	0.681	0.946 (0.816–1.096)	0.471	1.135 (1.016–1.268)	0.0256	7,906	

Data are OR for the risk allele (95% CI), unless otherwise indicated. The risk allele is indexed to the positive strand of the National Center for Biotechnology Information build 36. The nearest gene is defined as the gene closest to the SNP or within the boundary of the 100-kb window from the SNP. RAF (GDM) and RAF (control) refer to the risk allele frequencies in women with GDM and nondiabetic control women, respectively. P values for stage 1 and stage 2 were calculated using logistic regression under an additive genetic model. P values for the joint stage 1 plus stage 2 were calculated by METAL using the inverse-variance method under a fixed-effects model. CHR, chromosome. *These were imputed SNPs using MACH. The number of samples required to achieve 80% power ($\alpha = 5 \times 10^{-8}$) was calculated based on the OR in the joint stage 1 plus stage 2 analysis, the risk allele frequency in stage 1 control subjects, and a GDM prevalence of 5%.

of an association. In the joint analysis of stage 1 plus stage 2 results (Table 2), rs7754840 in *CDKAL1* (1.518 [1.372–1.680]; $P = 6.65 \times 10^{-16}$) and rs10830962 near *MTNR1B* (1.454 [1.315–1.608]; $P = 2.49 \times 10^{-13}$) reached genome-wide significance for an association with GDM. One additional variant, rs1470579 in *IGF2BP2* (1.332 [1.197–1.484]; $P = 1.67 \times 10^{-7}$), showed a near genome-wide

significant association with GDM. The regional association plot of SNPs near *CDKAL1* and *MTNR1B*, including those that have been imputed, are depicted in Fig. 2.

Association with glucose and insulin-related traits. To obtain additional insight into the role of these two variants, we performed an association analysis between these variants and quantitative traits of fasting glucose, fasting

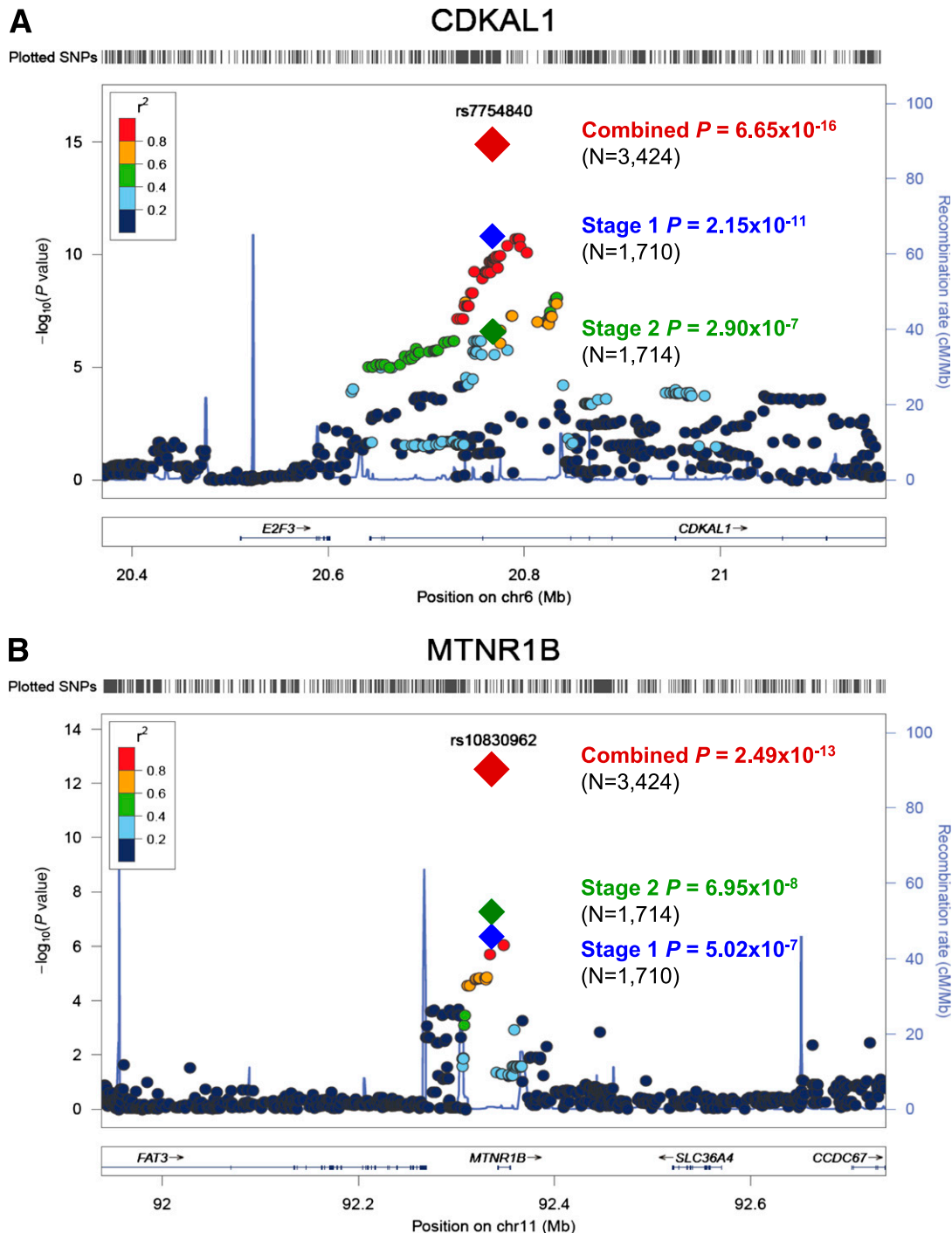


FIG. 2. Dense regional association plot near *CDKAL1* (A) and *MTNR1B* (B). The hash marks above the panel represent the position of each SNP that was genotyped or imputed. The negative \log_{10} of P values from logistic regression are shown in the panel. The blue diamond indicates the SNP with the most significant association in the stage 1 genome scan. The green and red diamonds represent the results of the SNP in stage 2 and joint stage 1 plus stage 2 analysis, respectively. Their corresponding P values are indicated on the right. Estimated recombination rates are plotted to reflect recombination hot spots. The SNPs in LD with the most significant SNP are color coded to represent their strength of LD. The locations of genes, exons and introns are shown in the lower panel (taken from the Human Genome hg18 build). (A high-quality color representation of this figure is available in the online issue.)

insulin, HOMA-IR, and HOMA-B in women with GDM and control subjects, separately (Table 3). The rs7754840 C allele of *CDKAL1*, which was the risk variant of GDM, was significantly associated with decreased fasting insulin concentration ($\beta = -0.026$; $P = 0.00051$) and decreased HOMA-B ($\beta = -0.034$; $P = 0.00085$) in women with GDM. The rs10830962 G allele near *MTNR1B* was nominally associated with decreased fasting insulin concentrations ($\beta = -0.018$; $P = 0.029$) in women with GDM. This variant was also marginally associated with increased fasting glucose concentrations ($\beta = 0.025$; $P = 0.041$) in control subjects.

Comparison of genetic risk loci between GDM and type 2 diabetes. Among 41 known type 2 diabetes loci, we were able to examine the association signals for 34 loci that were directly genotyped or imputed (Table 4). There was an excess of small P values compared with the expected distribution under the null hypothesis (Fig. 3). When these known type 2 diabetes risk variants and markers in LD ($r^2 > 0.8$) were excluded, the QQ plot of our stage 1 genome scan was similar to that expected under the null hypothesis (Fig. 1A).

We compared the β -coefficient in the logistic regression analysis of known type 2 diabetes variants in GDM (our stage 1 genome scan) and type 2 diabetes (AGEN type 2 diabetes stage 1 meta-GWA results [26]) (Fig. 4 and Supplementary Table 2). There was a significant positive correlation between the β -coefficients of GDM and type 2 diabetes (Pearson correlation coefficient 0.442; $P = 0.0062$). However, some variants, such as rs10830963 of *MTNR1B* and rs11708067 of *ADCY5*, showed considerable differences in effect size between GDM and type 2 diabetes.

Finally, we examined the association results for rs7754840 of *CDKAL1* and rs10830962 of *MTNR1B* in the stage 1 meta-GWA study of the AGEN type 2 diabetes report (26). The C allele of rs7754840 was significantly associated with an increased risk of type 2 diabetes ($n = 18,732$; OR 1.20 [95% CI 1.14–1.25]; $P = 9.63 \times 10^{-15}$). However, the G allele of rs10830962 was not associated with type 2 diabetes risk ($n = 10,754$; 1.040 [0.98–1.11]; $P = 0.209$).

DISCUSSION

The main finding of this study is that variants in *CDKAL1* and near *MTNR1B* are associated with GDM at a genome-wide significance level ($P < 5.0 \times 10^{-8}$). Our findings are confirmatory of previous candidate gene studies of GDM (22,34,35). Previously, we selected 18 SNPs, known to be associated with the risk of type 2 diabetes, for association testing in GDM (22). Among them, two SNPs (rs7756992 and rs7754840) in *CDKAL1* were associated with GDM risk at a genome-wide significance level, and one SNP (rs10811661) near *CDKN2A/2B* also was strongly associated with GDM. Another study in Europeans tested 11 known SNPs of type 2 diabetes and found a modest association between GDM and *TCF7L2*, *CDKAL1*, and *TCF2* variants (34). Recently, Kim et al. (35) reported that two SNPs in *MTNR1B* (rs1387153 and rs10830963) were strongly associated with GDM in Korean women. The study by Kim et al. was led by one of our investigators but was performed independently of this study, and 505 subjects with GDM in that study overlapped with our stage 2 follow-up subjects. However, only a limited number of genetic variants known to be associated with type 2 diabetes have been tested thus far, and a systematic approach such as GWA analysis has not been applied to GDM until now. To the best of our knowledge, this is the first study to

investigate the genetic association of GDM using dense SNP markers across the whole genome.

Variants in *CDKAL1* are known to be strongly associated with type 2 diabetes (8–10,36,37). In our previous studies, the variant rs7754840 in *CDKAL1* was significantly associated with GDM risk in 1,501 Koreans (22) as well as type 2 diabetes in 6,719 Asians including Koreans (38). The precise mechanism by which this variant confers type 2 diabetes and GDM risk is not yet clearly understood. *CDKAL1* (cyclin-dependent kinase 5 [CDK5] regulatory subunit-associated protein 1-like 1) has been suggested to interact with CDK5 because it has homology with CDK5RAP1, a known inhibitor of CDK5 (39). CDK5 is expressed in pancreatic β -cells, and it has recently been reported to promote β -cell survival (40). Pregnancy is a state in which increased insulin resistance results in stress that adversely affects β -cell survival (41). Therefore, it may be postulated that variants in *CDKAL1* might alter the function of CDK5 in β -cell compensation during pregnancy. The rs7754840 C variant of *CDKAL1* was significantly associated with decreased insulin concentration and HOMA-B in our subjects with GDM, which implies compromised β -cell compensation. It also should be noted that variants in *CDKAL1* are associated with decreased birth weight, which could be explained by reduced fetal insulin secretion (42).

Variants in *MTNR1B* were first identified as genetic risk factors strongly associated with elevated fasting glucose (43–45). Although it also was associated with type 2 diabetes risk in Europeans, the strength of association was rather weak compared with its association with fasting glucose (44,45). It is interesting that the rs10830962 variant in *MTNR1B* was the second most strongly associated loci in GDM but was not associated with type 2 diabetes risk in a large-scale meta-GWA study of East Asians (AGEN type 2 diabetes meta-GWA study [26]). The effect size of the *MTNR1B* rs10830962 variant was considerably different in our GDM GWA study ($\beta = 0.374$) compared with the AGEN type 2 diabetes meta-GWA study ($\beta = 0.040$). The risk allele of rs10830962 G was enriched in women with GDM (risk allele frequency 0.529 and 0.537 in stage 1 and stage 2 subjects, respectively) compared with AGEN type 2 diabetic subjects (risk allele frequency range for participating studies 0.40–0.46). It is not clear why this variant is more strongly associated with the risk of GDM compared with type 2 diabetes. However, it could be cautiously speculated that there might be differences in genetic susceptibility between type 2 diabetes and GDM conferred by the variants of *MTNR1B*. Additional investigations are required to confirm these findings.

It was recently revealed that *MTNR1B* is expressed in pancreatic β -cells (44). Melatonin is thought to inhibit insulin secretion from β -cells through the activation of *MTNR1B*, which is a G-protein-coupled receptor (46). The rs10830962 variant is in strong LD ($r^2 = 0.98$, in our study subjects) with rs10830963, which originally was reported to be associated with fasting glucose concentrations, insulin concentrations, and type 2 diabetes. It is interesting that the rs1083092 variant is located 4.36 kb upstream of *MTNR1B* and in the vicinity of the region where monomethylation of lysine 4 of histone 3 (H3K4Me1), known as an enhancer-associated histone mark, was enriched in the Encyclopedia of DNA Elements database (<http://genome.ucsc.edu/encode/>) (47). It would be of worth to study the functional role of this variant as well as to search for causative variants near this locus, especially in the promoter and/or enhancer region.

TABLE 3
Association of rs7754840 and rs10830962 with fasting glucose, fasting insulin, HOMA-IR, and HOMA-B in women with GDM and control subjects

	<i>n</i>	GDM						Nondiabetic control women						
		CC	CG	GG	β	SE	<i>P</i>	<i>n</i>	CC	CG	GG	β	SE	<i>P</i>
rs7754840 (CDKAL1)														
Fasting glucose (mmol/L)														
Stage 1	467	5.17 ± 0.80	5.21 ± 1.16	5.21 ± 1.10	-0.022	0.068	0.704	1,193	4.42 ± 0.35	4.43 ± 0.36	4.47 ± 0.37	-0.023	0.015	0.130
Stage 2	890	5.21 ± 1.16	5.08 ± 0.86	5.17 ± 0.92	0.033	0.044	0.458	736	4.91 ± 0.46	4.92 ± 0.4	4.91 ± 0.41	-0.005	0.022	0.817
Stage 1 plus stage 2	1,357				0.015	0.037	0.679	1,929				-0.017	0.012	0.169
log ₁₀ (fasting insulin [pmol/L])														
Stage 1	456	1.86 ± 0.19	1.85 ± 0.23	1.90 ± 0.22	-0.015	0.014	0.287	1,193	1.66 ± 0.34	1.67 ± 0.28	1.66 ± 0.27	0.0001	0.012	0.995
Stage 2	890	1.89 ± 0.21	1.93 ± 0.21	1.95 ± 0.19	-0.031	0.009	0.00083	470	1.70 ± 0.17	1.71 ± 0.16	1.74 ± 0.17	-0.010	0.010	0.314
Stage 1 plus stage 2	1,346				-0.026	0.008	0.00051	1,663				-0.006	0.008	0.445
log ₁₀ (HOMA-IR)														
Stage 1	456	0.38 ± 0.22	0.36 ± 0.26	0.42 ± 0.26	-0.015	0.016	0.358	1,193	0.11 ± 0.34	0.12 ± 0.28	0.12 ± 0.27	-0.002	0.012	0.861
Stage 2	886	0.41 ± 0.24	0.44 ± 0.24	0.46 ± 0.23	-0.030	0.011	0.0057	470	0.20 ± 0.18	0.21 ± 0.17	0.23 ± 0.18	-0.010	0.011	0.332
Stage 1 plus stage 2	1,342				-0.025	0.009	0.0048	1,663				-0.007	0.008	0.398
log ₁₀ (HOMA-B)														
Stage 1	455	2.15 ± 0.23	2.17 ± 0.33	2.20 ± 0.27	-0.021	0.019	0.275	1,191	2.19 ± 0.36	2.20 ± 0.32	2.17 ± 0.31	0.013	0.013	0.335
Stage 2	885	2.18 ± 0.27	2.25 ± 0.27	2.25 ± 0.25	-0.039	0.012	0.0012	470	2.04 ± 0.22	2.04 ± 0.19	2.08 ± 0.2	-0.009	0.013	0.481
Stage 1 plus stage 2	1,340				-0.034	0.010	0.00085	1,661				0.001	0.009	0.900
rs10830962 (MTNR1B)														
Fasting glucose (mmol/L)														
Stage 1	468	5.08 ± 0.95	5.18 ± 0.98	5.38 ± 1.25	-0.144	0.068	0.031	1,205	4.45 ± 0.37	4.45 ± 0.36	4.41 ± 0.37	0.020	0.015	0.163
Stage 2	891	5.19 ± 1.13	5.10 ± 0.92	5.19 ± 0.92	0.021	0.046	0.646	753	4.97 ± 0.44	4.91 ± 0.42	4.90 ± 0.38	0.034	0.022	0.115
Stage 1 plus stage 2	1,359				-0.028	0.038	0.405	1,958				0.025	0.012	0.041
log ₁₀ (fasting insulin [pmol/L])														
Stage 1	457	1.83 ± 0.24	1.86 ± 0.20	1.90 ± 0.21	-0.035	0.014	0.012	1,205	1.67 ± 0.30	1.66 ± 0.28	1.67 ± 0.28	0.001	0.011	0.917
Stage 2	891	1.91 ± 0.22	1.93 ± 0.19	1.92 ± 0.22	-0.009	0.010	0.377	479	1.73 ± 0.17	1.72 ± 0.16	1.71 ± 0.17	0.014	0.010	0.173
Stage 1 plus stage 2	1,348				-0.018	0.008	0.029	1,684				0.008	0.008	0.275
log ₁₀ (HOMA-IR)														
Stage 1	457	0.34 ± 0.26	0.38 ± 0.23	0.43 ± 0.24	-0.047	0.016	0.0039	1,205	0.12 ± 0.30	0.12 ± 0.29	0.12 ± 0.28	0.003	0.011	0.783
Stage 2	887	0.42 ± 0.25	0.44 ± 0.22	0.44 ± 0.25	-0.010	0.011	0.372	479	0.23 ± 0.18	0.21 ± 0.17	0.20 ± 0.18	0.018	0.011	0.090
Stage 1 plus stage 2	1,344				-0.022	0.009	0.017	1,684				0.011	0.008	0.150
log ₁₀ (HOMA-B)														
Stage 1	456	2.17 ± 0.30	2.17 ± 0.29	2.16 ± 0.29	0.005	0.019	0.776	1,203	2.19 ± 0.33	2.18 ± 0.31	2.21 ± 0.33	-0.012	0.013	0.354
Stage 2	886	2.20 ± 0.27	2.25 ± 0.26	2.21 ± 0.28	-0.010	0.013	0.411	479	2.04 ± 0.21	2.06 ± 0.20	2.05 ± 0.20	-0.003	0.012	0.830
Stage 1 plus stage 2	1,342				-0.006	0.010	0.595	1,682				-0.007	0.009	0.415

Associations were identified using linear regression analysis under an additive genetic model adjusted for age in women with GDM and age and BMI in nondiabetic control women. β , the regression coefficient is the per-allele effect size of the risk variant (rs7754840 C and rs10830962 G); *n*, number of subjects with nonmissing phenotypes. *P* values for joint stage 1 plus stage 2 were calculated by meta-analysis using the inverse-variance method under a fixed-effects model. *P* values < 0.05 in stage 1 plus stage 2 are indicated in boldface.

TABLE 4
Association of confirmed type 2 diabetes loci with GDM risk in stage 1 genome scan results

SNP	CHR	Nearest gene	Risk allele	Non-risk allele	RAF (GDM)	RAF (control)	OR (95% CI)	<i>P</i>	Type
rs10923931	1	<i>NOTCH2</i>	G	T	0.972	0.970	1.071 (0.685–1.673)	0.764	Typed
rs340874	1	<i>PROX1</i>	C	T	0.367	0.352	1.073 (0.913–1.206)	0.390	Imputed
rs11899863	2	<i>THADA</i>	—	—	—	—	—	—	Filtered
rs780094	2	<i>GCKR</i>	C	T	0.509	0.478	1.133 (0.974–1.318)	0.104	Typed
rs243021	2	<i>BCL11A</i>	A	G	0.665	0.661	1.019 (0.870–1.195)	0.819	Imputed
rs7578326	2	<i>IRS1</i>	G	A	0.181	0.178	1.033 (0.782–1.364)	0.821	Imputed
rs13081389	3	<i>PPARG</i>	G	A	0.042	0.041	1.025 (0.706–1.486)	0.898	Imputed
rs7612463	3	<i>UBE2E2</i>	C	A	0.833	0.805	1.211 (0.991–1.478)	0.061	Imputed
rs6795735	3	<i>ADAMTS9</i>	T	C	0.802	0.785	1.106 (0.920–1.330)	0.283	Typed
rs11708067	3	<i>ADCY5</i>	G	A	0.002	0.001	1.372 (0.09–20.996)	0.820	Imputed
rs1470579	3	<i>IGF2BP2</i>	C	A	0.377	0.293	1.465 (1.244–1.709)	3.03 × 10⁻⁶	Typed
rs1801214	4	<i>WFS1</i>	T	C	0.978	0.977	1.041 (0.627–1.724)	0.879	Imputed
rs4457053	5	<i>ZBED3</i>	—	—	—	—	—	—	Filtered
rs10440833	6	<i>CDKAL1</i>	A	T	0.561	0.439	1.722 (1.466–2.021)	4.36 × 10⁻¹¹	Imputed
rs2191349	7	<i>DGKB/TMEM195</i>	T	G	0.674	0.672	1.009 (0.856–1.190)	0.917	Imputed
rs849134	7	<i>JAZF1</i>	A	G	0.750	0.735	1.114 (0.909–1.366)	0.298	Imputed
rs4607517	7	<i>GCK</i>	A	G	0.214	0.204	1.062 (0.881–1.279)	0.530	Imputed
rs972283	7	<i>KLF14</i>	A	G	0.304	0.297	1.034 (0.877–1.219)	0.692	Imputed
rs896854	8	<i>TP53INP1</i>	C	T	0.700	0.693	1.034 (0.873–1.223)	0.700	Imputed
rs3802177	8	<i>SLC30A8</i>	G	A	0.625	0.575	1.248 (1.065–1.463)	0.0064	Imputed
rs17584499	9	<i>PTPRD</i>	—	—	—	—	—	—	Filtered
rs10965250	9	<i>CDKN2A/2B</i>	G	A	0.602	0.535	1.319 (1.130–1.540)	0.00042	Imputed
rs13292136	9	<i>CHCHD9</i>	T	C	0.108	0.100	1.096 (0.856–1.408)	0.467	Imputed
rs12779790	10	<i>CDC123/CAMK1D</i>	G	A	0.147	0.130	1.186 (0.943–1.492)	0.146	Imputed
rs5015480	10	<i>IDE/HHEX</i>	C	T	0.220	0.188	1.219 (1.012–1.468)	0.037	Typed
rs7903146	10	<i>TCF7L2</i>	T	C	0.041	0.027	1.499 (0.998–2.247)	0.051	Imputed
rs231362	11	<i>KCNQ1</i>	G	A	0.877	0.871	1.070 (0.839–1.363)	0.585	Imputed
rs163184	11	<i>KCNQ1</i>	G	T	0.451	0.414	1.188 (1.009–1.397)	0.038	Imputed
rs5215	11	<i>KCNJ11</i>	C	T	0.412	0.382	1.132 (0.972–1.319)	0.115	Typed
rs1552224	11	<i>CENTD2</i>	A	C	0.950	0.920	1.692 (1.206–2.375)	0.0024	Imputed
rs1387153	11	<i>MTNR1B</i>	T	C	0.511	0.433	1.401 (1.196–1.637)	2.69 × 10⁻⁵	Imputed
rs1531343	12	<i>HMGA2</i>	G	C	0.887	0.887	1.003 (0.793–1.269)	0.982	Typed
rs4760790	12	<i>TSPAN8/LGR5</i>	G	A	0.760	0.743	1.098 (0.919–1.313)	0.305	Imputed
rs7957197	12	<i>HNF1A</i>	—	—	—	—	—	—	Filtered
rs7172432	15	<i>C2CD4A/4B</i>	A	G	0.550	0.542	1.035 (0.886–1.208)	0.664	Imputed
rs11634397	15	<i>ZFAND6</i>	G	A	0.094	0.090	1.089 (0.779–1.524)	0.616	Imputed
rs8042680	15	<i>PRC1</i>	—	—	—	—	—	—	Filtered
rs11642841	16	<i>FTO</i>	A	C	0.039	0.038	1.027 (0.648–1.628)	0.909	Imputed
rs391300	17	<i>SRR</i>	C	T	0.745	0.741	1.024 (0.854–1.229)	0.800	Imputed
rs4430796	17	<i>HNF1B</i>	—	—	—	—	—	—	Filtered
rs5945326	X	<i>DUSP9</i>	—	—	—	—	—	—	Filtered

Data are OR for the risk allele (95% CI), unless otherwise indicated. The SNPs were selected from the results of DIAGRAM+ study (2), the MAGIC study (3), and recent GWA studies of type 2 diabetes in Asians (4,5). The risk allele is indexed to the positive strand of the National Center for Biotechnology Information build 36. RAF (GDM) and RAF (control) refer to the risk allele frequencies in women with GDM and control women, respectively. Type denotes whether the variant was directly genotyped in the genome scan (Typed), imputed using MACH (Imputed), or excluded after postimputation quality control (Filtered). ORs and *P* values were calculated using logistic regression under an additive model. CHR, chromosome. *P* values < 0.05 are indicated in boldface.

Because we performed a GWA analysis using 2.19 million genotyped or imputed markers, we were able to test the hypothesis that GDM and type 2 diabetes might share a similar genetic background. Among 11 variants listed in Table 2, five SNPs were located near the known type 2 diabetes loci. Three variants that showed an association at or near genome-wide significance, rs7754840 in *CDKAL1*, rs10830962 near *MTNR1B*, and rs1470579 in *IGF2BP2*, were identical or in strong LD with known type 2 diabetes variants. Two other variants, rs10757261 in *CDKN2A/2B* and rs10882066 in *IDE*, were not in LD with known type 2 diabetes variants ($r^2 = 0.013$ between rs10757261 and rs10811661 and $r^2 = 0.002$ between rs10882066 and rs5015480). Given that many of the type 2 diabetes risk loci

were associated with GDM, unbiased GWA studies of GDM showed genome-wide significant associations confined to known type 2 diabetes loci, and the effect size of the type 2 diabetes risk variants in GDM and type 2 diabetes were mostly comparable, it would be acceptable to state that GDM and type 2 diabetes share a similar genetic background.

Among the known type 2 diabetes-related genes, those that are thought to modulate pancreatic β -cell function were preferentially associated with GDM (*CDKAL1*, *MTNR1B*, *IGF2BP2*, *CDKN2A/2B*, *SLC30A8*, *IDE*, *KCNQ1*, and *CENTD2*) (Table 4). In contrast, loci relevant to insulin resistance, such as *FTO*, *PPARG*, *IRS1*, *KLF14*, and *GCKR* were not significantly associated with GDM (Table 4). These

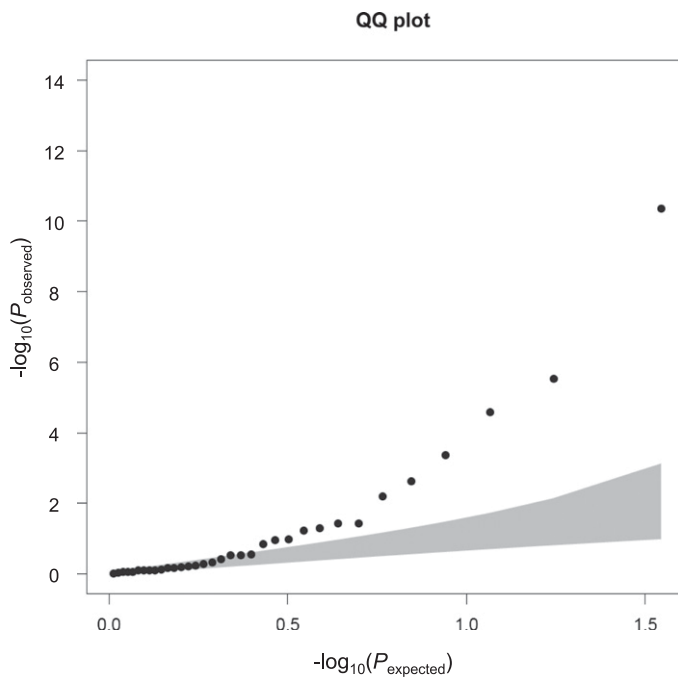


FIG. 3. QQ plot of the association between known type 2 diabetes variants and GDM. Comparison of the effect size of known type 2 diabetes variants in GDM and type 2 diabetes. The gray zone indicates the 95% CI. The known type 2 diabetes variants tested for association are listed in Table 4.

findings support the previous notion that defective pancreatic β -cell compensation to overcome increased insulin resistance during pregnancy might be the core pathophysiology of GDM.

There are certain limitations in our study. First, the sample size was relatively modest for a GWA, and we had limited power in detecting possible variants with small effect size. Assuming a 2.2% prevalence of GDM (48), a disease allele frequency of 0.3, and a genotype relative risk of 1.5 in a multiplicative model, our study had 93% power. However, when genotype relative risk was assumed to be 1.4, the power dropped to 68%. It is possible that variants with a small effect would not have been detected in our study. Therefore, we might have missed genetic variants that have a small effect but are specific to GDM. Regarding the study design, the control group was not fully evaluated for their glucose tolerance status during pregnancy and parity. It is possible that women with GDM might have been included in the control group. However, because the prevalence of GDM was estimated to be 2.2% in Korean women (48), and approximately one-half of these women progress to type 2 diabetes within 10 years (22), only $\sim 1\%$ of women with GDM would have been included in the control group. Therefore, we speculate that the ascertainment bias would have been minimal. In studying genetic variants of GDM, the ideal control group would be pregnant women with normal glucose tolerance. However, we were only able to use nondiabetic control women as control subjects. This could be one of the reasons that variants in *CDKAL1* and *MTNR1B* were most significantly associated with GDM in our study. In this regard, the results of our study should be interpreted cautiously.

In conclusion, we have performed a GWA study in the context of GDM for the first time and confirmed that variants in *CDKAL1* and *MTNR1B* confer the risk of GDM with genome-wide significance. By comparing genetic variants in GDM and type 2 diabetes, we provide evidence that they share a similar genetic background. We hope that even larger GWA studies of GDM and meta-GWA studies could further reveal the genetic risk loci for GDM.

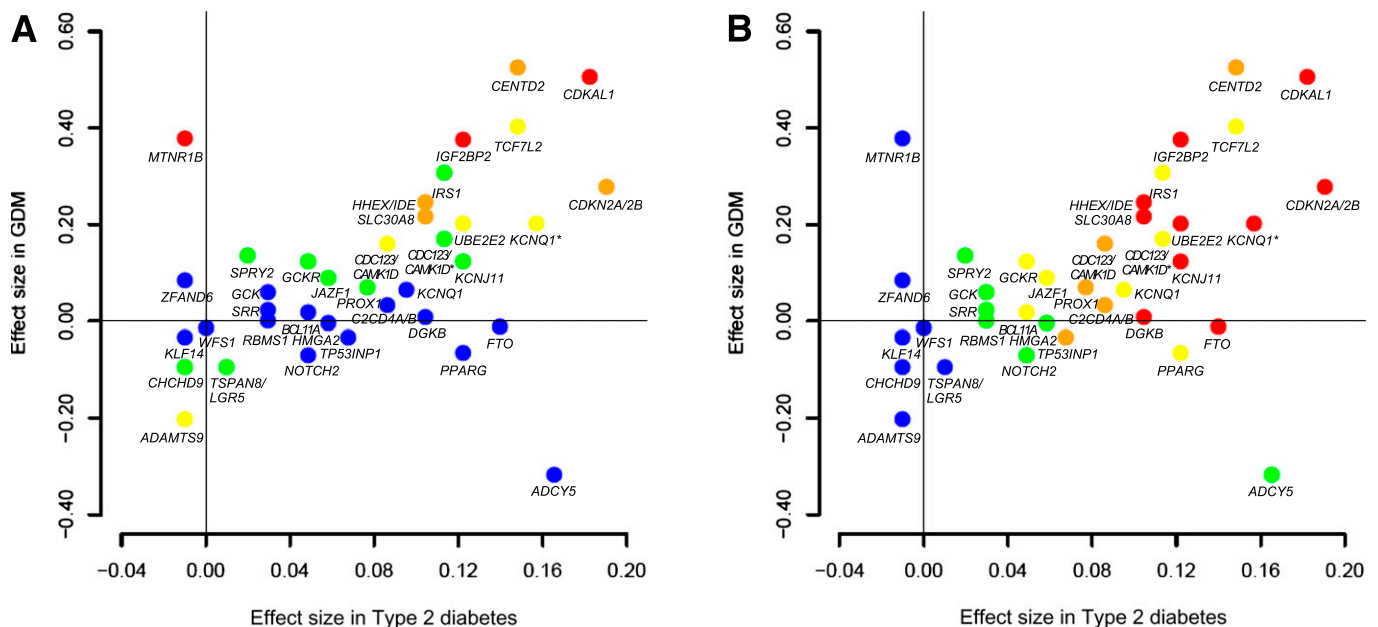


FIG. 4. Comparison of the effect size of known type 2 diabetes variants in GDM and type 2 diabetes. Effect size (β -coefficient from logistic regression analysis) of the known type 2 diabetes variants in GDM (y-axis) and type 2 diabetes (x-axis) are plotted with their corresponding P values (A: P values in GDM; B: P values in type 2 diabetes): red, $P < 0.0001$; orange, $0.0001 \leq P < 0.01$; yellow, $0.01 \leq P < 0.10$; green, $0.10 \leq P < 0.50$; blue, $0.50 \leq P$. The β -coefficient for GDM was derived from our stage 1 genome scan and that for type 2 diabetes was derived from the AGEN type 2 diabetes study (26). The two *CDC123/CAMK1D* variants are distinguished by *CDC123/CAMK1D* for rs10906115 and *CDC123/CAMK1D** for rs12779790. The two *KCNQ1* variants are distinguished by *KCNQ1* for rs231362 and *KCNQ** for rs2237892. (A high-quality color representation of this figure is available in the online issue.)

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

S.H.K. researched data, contributed to the discussion, and drafted the manuscript. S.-H.K., N.H.C., I.K.L., and B.-G.H. provided study samples. Y.M.C., S.H.C., M.K.M., H.S.J., H.D.S., and S.Y.K. contributed to the discussion. M.J.G. researched data. Y.S.C. provided study samples and researched data. H.M.K. provided statistical advice, contributed to the discussion, and edited the manuscript. H.C.J. and K.S.P. provided study samples, researched data, contributed to the discussion, and reviewed the manuscript. H.C.J. and K.S.P. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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