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Assessment of the association between the polymorphism rs1256031 of the estrogen receptor β gene and GDM susceptibility

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ABSTRACT

Estrogen has an important role in regulating glucose homeostasis, and existing evidence indicates that it might be involved in the development of hyperglycemia in pregnancy. It mediates its effect through estrogen receptors including the nuclear receptor ERβ encoded by *ESR2*. The association between the *ESR2* polymorphism rs1256031 and GDM susceptibility has not been investigated yet. This study aimed to evaluate the relationship between rs1256031 and GDM risk in Chinese population. A total of 241 GDM patients and 139 healthy pregnant women were recruited for this study. The rs1256031 genotype was examined by time-of-flight mass spectrometry and the association between rs1256031 and GDM susceptibility was assessed by binary logistic regression in three different genetic models. The polymorphism rs1256031 was not associated with GDM susceptibility in additive [OR (95% CI) = 0.871 (0.453,1.675); P = 0.680], dominant [OR (95% CI) = 0.908 (0.495,1.665); P = 0.755] or recessive [OR (95% CI) = 0.912 (0.591,1.408); P = 0.677] models after adjusting for confounding factors. We observed no association between the polymorphism rs1256031 in the ESR2 gene and GDM susceptibility in Chinese pregnant women.

Keywords: gestational diabetes mellitus; estrogen receptor β; single nucleotide polymorphism

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as abnormal glucose intolerance with onset or first recognition during pregnancy. It is a prevalent and perilous disease threatening both the mother and the offspring.[1-4] The incidence of GDM in China was estimated to be as high as 12.1% in 2013 and is rapidly increasing each year.[5] GDM not only brings about adverse pregnant outcomes such as stillbirth, macrosomia, shoulder dystocia, neonatal hypoglycemia and neonatal respiratory distress syndrome,[6,7] but also has substantial long-term negative effects on

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the health of both the patients and their offspring including increased risk of developing type 2 diabetes (T2D), obesity, metabolic syndromes and cardiovascular diseases in later life.[8,9] Therefore, developing effective early detection and intervention strategies to protect pregnant women against maternal and fetal complications has become an urgent need.

Estrogen plays a fundamentally important role in glucose metabolism as illustrated by numerous previous studies in rodent models as well as in human.[10-12] Mutation in the aromatase which catalyzes the biosynthesis of estrogens leads to insulin insensitivity and glucose intolerance,[13-15] and the insulin-resistant state can be reversed by estradiol treatment in male aromatase knockout (ArKO) mice.[16] The exact mechanisms by which estrogen regulates glucose homeostasis have not been fully elucidated yet. Existing evidence suggested that estrogen improves glucose-stimulated insulin secretion,[17] protects pancreatic beta-cells from apoptosis,[18] and enhances the expression of insulin signaling molecules in skeletal muscle, preventing against the development of insulin resistance.[19]

Estrogen is mainly produced by the placenta during pregnancy,[20,21] and its levels dramatically increase during gestation.[22] Estrogen is vitally important in developing and maintaining proper placental structure and function,[23] and in regulating the differentiation of T cells involved in gestational immune activities.[24] Montelongo et al found that plasma estrogen levels were lower in diabetic pregnant women than in healthy pregnant controls.[25] Recently, Qi et al demonstrated that decreased cord blood estrogen levels were associated with GDM. These facts indicate that estrogen might be involved in the development of hyperglycemia during pregnancy.[26]

Estrogens mediate their effects through estrogen receptors including the nuclear receptors (ERα and ERβ) and the G protein-coupled estrogen receptor (GPER).[27] These receptors are expressed in a variety of tissues and cells such as skeletal muscle, adipose tissue and pancreatic β cells.[28] Activation of ERβ leads to closure of K_{ATP} channels and therefore enhancement of glucose-induced insulin secretion.[29] It has been reported that polymorphism of *ESR2* was associated with metabolic syndrome.[30] Recently, rs1256031 located within the intron of *ESR2* has been identified as a type 2 diabetes (T2D) susceptible locus in a Mexican population.[31] Considering the evidence that GDM and T2D share a common genetic background and similar pathogenic mechanisms,[32] and that estrogen might be implicated in GDM pathogenesis, in the present study we for the first time investigated whether the polymorphism rs1256031 in *ESR2* gene is associated with GDM susceptibility.

METHODS AND MATERIALS

Subjects

A total of 380 unrelated Chinese pregnant women including 241 GDM patients and 139 controls were recruited from Buji People's Hospital and Songgang People's Hospital, Shenzhen, China, between July 2012 and May 2013. All pregnant women were routinely required to undertake the one-step GDM screening: a 2h 75g oral glucose tolerance test (OGTT) at 24–28 gestational weeks. The diagnosis of GDM was made according to the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) criteria: fasting plasma glucose (FPG) ≥ 5.1 mmol/L, or 1h plasma glucose (1h-PG) ≥ 10.0 mmol/L, or 2h plasma glucose (2h-PG) ≥ 8.5 mmol/L.[33] Subjects meeting the following criteria were excluded from our study: 1) pregnant women with a past medical history of diabetes; 2) pregnant women with a family history of diabetes or hypertension; 3) pregnant women without a medical history of diabetes but who were diagnosed as pre-gestational diabetes mellitus (PGDM) at their first antenatal examination.

The institutional review board of the Clinical Research Institute at Buji People's Hospital

and Songgang People's Hospital approved the study protocol, and written informed consent was obtained from each subject. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Clinical and biochemical measurements

Anthropometric variables including height and weight before pregnancy were recorded at 24–28 weeks of gestation. Pre-pregnancy body mass index (pre-BMI) (kg/m^2) was calculated from these data. Other clinical information, such as age, educational background and family history of hypertension and diabetes, was also collected.

Genotype analysis

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). The polymorphism rs1256031 was genotyped using the Agena MassARRAY iPLEX platform (Agena Inc., CA). Primers are listed in Supplementary Table S1. For quality control we randomly tested 5% of samples using the same sets of primers, and the results were 100% consistent.

Statistical Analysis

Descriptive statistics were applied to summarize the characteristics of the GDM group and the control group in our study population. Independent sample t-test was performed to compare the differences between groups of continuous variables with normal distribution, and Pearson chi-squared test or Fisher's exact test was performed for categorical variables. Hardy–Weinberg equilibrium (HWE) for each SNP was examined by Chi-square test. The association between rs1256031 and GDM risk was analyzed by binary logistic regression and estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) using additive, dominant and recessive genetic models with confounder factors adjusted. Pre-pregnancy BMI (kg/m^2), age (years), parity (nulliparous, multiparous), educational levels (high school or lower, college or above), history of delivering infants with RDS (yes, no) and history of macrosomia delivery (yes, no) were selected as the covariates included in the final models based on their biological plausibility reported by previous studies. All of the statistical tests were performed with SPSS 16.0 software and were two-sided. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population

The selected characteristics of 380 participants are presented in Table 1. Among 380 participants, 241 (63.42%) women were diagnosed with GDM, and 139 (36.58%) women were healthy controls. GDM patients exhibited significantly higher pre-pregnancy BMI (22.15 vs. 21.69, kg/m^2) than healthy controls. No statistically significant differences were observed in any other listed clinical characteristics between the two groups.

rs1256031 and GDM susceptibility

The observed genotype distribution of rs1256031 in both cases and controls was consistent with Hardy-Weinberg equilibrium ($P > 0.05$, Table 2). Three different genetic models were applied in the logistic regression analysis with adjustment for confounding factors. As presented in Table 2, no significant association was identified between rs1256031 and GDM susceptibility.

Table 1 Clinical and biomedical characteristics of the study population

Characteristics	All Participants (*n*=380)	Cases (*n*=241)	Controls (*n*=139)	*P*-value
Age (year)	28.55 ± 4.96	28.52 ± 5.06	28.59 ± 4.80	0.899
Pre-pregnancy BMI (kg/m^2)	21.98 ± 1.95	22.15 ± 1.56	21.69 ± 2.47	0.047
History of Delivering Infants with RDS				0.366
Yes	1 (0.003%)	0 (0%)	1 (0.01%)	
No	379 (0.997%)	241 (100%)	138 (0.99%)	
History of Macrosomia Delivery				0.531
Yes	5 (1.3%)	2 (0.01%)	3 (0.02%)	
No	375 (98.7%)	239 (0.99%)	136 (0.98%)	
Parity				0.594
0	178 (46.84%)	110 (45.64%)	68 (48.92%)	
≥ 1	202 (53.16%)	131 (54.36%)	71 (51.08%)	
Educational Level				0.363
High School or Lower	344 (90.53%)	221 (91.70%)	123 (88.49%)	
College or Higher	36 (9.47%)	20 (8.30%)	16 (11.51%)	

BMI, body mass index; RDS, respiratory distress syndrome

Table 2 Genotype distributions of rs1256031 and the association with GDM in Chinese pregnant women

Model	Genotypes	GDM		Controls		OR (95% CI)	*P*-value
		Number	%	Number	%		
Additive	GG	38	15.8	20	14.4	1	
	GA	110	45.6	62	44.6	0.942 (0.494,1.794)	0.855
	AA	93	38.6	57	41.0	0.871 (0.453,1.675)	0.680
Dominant	GG	38	15.8	20	14.4	1	
	GA + AA	203	84.2	119	85.6	0.908 (0.495,1.665)	0.755
Recessive	GG + GA	148	61.7	82	59.0	1	
	AA	93	38.3	57	41.0	0.912 (0.591,1.408)	0.677

Hardy-Weinberg equilibrium: GDM $\chi^2 = 0.172$; $p = 0.918$. Control $\chi^2 = 0.132$; $p = 0.936$.

DISCUSSION

In the present study we for the first time investigated the association between the *ESR2* polymorphism rs1256031 and GDM susceptibility, and we observed no significant correlation of rs1256031 and GDM risk in our study subjects. In a recent study conducted in a Mexican cohort of 597 T2D patients and 605 controls, Herrera-Lopez et al reported that T2D patients with AA genotype of rs1256031 showed reduced disease risk compared with patients with GG genotype [OR (95% CI) = 0.492 (0.307,0.789); $P = 0.003$], indicating that the A allele was protective.[31] In concordance with their study, although we did not detect any statistically significant association between rs1256031 and GDM risk in the logistic regression analysis, we noticed that the OR value was below 1.0 in all three genetic models, suggesting that the AA and GA genotypes tend to play a protective role. Given the evidence implicating a critical role of estrogen receptors

Supplementary Table S1 Information of primers for MassARRAY iPLEX assays

SNP	Primer	Sequence (5'-3')
rs1256031	2nd-PCR Primer	ACGTTGGATGTTCTTCCCCTAGGCTAGGAG
	1st-PCR Primer	ACGTTGGATGGCGTTTAGAGAAAGTTAGGG
	Extend Primer	AGGGTCTCAGTTCACAATC

in the regulation of glucose homeostasis, there are several possible explanations for the lack of association with GDM susceptibility in the present study. First, as suggested by previous studies, there exist distinct pathogenic mechanisms for the development of GDM,[34] therefore susceptible variants of T2D may not necessarily be associated with GDM. Second, the functional effect of rs1256031 in GDM etiology may be weak so that the association could not be easily detected. Third, the lack of association with GDM risk may be due to lack of statistical power in only 241 patients and 139 controls. Fourth, the association between rs1256031 and GDM susceptibility may be occasioned by several factors that were not included and analyzed in this study such as differences in fetal sex, paternally transmitted variants of the fetus, dietary, physical activity habits, and psychological states.

The polymorphism rs1256031 residing in intron 2 of *ESR2* is close to the promoter region of this gene. Borgquist et al showed that the A allele of rs1256031 was associated with a less favorable prognosis for breast cancer after hormone therapy, implying that rs1256031 is functional.[35] It is currently not clear whether and how this nucleotide exchange influences the expression or activity of the receptor.

The minor allele frequency (MAF) of rs1256031 displays ethnic variations. According to the 1000 Genomes data, it is lower in East Asian (38%) and African (34%) populations, and higher in other populations such as American (53%) and European (43%) populations. In the present study, it is 37% in the control group, which is close to the MAF in the East Asian population (38%).

In conclusion, our results suggest that the rs1256031 of *ESR2* is not associated with GDM susceptibility in Chinese pregnant women. Future multi-centered studies with larger sample sizes are needed to validate this finding.

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CONFLICT OF INTEREST

No potential conflicts of interest were disclosed.

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