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A genetic risk score that includes common type 2 diabetes risk variants is associated with gestational diabetes

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SUMMARY

Objective—Gestational diabetes (GDM) is characterized by maternal glucose intolerance that manifests during pregnancy. Because GDM resembles type 2 diabetes (T2DM), shared genetic predisposition is likely but has not been established. We tested the hypothesis that a genetic risk score (GRS) that included variants known to be associated with T2DM is associated with GDM.

Study design—We conducted a case-control study using the Vanderbilt Medical Center biobank (BioVU), and calculated a simple-count GRS using 34 variants previously associated with T2DM or fasting glucose in the general population, or with GDM or glucose intolerance in pregnancy. We assessed the association of the GRS with GDM adjusting for maternal age, parity, and body mass index (BMI) and calculated the area under the curve for the receiver-operating characteristic curve (c-statistic).

Study population—Among Caucasian women, we identified 458 cases of GDM and 1538 pregnant controls with normal glucose tolerance.

Results—Cases of GDM had a higher number of risk alleles compared to controls (38.9 ± 4.0 vs. 37.4 ± 4.0 risk alleles, $P = 1.6 \times 10^{-11}$). The GRS was significantly associated with GDM; the adjusted odds ratio associated with each additional risk allele was 1.10 (95% CI, 1.07–1.13, $P = 6 \times 10^{-11}$). Clinical variables predicted the risk for GDM (c-statistic 0.67, 95% CI: 0.64–0.70), and adding the GRS modestly improved prediction (0.70, 95% CI: 0.67–0.73).

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Conclusions—Among Caucasian women, a GRS that included common T2DM genetic risk variants was associated with increased risk of GDM but showed limited utility in the identification of GDM cases.

Keywords

Gestational diabetes; risk assessment; type 2 diabetes

INTRODUCTION

Gestational diabetes (GDM) is characterized by maternal glucose intolerance that manifests during pregnancy. It occurs in approximately 9% of all pregnancies and is estimated to affect more than 200,000 pregnant women annually in the US.¹ GDM has important clinical implications because it is associated with significant short- and long-term adverse health consequences for women and their children.²

Normal pregnancy is characterized by a progressive decrease in insulin sensitivity that begins in the second trimester and can reach levels observed in type 2 diabetes (T2DM) during the third trimester. To compensate for this pregnancy-induced insulin resistance, pancreatic beta cells increase insulin release.³ However, in some women the amount of insulin released cannot meet the increased requirements, resulting in GDM.^{2,3} The pathogenesis of GDM is similar to T2DM, which is also characterized by insulin resistance followed by a compensatory increase in insulin secretion that is unable to meet requirements.⁴

GDM and T2DM share not only similar pathophysiology but also similar risk factors; GDM increases the likelihood of developing T2DM later in life,⁵ and women with a family history of T2DM have higher risk of developing GDM.⁶ Thus, it is likely that both conditions, to some degree, have a common genetic susceptibility.

Indeed, some genetic variants in selected T2DM risk loci have been associated with the risk of GDM with modest effects.^{7–9} Thus, similar to T2DM, it is possible that variants in several loci, each with small individual effects, contribute to the risk of GDM in a cumulative fashion. Thus, measuring their joint contribution may result in a stronger genetic signal than that provided by single variants.

We therefore examined the hypothesis that a genetic risk score that included variants known to be associated with T2DM and glucose in the general population, as well as variants previously associated with GDM and glucose intolerance during pregnancy, is associated with risk of GDM. The risk score included variants in loci known to affect β cell function (*KNCQ1*, *KCNJ11*, *SLC30A8*, etc.) or insulin sensitivity (e.g. *FTO*, *IRS1*, *PPARG*) and in loci associated with T2DM through mechanisms that are unclear (e.g. *DUSP9* and *HMG20A*).¹⁰

To test this hypothesis we conducted a case-control study examining the combined effect of these variants on the risk of GDM.

METHODS

Study design

The study was performed using the Vanderbilt University Medical Center (VUMC) DNA Biobank - BioVU. A full description of BioVU has been published.¹¹ Briefly, BioVU accrues DNA from blood samples obtained during routine clinical care from patients who have consented to have a DNA sample collected. DNA is extracted from samples that would otherwise be discarded, de-identified, and linked to a de-identified version of the electronic medical record (EMR) at VUMC. Approval for the present study was obtained from the Vanderbilt Institutional Review Board.

Definition of cases and controls

We first selected women in BioVU that met the following criteria: 1) aged ≥ 18 years old, 2) Caucasian, 3) one or more pregnancy-related ICD9 or CPT codes, 4) no diabetes-related ICD9- (250.***) codes before a pregnancy code. The study population included pregnant women with a DNA sample who had delivered or received pregnancy care between 1994 and 2016 at VUMC. Then, to ascertain potential cases of GDM, we used bioinformatic algorithms to search for women who had a 100g oral glucose tolerance test (100g OGTT) diagnostic of GDM using Carpenter and Coustan criteria¹² or had two or more ICD9 codes for glucose intolerance during pregnancy (648.83) (Figure 1). We then reviewed the de-identified EMRs of these potential cases to identify confirmed cases of GDM, defined as a pregnant woman who: a) had a 100g OGTT diagnostic of GDM using Carpenter and Coustan criteria,¹² b) had diagnosis of GDM made by a physician, or c) had received an intervention for GDM (nutritional or antidiabetic medication). In the US, the National Institutes of Health decision-making panel recommends a two-step approach to rule out GDM. This includes a 1-h 50g glucose challenge test (50g GCT) followed by the 3-h 100g OGTT for those who screen positive in the 50g GCT.¹³

We excluded potential cases that did not have enough clinical information to support the diagnosis of GDM, and those with diabetes before pregnancy. We also excluded cases receiving an antidiabetic medication at the time of screening for GDM, and those with a drug exposure or disease that may have affected the 100g OGTT (e.g., high dose glucocorticoids, cystic fibrosis, and pancreatic insufficiency). For cases with more than one qualifying pregnancy with GDM, we used the first.

Screening for GDM is performed routinely between 24 and 28 weeks of pregnancy with a 50g GCT. To identify potential controls, we selected pregnant women who had a normal 50g GCT (<140 mg/dL) (Figure 1). We frequency-matched cases and controls by age (± 5 years) and gestation type (singleton and multiple pregnancy). We excluded potential controls with inadequate clinical information and those with a previous history of GDM.

Clinical covariates

Using bioinformatic approaches and manual chart review, we extracted clinically relevant covariates such as: parity, height, pre-pregnancy weight, pre-delivery weight, and gestational age at delivery. We calculated the date of conception by subtracting the gestational age at

delivery from the delivery date. If the gestational age at delivery was not recorded, we used the gestational age at the earliest pre-natal care visit to calculate the date of conception. We defined pre-pregnancy weight as the weight measured closest to the date of conception within the 2 years before and 12 weeks after the date of conception. If the closest weight was measured between weeks 8 and 12 after conception, we subtracted 0.45 kg from this weight.¹⁴ Weights that overlapped with a previous pregnancy were excluded. We considered pre-delivery weight to be a weight recorded in the 15 days before delivery. We calculated body mass index (BMI) by dividing weight (in kilograms) by the square of height (in meters).

Genotyping

We genotyped 34 SNPs that included: 18 SNPs for loci that had been significantly associated ($P < 5 \times 10^{-8}$) with T2DM or fasting glucose in two or more genome wide association studies (GWAS) in the general population, 6 SNPs previously associated with GDM or glycemic traits in pregnancy, and 10 SNPs previously associated with both T2DM/ fasting glucose in the general population and GDM/glycemic traits in pregnant women (Supplement Table S1). The selected SNPs were from studies in the general population published up to 2013, from two large-meta-analysis of GDM,^{7, 8} and from one GWAS of glycemic traits in pregnant women.¹⁵ If two or more variants were in linkage disequilibrium ($r^2 > 0.5$), we selected the variant that was directly associated with GDM or tag variants. Genotyping was performed by the Vanderbilt Technologies for Advance Genomics (VANTAGE) at Vanderbilt University Medical Center according to standard protocols using the Sequenom platform. SNPs that failed or did not pool well in Sequenom were genotyped using TaqMan assays. For quality control, we required genotyping call rates above 90%. We examined significant departures from Hardy-Weinberg equilibrium (HWE) in controls using a threshold of $P < 0.001$.

Statistical analysis

Demographic and clinical characteristics were described as frequencies and percentages for categorical variables, or median and interquartile range for continuous variables. Clinical characteristics and genotypes were compared in cases and controls using Student's t-test or Pearson's chi-squared test, as appropriate. To estimate the individual effect of each variant on the risk of gestational diabetes, odds ratios (OR) and 95% confidence interval (95% CI) were calculated with logistic regression analysis assuming an additive genetic model.

Because pre-delivery BMI showed a strong linear correlation ($R^2 = 0.86$, $P < 0.001$) with pre-pregnancy BMI and more patients had pre-delivery BMI values recorded, we used this covariate in the adjusted model. The distribution of pre-delivery BMI was skewed and was natural log-transformed for analysis. Maternal age and parity (0, 1, or 2) were also included as covariates in the logistic regression model.

To study the combined effect of all SNPs on the risk of GDM, a simple-count (0, 1, 2 for each risk allele) GRS was used in the logistic regression analysis (Supplemental Table S1). Patients missing information for 4 or more SNPs were excluded from the risk score calculation. In patients with incomplete genotypes for 3 or fewer SNPs, we took the

conservative approach of assuming that they did not carry the risk allele (i.e., we assigned a score of 0 for the missing genotype). In a sensitivity analysis, we excluded all patients with missing genotypes. Based on quintiles derived from the number of risk alleles, ORs and 95% CI were calculated using the lowest quintile as reference. The c-statistic (the area under the receiver-operating characteristic curve) for the logistic regression model was calculated including clinical co-variables with and without the GRS. To determine if the prediction accuracy of the GRS would be improved by weighting, we constructed an OR-weighted GRS using the individual's effect size for each variant observed in the single SNP analysis, and calculated the c-statistics for the single count and OR-weighted GRS.

Assuming an additive model, a two tailed alpha level of 0.05 and a 3:1 ratio for controls cases, at least 440 cases and 1320 controls were required to provide 80% power to detect an OR of 1.30 for variants with an allele frequency of 20% in single SNP analysis, with greater power for more frequent variants and for GRS analysis.

Statistical analyses were performed using R software version 3.2.3 (www.R-project.org) and SPSS (v. 21, IBM® SPSS® Inc., Chicago, IL). The primary analysis was for the GRS; a two sided P-value <0.05 was considered statistically significant. Secondary analyses for individual SNPs were hypothesis-generating and are presented as OR and 95% CI with both unadjusted and Bonferroni adjusted P-values.

RESULTS

Population characteristics

Among Caucasian pregnant women, we identified 619 cases of GDM and 1687 controls (Figure 1), 458 cases and 1538 controls had genotype data and were included in the analysis. (Figure 1). Cases of GDM had higher parity, pre-pregnancy BMI, and pre-delivery BMI compared to controls ($P < 0.01$ for all comparisons, Table 1). Pre-pregnancy BMI was missing in 34% of cases and 7% of controls and pre-delivery BMI was missing in 8% of cases and 3% of controls.

Single SNP analysis

Genotypes were all in Hardy-Weinberg equilibrium and within expected frequency (Supplement Table S1). In single SNP analyses, the OR adjusted for age, parity (0, 1, or 2) and BMI ranged from 0.85 to 1.40 in the 34 SNPs (Figure 2); the OR point estimate was >1.0 in 24 SNPs. Four of these SNPs were nominally ($P < 0.05$) associated with the risk of GDM (rs1801282 in *PPARG*, rs4746822 in *HKDC1*, rs10830963 in *MTNR1B*, and rs7756992 in *CDKAL1*), and 4 were significantly ($P < 0.001$, Bonferroni adjustment for 34 SNPs) associated with GDM (rs5015480 in *HHEX*, rs44030796 in *IGF2BP2*, rs7936247 in *MTNR1B*, and rs4506565 in *TCF7L2*) (Supplement Table S2).

Genetic risk score

There was significant overlap in the distribution of the GRS between cases and controls. However, the mean $\pm$ standard deviation (SD) of 38.9 ± 4.0 risk alleles in cases was significantly higher compared to 37.4 ± 4.0 in controls ($P = 1.6 \times 10^{-11}$). The GRS was

significantly associated with GDM; the OR associated with each additional risk allele was 1.10 (95% CI: 1.07-1.13, $P=6 \times 10^{-11}$) for the single count GRS and 1.11 (95% CI: 1.08-1.14, $P=3 \times 10^{-13}$) for the OR-weighted GRS adjusted for age, parity, and BMI. Similar results were obtained when analysis was restricted to patients with complete genotype information (390 cases and 1389 controls). Odds ratios increased across quintiles of GRS count (Figure 3). Women in the highest quintile of the GRS (42 risk alleles) had an OR=3.19 (95% CI: 2.17-4.71) for GDM compared with women in the lowest quintile (34 risk alleles). The c-statistic for logistic regression models were as follows: clinical covariates only 0.67 (95% CI: 0.64 - 0.70); simple count GRS only 0.60 (95% CI: 0.57 - 0.63); OR-weighted GRS only 0.61 (95% CI 0.58 - 0.64); combination of clinical covariates and simple or OR-weighted GRS 0.70 (95% CI 0.67 – 0.73).

DISCUSSION

The main finding of this study is that a GRS, which included SNPs known to be associated with T2DM, was associated with the risk of GDM in Caucasians but had limited discriminatory ability to identify cases of GDM. Additionally, several T2DM risk variants were associated with GDM for the first time.

The genetic contribution to the risk of GDM remains poorly defined. There have been several studies focused on a small number of known T2DM risk variants, summarized in two meta-analyses,^{7, 8} as well as a single GWAS in GDM.⁹ These studies suggested that some known T2DM risk variants are associated with GDM.

GDM is a precursor of T2DM in some women. Accordingly, we anticipated that a GRS that incorporated T2DM risk alleles would be associated with the risk of GDM. Cases of GDM indeed had a higher GRS than controls, but the discriminative ability of the GRS for GDM was modest. In T2DM, the overall discriminative accuracy (measured by the c-statistic) for genetic models are typically ~0.60 and for models using only clinical information range from 0.7 to 0.96.¹⁶ Similar to our observations in GDM, others have reported that the addition of genetic information to clinical variables improved the prediction of T2DM modestly.¹⁶ A previous study in GDM of a GRS composed of 11 T2DM variant,¹⁷ found a modest c-statistic for the GRS (0.68) that improved the clinical model (0.73). Although we included several of the loci from that study and added loci that were subsequently associated with T2DM, the predictive ability of our GRS (single count and weighted) for GDM was ~0.60; which suggests that adding additional common T2DM variants with small effect sizes has small impact on genetically predicted risk of GDM, an observation also made in T2DM.¹⁶ Thus, the observation that clinical risk factors themselves have a strong genetic component that is partially captured by the GRS in T2DM may also apply to gestational diabetes.

We used a simple GRS because for some of the risk alleles included there was inadequate information in the literature to assign a weight. One could argue that a weighted risk score could have performed better. To begin to address this question, we used the effect sizes from the single SNP analysis to construct an OR-weighted GRS. The OR-weighted GRS and the simple count GRS performed similarly; which has also been observed in T2DM.¹⁸

Although the GRS overall has limited discriminative value for GDM at the population level, it may improve risk stratification for some women. For example, each additional risk allele was associated with a 10% increased risk for GDM, and women with 42 or more risk alleles had more than 3-fold increased risk for GDM compared to women with 34 or fewer risk alleles. Thus, the GRS could provide information about the genetic burden in women at high risk for GDM in order to assist interventions aimed at reducing the impact of metabolic disorders in pregnancy.

Concordant with previous studies, we observed associations between GDM and variants in *IGF2BP2*, *TCF7L2*, *MTNR1B*, *HHEX/IDE* and *CDKAL1*.^{7–9, 17} Risk alleles in these loci have shown to decrease insulin response after glucose administration.^{19–25} This suggests that the signals for these loci are more likely to be significant in conditions where β -cell response is impaired after glucose stimulation, such as occurs in GDM. Current evidence indicates that these variants are likely to influence β -cell function through several molecular mechanisms (e.g. direct regulation of the corresponding gene or genes known to play a role in insulin activity and metabolic control;^{26, 27} interaction with local enhancers;²⁸ alteration of splicing patterns;²⁹ or normal development of β -cells^{30, 31}).

Additionally, we report for the first time associations between variants in *PPARG* and *HKDC1* and the risk of GDM in Caucasians. The *PPARG* rs1801282 (C34G substitution ~ Pro12Ala) variant was associated with the risk of GDM. In the general population, the G allele (12Ala) is associated with reduced activity of PPAR γ , improved insulin sensitivity,³² and reduced risk of T2DM.³³ Concordant with these findings, we found that carriers of the C allele (Pro12) had a higher risk of GDM compared to the G allele (12Ala). In the EDEN study, the C allele of rs1801282 was associated with the risk of GDM only when the T allele of another *PPARG* variant, rs3856806 (C1431T), that is in weak linkage disequilibrium with rs1801282 ($D'=0.68$, $R^2=0.42$) was present.³⁴ We did not genotype for this haplotype because it is uncommon (~ 4% in Caucasians).^{34, 35}

We also found that *HKDC1* rs4746822 variant was associated with GDM. This variant was significantly associated with plasma glucose levels 2 hours after a glucose load in pregnant women in the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study,¹⁵ and nominally associated with 2-hour glucose levels in the general population,³⁶ but has not previously been associated with T2DM. A recent study have found for the first time that rs4746822 was significantly associated with the risk of GDM in Indian women.³⁷ The function of several variants in the *HKDC1* region that are associated with gestational hyperglycemia and are in linkage disequilibrium with rs4746822 has been studied *in vitro*. These variants, which are organized in different haplotypes, disrupt regulatory element activity and reduce expression on *HKDC1*, a gene that encodes for a novel form of hexokinase³⁸ that plays an important role in glucose utilization during pregnancy and aging in animal studies.³⁹

The GRS was significantly associated with the risk of GDM but the risk associated with individual SNPs varied; of the 34 SNPs in the GRS, 24 had an OR greater than 1, but only 8 were associated with GDM with a P value <0.05. Considering that the SNPs selected for the GRS were derived from large GWAS studies with thousands of participants and had small

effects, it is not surprising that some SNPs were not significantly associated with GDM. Thus, a larger study would be needed to detect the contribution of variants with small effect sizes, since the current study was powered to detect ORs of 1.3 or larger.

Our study had limitations. First, we selected cases of GDM and controls using EMRs from patients seen at a tertiary care hospital. Thus, the findings may not be applicable to all populations. However, cases and controls were carefully phenotyped and represent a broad spectrum of pregnant women. Second, although the two step-approach with 100g OGTT is the gold standard for GDM diagnosis in the US;¹³ the WHO recommends a one-step 75g OGTT to screen for GDM in developing countries in order to decrease costs with acceptable sensitivity.² Use of a different approach to ascertain cases of GDM could affect results. Third, we included only those SNPs most robustly associated with T2DM (i.e., significant in 2 or more GWAS studies). Although it is possible that including more SNPs could improve the performance of the GRS, it is likely that the largest improvement will result from the inclusion of fewer, more discriminatory SNPs with large effect size rather than multiple common variants with modest effects. Fourth, we did not have information about family history of T2DM. The addition of information about family history of T2DM might have improved the predictive ability of the clinical model and further reduced the impact of the genetic score by incorporating some of the information present in the GRS. Fifth, although we adjusted for parity in our analysis to account for differences between cases and control women, we cannot exclude the possibility that some controls may have developed GDM in future pregnancies and were therefore misclassified in our study. Such misclassification would have acted to impair our ability to detect genetic differences between cases and controls. Sixth, we cannot exclude the possibility that a low frequency or rare variants with large effects may contribute to the risk of GDM, as has been shown in T2DM.⁴⁰

CONCLUSIONS

In summary, we found that a GRS that includes T2DM variants was associated with increased risk of GDM, but the improvement in the discriminatory accuracy for genetics added to clinical factors for predicting GDM was moderate and similar to that observed in T2DM. We also report for the first time that polymorphisms in *PPARG* and *HKDC1* were associated with increased risk of GDM in Caucasians.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Flow diagram of the selection of cases of gestational diabetes and controls

100g OGTT: 100 gr oral glucose tolerance test; 50g GCT: 50 gr glucose challenge test;

*not genotyped because lack of DNA sample or inadequate DNA sample

Figure 2. Association of risk loci and gestational diabetes in Caucasian women
 Regression model adjusted by age, parity (0,1 or 2) and ln(pre-delivery BMI). ^a T2DM variants and ^b fasting glucose variants in the general population, ^c GDM variants and ^d glycemic trait variants in pregnant women

Figure 3. Distribution of the genetic risk score (in quintiles) and risk of gestational diabetes in Caucasian women

Table 1

Demographic and clinical characteristics of Caucasian women with gestational diabetes (GDM) and controls

Characteristics	Controls (n=1538)	GDM (n=458)	P-values
Age at pregnancy (years)	29 (26, 33)	30 (26, 34)	<0.001
Parity	0 (0, 1)	1 (0, 2)	<0.001
Previous pregnancies < 20 weeks			<0.001
0	859 (55.9)	200 (43.7)	
1	451 (29.3)	131 (28.6)	
2	228 (14.8)	27 (27.7)	
Primigravida	670 (43.6)	140 (30.6)	<0.001
Type of current gestation			
Singleton	1509 (98.1)	449 (98.0)	0.644
Twin	26 (1.7)	7 (1.5)	
Multiple	3 (0.2)	2 (0.4)	
Height (m)	1.65 (1.60, 1.70)	1.63 (1.57, 1.67)	<0.001
Pre-pregnancy weight (kg) ^a	65.3 (58.1, 74.8)	75.7 (62.7, 90.7)	<0.001
Pre-pregnancy body mass index (kg/m ²) ^a	23.8 (21.4, 27.4)	28.6 (23.8, 34.7)	<0.001
Underweight (<18.5 kg/m ²)	51 (3.5)	9 (2.6)	<0.001
Normal weight (18.5–24.9 kg/m ²)	830 (57.6)	100 (29.0)	
Overweight (25–30 kg/m ²)	340 (23.6)	88 (25.5)	
Obese (≥ 30 kg/m ²)	221 (15.3)	148 (42.9)	
Pre-delivery weight (kg) ^b	81.2 (73.2, 91.3)	86.2 (75.8, 103.9)	<0.001
Pre-delivery body mass index (kg/m ²) ^b	29.8 (26.9, 33.7)	33.0 (29.0, 38.4)	<0.001
Gestational age at delivery (weeks) ^c	39.3 (38.6, 40.3)	39.0 (37.6, 39.6)	<0.001

Data are expressed as median (interquartile range) or number (percent)

^a pre-pregnancy weight and pre-pregnancy BMI were missing in 96 (6.2%) controls and in 113 (24.7%) cases.^b pre-delivery weight and pre-delivery BMI were missing in 45 (2.9%) of controls and 36 (7.9%) of cases.^c gestational age at delivery was not available in 10 (0.7%) controls and in 8 (1.7%) cases