Short Communication

Pharmacogenomics

Variation in the α_{2A}-adrenergic receptor gene and risk of gestational diabetes

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Aim: Sympathetic activation suppresses insulin secretion via pancreatic ADRA2A. Because sympathetic activity and insulin demand increase during pregnancy, we tested the hypothesis that *ADRA2A* variants are associated with gestational diabetes (GDM). **Patients & methods:** Among Caucasian pregnant women without pre-existing diabetes, we genotyped 458 who had GDM and 1537 without GDM for seven *ADRA2A* variants. **Results:** rs1800038 (OR: 2.34; p = 0.020) and rs3750625 (OR: 1.56; p = 0.010) increased the risk of GDM, and rs11195418 decreased it (OR: 0.62; p = 0.025). The associations remained significant after adjustment for maternal age, maternal BMI, parity and a genetic risk score that included variants previously associated with Type 2 diabetes mellitus and GDM. **Conclusion:** *ADRA2A* genetic variation contributes independently to the risk of GDM in Caucasian women.

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Normal pregnancy is characterized by a state of relative insulin resistance that results from several hormonal and metabolic changes and ensures adequate glucose supplies to the fetus while the mother is fasting [1–3]. In compensation, insulin release increases two- to threefold in pregnancy [3]; however, in some women the amount of insulin released cannot counteract the degree of insulin resistance and they develop gestational diabetes (GDM) [4].

During normal pregnancy, in addition to increased insulin release, there is also an increase in sympathetic activity [5]. Sympathetic activation suppresses insulin secretion via activation of pancreatic ADRA2A. In the general population, hyperfunctional genetic variants in the *ADRA2A* gene have been associated with reduced insulin secretion and increased the risk of Type 2 diabetes mellitus (T2DM) [6,7]. Recently, our group found that *ADRA2A* variants rs553668 and rs10885122 were significantly associated with stress-induced hyperglycemia in patients with an acute myocardial infarction [8], a condition that is accompanied by increased sympathetic drive [9,10].

We previously studied the combined effect of genetic variants associated with T2DM or blood glucose in at least two genome-wide association studies (GWAS) and those associated with glucose intolerance during pregnancy on the risk of GDM using a genetic risk score (GRS) composed of 34 variants in 33 genes, including one *ADRA2A* variant (rs10885122) [11]. The GRS was significantly associated with the risk of GDM, but it had limited predictive ability to distinguish cases of GDM; thus, we sought to identify additional variants that increased the risk of GDM.

Accordingly, we hypothesized that a setting of physiologically increased insulin secretion accompanied by increased sympathetic activation (such as what occurs in pregnancy) would be a state in which the contribution of *ADRA2A* variation is particularly important. Thus, we tested the hypothesis that *ADRA2A* variants are associated with altered risk of GDM in a case–control study.

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Table 1. Clinical characteristics of women with gestational diabetes and pregnant controls.

Clinical characteristics	Controls (n = 1538)	Cases (n = 458)
Age at pregnancy (years)	29 (26–33)	30 (26234)
Parity (n)	0 (0–1)	1 (0–2)
Singleton (n, %)	1509 (98.1)	449 (98.0)
Height (m)	1.65 (1.60–1.70)	1.63 (1.57–1.67)
Maternal BMI (kg/m^2)†	29.8 (26.9–33.7)	33.0 (29.0– 38.4)

Categorical variables are described as frequencies and percentages and continuous variables as median and interquartile ranges.
†Maternal BMI was missing in 45 (2.9%) of controls and 36 (7.9%) of cases.

Materials & methods

Study population

We have previously defined the association of genetic variants known to be associated with T2DM and glucose intolerance during pregnancy with the risk of GDM in a study performed using Vanderbilt University Medical Center's DNA Biobank, BioVU [11]. For the current study, we used the same study population to determine the association of *ADRA2A* variants with the risk of GDM. The study was approved by the Vanderbilt Institutional Review Board, and the detailed methods have been described previously [11]; a brief overview is provided here. Using bioinformatic algorithms and manual review, we identified and confirmed cases of GDM (defined as a positive oral glucose tolerance test using Carpenter and Coustan criteria [12] or a diagnosis of GDM by a physician accompanied by a prescribed dietary or therapeutic intervention and controls who had a normal glucose challenge test in pregnancy). Cases and controls were all of European descent and groups were frequency matched by age (±5 years) and gestation type (singleton and multiple pregnancy).

Genotyping

We genotyped seven tagging *ADRA2A* SNPs (rs1800038, rs3750625, rs553668, rs2484516, rs1800545, rs1800544 and rs11195418) additional to the one *ADRA2A* variant associated with fasting glucose in the general population (rs10885122). Genotyping was performed by the Vanderbilt Technologies for Advance Genomics according to standard protocols using the Sequenom platform for all SNPs except for rs2484516 which was genotyped using TaqMan assays. For quality control, we required genotyping call rates >90% and calculated Hardy–Weinberg equilibrium of genotype distributions.

Statistical analysis

Categorical variables were described as frequencies and percentages and continuous variables as median and interquartile ranges. Clinical characteristics and genotypes were compared in cases and controls using Student's *t*-test or Pearson χ^2 test, as appropriate. The individual effect of *ADRA2A* variants on the risk of GDM was evaluated in an additive genetic model using logistic regression analysis and odds ratios with 95% confidence intervals (OR; 95% CI) were calculated. The regression model included the following covariates: maternal age, maternal BMI, parity and a GRS that included 33 nonadrenergic variants that were previously associated with T2DM, GDM and glucose intolerance during pregnancy and which we studied before in GDM [11].

Statistical analyses were performed using R software version 3.2.3 (www.R-project.org) and SPSS (v. 24, IBM® SPSS® Inc., IL, USA). All p-values are two-sided and p-values <0.05 were considered statistically significant.

Results

Population characteristics

We identified 458 cases of GDM and 1538 control women with a normal glucose tolerance test (NGT) in pregnancy. Cases had higher parity and maternal BMI compared with controls (Table 1).

ADRA2A variants & risk of gestational diabetes

Minor allele frequencies for the *ADRA2A* variants studied were within expected ranges (Table 2). In the unadjusted single SNP analysis, two variants were associated with the increased risk of GDM: rs1800038 (OR: 2.34; 95% CI: 1.14, 4.77; p = 0.020) and rs3750625 (OR: 1.56; 95% CI: 1.11, 2.20; p = 0.010) and one variant (rs11195418) with decreased risk (OR: 0.62; 95% CI: 0.40, 0.94; p = 0.025). The same variants remained significantly associated with the risk of GDM once we adjusted for maternal age, parity, maternal BMI and GRS (Figure 1): rs1800038

Table 2. Distribution of *ADRA2A* alleles and risk of gestational diabetes.

Genetic variant	Minor allele	Minor allele frequency		Odds ratio (95% CI)	p-value
		Controls (%)	Cases (%)		
rs1800038	A	0.6	1.4	2.34 (1.14–4.77)	0.020
rs3750625	A	3.9	5.9	1.56 (1.11–2.20)	0.010
rs553668	A	16.8	17.8	1.08 (0.89–1.31)	0.460
rs2484516	G	5.8	5.8	1.00 (0.73–1.38)	0.995
rs1800545	A	10.6	10.6	1.00 (0.79–1.27)	0.994
rs1800544	C	27.7	28.8	0.95 (0.81–1.12)	0.523
rs10885122	T	13.3	13.8	1.04 (0.84–1.28)	0.752
rs11195418	G	4.6	2.8	0.62 (0.40–0.94)	0.025

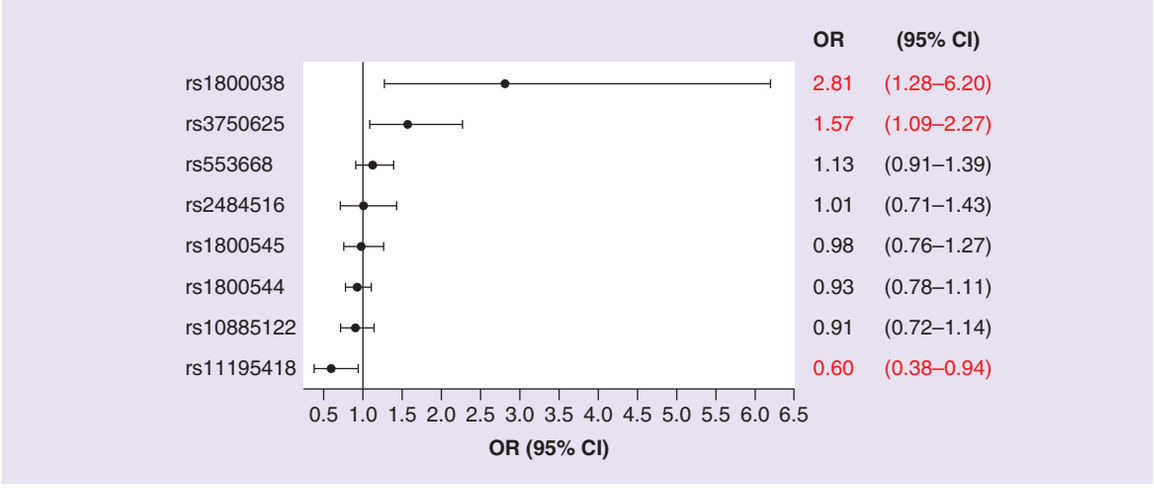

Figure 1. *ADRA2A* variants and risk of gestational diabetes. OR and 95% CI are adjusted for maternal age, parity, maternal body mass index and a genetic risk score for Type 2 diabetes mellitus and gestational diabetes.
OR: Odds ratio.b

(OR: 2.81; 95% CI: 1.28–26.20; p = 0.010), rs3750625 (OR: 1.57; 95% CI: 1.09, 2.27; p = 0.016) and rs11195418 (OR: 0.60; 95% CI: 0.38–30.94; p = 0.026).

Discussion

This study evaluated the association of *ADRA2A* variants and the risk of GDM in women of European ancestry and found that carriers of the minor allele for rs1800038 and rs3750625 had increased risk for GDM, while carriers of rs11195418 had lower risk. These findings remained significant after adjusting for other genetic variants known to affect the risk of GDM and T2DM and for clinical characteristics likely to increase the risk of GDM, supporting the hypothesis that *ADRA2A* variants play an important role in glucose regulation during pregnancy.

We did not find an association between GDM and the two *ADRA2A* variants most frequently associated with T2DM (rs553668 and rs10885122). Rosengren found that the 3′UTR *ADRA2A* variant, rs553668 was associated with reduced insulin secretion and increased risk of T2DM [7] but the variant did not achieve genome-wide significance in GWAS studies. In our study, GDM was most often diagnosed using the glucose response to an oral glucose load (OGTT) and in population studies the rs553668 variant was associated with fasting glucose levels and T2DM [13], but not with OGTT measures [13,14]. One possible explanation for this observed association with T2DM but not with OGTT measurements is that the effect of rs553668 on glucose control may manifest only once pancreatic β-cell function is impaired enough to cause hyperglycemia, but it may have less impact in 'prediabetic' states that are detected with the OGTT. The rs10885122 is a variant located 0.2 Mb from the *ADRA2A* gene that has been associated with fasting glucose and reduced glucose-stimulated insulin release in the general population [6,15]. As we reported previously [11], there was no significant association between rs10885122 and GDM. Although carriers of rs10885122 showed a consistent but modest decrease in insulin release after

OGTT in nondiabetic Danish subjects [6] and the variant was associated with a small increase in fasting glucose levels in the MAGIC study [15], it was not associated with T2DM, HOMA-B and response to glucose challenge at genome-wide significance in large studies [14,15], which is compatible with our findings. It is also possible that the clinical effects of rs10885122 and rs553668 manifest during conditions of extreme sympathetic activation since we have previously reported that in patients with acute myocardial infarction, both variants are associated with stress-induced hyperglycemia [8].

Less is known about the three *ADRA2A* variants we found to be associated with GDM. The rs1800038 is rare in populations of European descent (<1%), which may explain why previous GWAS studies did not report an association between this variant and metabolic phenotypes. The only phenotype previously associated with this variant was pain sensitivity to cold after the infusion of dexmedetomidine (a selective α_2-adrenoceptor agonist) in a candidate gene study reported by our group [16]. Because rs1800038 is a synonymous substitution, it is possible that this variant was excluded in previous studies. The functional importance of synonymous variants, once considered 'silent', is increasingly appreciated in recent years, since they may affect several physiological mechanisms [17] and drug response [18] through effects on mRNA splicing, translation fidelity, mRNA structure and protein folding [17,19]. Chromatin state markers from Haploreg version 4.1 [20] indicate that the genomic location of rs1800038 is close to an active promoter in several cell types, including cells that are metabolically relevant (e.g., adipose cell nuclei, stem cell derived cultured adipocytes and pancreatic islet cells). Thus, it is possible that rs1800038 (or a rare functional variant tagged by rs1800038) conferred higher promoter activity and thus higher *ADRA2A* expression, resulting in lower insulin release and increased risk of GDM, a theory that would needs to be further explored in future studies.

rs3750625 is a 3′UTR *ADRA2A* variant that was previously associated with postprandial gastric volume in overweight and obese European–Americans [21]. Because 3′UTR variants are known to regulate gene expression post-transcriptionally through microRNA (miRNA) binding, we searched for potential miRNA targets of rs3750625 using miRdSNP, a comprehensive data source of variants affecting post-transcriptional regulation [22]. We found that this variant is within or close to the seed region for 23 miRNAs. This suggests that rs3750625 could potentially increase *ADRA2A* expression through its effect on miRNA binding. Recently, Linnstaedt *et al.* reported that rs3750625 increased the binding affinity of miR-34a *in vitro* decreasing *ADRA2A* expression [23], which would therefore be expected to increase insulin release and thus reduce the risk of GDM. Although this is the opposite of our finding, we cannot exclude that rs3750625 could affect the binding affinity of the other miRNAs through structural accessibility [24], or through *cis*-effects [25] and result in a net increase of *ADRA2A* expression.

The rs11195418 is an upstream variant that was marginally associated with systolic blood pressure but not with any glycemic traits in the British Women's Health and Heart Study cohort in the meta-analysis performed by Talmud *et al.* [13]. In Haploreg, rs11195418 is reported to be associated with differential expression of the *PDCD4* gene in whole blood ($p = 8.20 \times 10^{-5}$), *PDCD4* is located approximately 200 kb upstream from *ADRA2A* and plays an important role in metabolic phenotypes. For example, *PDCD4*-deficient mice do not develop obesity and insulin resistance when fed with high-fat diet compared with wild-type mice [26]. In women with polycystic ovary syndrome, the expression of *PDCD4* was correlated with BMI and insulin resistance [27]. In our study, rs11195418 was associated with reduced risk of GDM; thus, carriers of the rs11195418 variant may have decreased *PDCD4* expression, resulting in decreased risk of insulin resistance and GDM. The variant has been nominally associated with reduced risk of T2DM in the SIGMA cohort.

rs521674, an *ADRA2A* variant that is in complete linkage disequilibrium with rs1800544 ($D` = 1$ and $R^2 = 1$), was associated with T2DM in obese women when compared with lean women with NGT in the Stockholm Diabetes Prevention Program [28]. In the same study, rs553668 was associated with increased risk of T2DM in males. However, the association for both variants disappeared when BMI was included in the analysis and there was no association when lean T2DM subjects were compared with lean NGT subjects. Moreover, the expression of *ADRA2A* in pancreatic tissues from a small subset of these T2DM subjects and controls was not significantly different, suggesting that the association of both variants with T2DM was likely driven by BMI. In our study, although cases of GDM had higher BMI than control pregnant women, rs1800544 and rs5853668 were not associated with the risk of GDM, which is not surprising given that the difference in BMI between cases and controls was relatively small compared with the differences between NGT lean subjects and T2DM subjects in the Stockholm Diabetes Prevention Program study.

The study had limitations. Because the GDM cohort was relatively small, we were unable to divide our group into a discovery and replication cohort; additional studies to address our findings in other cohorts will be of interest. Because the selected variants were prespecified and located close or in the same genomic location, we did

not correct for multiple testing. It is also possible that some control pregnant women were misclassified from a phenotypic standpoint since some might develop GDM in a subsequent pregnancy. It is possible that different *ADRA2A* variants may influence different components of glucose regulation such as fasting levels or levels after a glucose load. The National Institutes of Health's decision-making panel recommends a two-step approach for the screening of GDM diagnosis [29] and that is what is used in clinical practice; a measurement is obtained 1 h after a 50 g glucose challenge test (with no fasting value) to screen all pregnant women, then the 3 h 100 g OGTT is performed only in those who previously screened positive in the 50 g test. Therefore, in our study we have data on fasting, 1, 2 and 3 h plasma glucose levels only in cases and are thus unable to compare the role of the *ADRA2A* variants on these measures of glucose regulation in cases and controls.

Conclusion

In summary, our exploratory study suggests that *ADRA2A* variants may play an important role in the regulation of glucose during pregnancy.

Future perspective

This is the first study that explores the role of *ADRA2A* variants on the risk of GDM. Further studies are required to understand the mechanism through which these variants affect the risk of GDM in order to identify potential preventive and therapeutic targets to improve prenatal care.

Financial & competing interests disclosure

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Summary points

- Animal and clinical studies have shown that genetic variability in the gene that encodes for the adrenergic receptor α2A gene (*ADRA2A*) is associated with decrease insulin secretion and increased the risk of Type 2 diabetes mellitus in the general population.
- Because normal pregnancy is characterized by an increase in sympathetic activity and increased insulin resistance, the aim of this study was to explore the role of *ADRA2A* variants in the risk of gestational diabetes.
- We found that variation in *ADRA2A* play a role in glucose regulation during pregnancy. However, our findings need to be further studied to determine its potential role in prenatal care.

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