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ORIGINAL ARTICLE

Novel association between a transient receptor potential cation channel subfamily M member 5 expression quantitative trait locus rs35197079 and decreased susceptibility of gestational diabetes mellitus in a Chinese population

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Keywords
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ABSTRACT

Aims/Introduction: Emerging evidence suggests that expression quantitative trait loci (eQTLs) are more likely to associate with complex diseases. Transient receptor potential cation channel subfamily M member 5 (TRPM5) is a ubiquitously expressed voltage-gated cation channel that acts indispensably to trigger insulin secretion in pancreatic β-cells. The present study evaluated the association between *TRPM5* eQTL single-nucleotide polymorphisms and the risk of gestational diabetes mellitus (GDM) in a Chinese population.

Materials and Methods: A total of 380 unrelated Chinese pregnant women including 241 GDM patients and 139 controls were included in this study. The eQTL single-nucleotide polymorphisms of *TRPM5* were obtained from the GTEx eQTL Browser, and were subsequently genotyped using the Agena MassARRAY iPLEX platform.

Results: Logistic regression analysis and linear regression analysis showed that rs35197079 and rs74848824 were significantly associated with reduced GDM risk and lower fasting plasma glucose levels after adjusting confounder factors in dominant genetic models. Stratification analysis based on pre-pregnancy body mass index validated a strong association between rs35197079 and GDM susceptibility in underweight and normal weight individuals. Luciferase and electrophoretic mobility shift assays carried out in rat pancreatic β-cells showed that rs35197079 was functional.

Conclusions: The *TRPM5* eQTL single-nucleotide polymorphism rs35197079 was associated with decreased GDM susceptibility in a Chinese population, especially in underweight and normal weight pregnant women, and it was functional in modulating gene transcription.

INTRODUCTION

Gestational diabetes mellitus (GDM), defined as abnormal glucose tolerance with onset or first recognition during pregnancy, is a major perilous disease during pregnancy threatening both the mother and the offspring[1–4]. The prevalence of GDM in recent years worldwide ranges from 8.9 to 53.4% and is rapidly increasing each year. GDM not only brings about adverse pregnant outcomes, such as stillbirth, macrosomia, shoulder dystocia, neonatal hypoglycemia and neonatal respiratory distress syndrome, but also has substantial long-term negative effects on the health of both patients and their offspring in that they

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might have considerable risk of developing type 2 diabetes mellitus, obesity, metabolic syndromes and cardiovascular diseases in later life[5–9]. Therefore, developing effective early detection and intervention strategies to protect pregnant women from maternal and fetal complications has become an urgent need.

It is well established that genetic components play a major role in the etiology of this multifactorial disease[10,11]. However, to date, published studies of the genetic susceptibility to GDM are very limited, and most susceptible loci were identified through analyzing the risk variants for type 2 diabetes mellitus, because the genetic background of the two diseases are similar[12,13]. Transient receptor potential cation channel subfamily M member 5 (TRPM5) is a voltage-modulated and calcium-activated cation channel expressed in a wide variety of cells and tissues, including taste cells, pancreatic β-cells, gastrointestinal cells, neurons and myocytes[14–16]. A study carried out by Ketterer *et al.* in a German population in which researchers selected nine single-nucleotide polymorphisms (SNPs) covering 100% variations of the region of interest, namely the coding region of *TRPM5*, 3 kb of its 5′-flanking region and 0.5 kb of its 3′-flanking region, showed that polymorphisms of *TRPM5* were associated with prediabetes phenotypes, including pancreatic β-cell dysfunction, suggesting a link between type 2 diabetes mellitus and TRPM5[17]. Furthermore, TRPM5 knockout mice show impaired glucose tolerance and reduced glucose-induced insulin secretion, suggesting that it acts indispensably to modulate this process in β-cells[18,19]. Additionally, TRPM5 is expressed in gastrointestinal cells and was shown to regulate secretion of glucagon-like peptide-1, which is important for controlling proper glucose homeostasis[14]. Given the aforementioned knowledge, it is reasonable to believe that TRPM5 might also be implicated in GDM pathogenesis.

Genomic loci explaining variations in gene transcription levels are commonly known as expression quantitative trait loci (eQTLs). Previous genetic studies assessing associations between candidate genes and disease susceptibility mostly focused on polymorphisms close to the gene coding region, and the functional impact of the identified susceptible SNPs, in most cases non-coding, is usually unclear. In contrast, eQTLs provide direct clues to how genetic variations affect gene expression. In recent years, accumulating evidence suggests that genetic variants associated with various complex traits and diseases are enriched in eQTLs[20–29]. Thus, in the present study, we investigated the association between *TRPM5* eQTL SNPs and GDM susceptibility, and further studied the functional roles of the identified susceptible variant.

MATERIALS AND METHODS

Participants

The Chinese pregnant women enrolled in this study were recruited between July 2012 and May 2013, from Buji people's hospital and Songgang people's hospital, Shenzhen, China. The diagnosis of GDM was made based on the one-step GDM screening: a 2-h 75-g oral glucose tolerance test at 24–28 gestational weeks, and we used the International Association of the Diabetes and Pregnancy Study Groups criteria: fasting plasma glucose (FPG) ≥5.1 mmol/L or 1-h plasma glucose (1 h-PG) ≥10.0 mmol/L or 2-h plasma glucose (2 h-PG) ≥8.5 mmol/L[30]. Normal glucose tolerance was defined as plasma glucose levels in the 75-g oral glucose tolerance test below all three of the following values: FPG 5.1 mmol/L, 1-h plasma glucose 10.0 mmol/L and 2-h plasma glucose 8.5 mmol/L.

We first recruited 241 women diagnosed with GDM into the patient group, and then later we randomly collected another 139 healthy pregnant women with normal glucose tolerance and without any maternal or fetal disorders admitted to the hospitals at the same time period as the control group. Women meeting the following criteria were excluded in our study: (i) pregnant women who had a past medical history of diabetes or had a family history of diabetes; (ii) pregnant women who were diagnosed as pre-gestational diabetes mellitus in their first antenatal examination; and (iii) pregnant women who had the following conditions or diseases: multiple gestation, pre-eclampsia, previous polycystic ovary syndrome, hypertension and autoimmune diseases.

The Ethics Committee of Shenzhen Research Institute of Population and Family Planning Science approved the study protocol on 31 March 2017 (Approval number: 2017JSKYS022), and written informed consent was obtained from each participant. All clinical investigations were carried out 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. The plasma glucose concentrations were measured with the glucose oxidase-peroxidase method using an automated biochemical instrument (Beckman Coulter AU5800, Brea, CA, USA).

Selection of candidate eQTL SNPs

We extracted cis-eQTL SNPs located within ±1 Mb of the transcriptional start site of *TRPM5* in the pancreas from the GTEx (Genotype-Tissue Expression) portal (http://gtexportal.org)[31]. Trans-eQTLs, which are distant from the *TRPM5* locus or on other chromosomes, or eQTLs affecting *TRPM5* expression in other tissues, were not included in the present study. We downloaded the genotypes of a 500-kb region covering all the retrieved TRPM5 eQTL SNPs (chr11: 2214044-2714044, hg19) in CHB from the 1,000 Genomes project website, and selected informative tag-SNPs based on linkage disequilibrium (LD) using ldSelect[32]. We binned SNPs with minor allele frequency ≥0.05 that were pairwise correlated at $r^2 > 0.8$.

Genotype analysis

Genomic DNA was isolated from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Germantown, MD, USA). All candidate eQTLs were genotyped using the Agena MassARRAY iPLEX platform (Agena Inc., San Diego, CA, USA). Primers are listed in Table S1. For quality control we randomly tested a 5% sample of cases and controls using the same sets of primers, and the results were 100% consistent.

Plasmid constructs, cell culture and luciferase assays

A 685-bp fragment flanking rs35197079 was amplified from a Chinese blood deoxyribonucleic acid sample homozygous for the major allele of rs35197079 by primers in Table S2, and cloned into HindIII and XhoI restriction sites upstream of luciferase gene in the pGL4.23 [luc2/minP] vector (Promega, Madison, WI, USA). Polymerase chain reactions were carried out with Phusion High-fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) to prevent artificial mutations. The construct containing the minor allele of rs35197079 was generated using Q5 Site-directed mutagenesis Kit (NEB, Ipswich, MA, USA) with primers listed in Table S2. The constructed plasmids were sequenced and results were as we expected.

Mouse insulinoma β-TC-6 cells were maintained in Dulbecco's modified Eagle medium (high glucose; Thermo Fisher Scientific) containing 15% fetal bovine serum, 50 μmol/L β-mercaptoethanol, 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in an atmosphere containing 5% CO_2, and the medium was changed every 2 days. For transfection analysis, $10^5/cm^2$ β-TC-6 cells were seeded onto 24-well plates in Dulbecco's modified Eagle medium 1 day before transfection. Cells were transfected with 950 ng plasmid constructs per well using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's recommendations. pRL-TK plasmids (50 ng; Promega) were co-transfected as an internal control. A total of 48 h after transfection, cells were harvested and luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega), and data were normalized to Renilla luciferase activity. Experiments were carried out three independent times and data are presented as the mean ± standard deviation of six replicates.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) probes labeled by biotin containing both alleles of rs35197079 were designed, namely, the A-probe with the major allele and the C-probe with the minor allele. The probe sequences are as follows: 5′-TTGCATTTTTCTTGTT**A**TAAATGAGGTTGAACATT-3′ (A-probe); 5′-TTGCATTTTTCTTGTT**C**TAAATGAGGTTGAACATT-3′ (C-probe). Nuclear extracts were isolated from β-TC-6 cells using the NE-PER nuclear and cytoplasmic extraction kit (Thermo Fisher Scientific) according to manufacturer's instructions and stored at −80°C. The biotin-labeled probes were incubated with or without the nuclear protein extract and in the presence or absence of unlabeled probes. EMSA was carried out using the Light-Shift chemiluminescent EMSA kit according to the manufacturer's instructions (Thermo Fisher Scientific).

Statistical analysis

Descriptive statistics were carried out to summarize the characteristics of the GDM group and the control group in our study population. Independent samples *t*-test was carried out to compare the differences between groups of continuous variables with normal distribution, and Pearson's χ^2-test or Fisher's exact test were carried out for categorical variables. The Hardy–Weinberg equilibrium for each SNP was examined by the χ^2-test. Associations between eQTL SNPs and GDM risk were analyzed by binary logistic regression and estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) using dominant genetic models with confounder factors adjusted. Pre-BMI (kg/m^2), maternal age at delivery (years), parity (nulliparous, multiparous) and educational levels (high school or lower, college or above) were selected as the covariates included in the final models based on their biological plausibility reported by previous studies. False positive report probability (FPRP) was estimated to evaluate the robustness of the statistically significant association using the method described by Wacholder *et al.*[33]. The FPRP threshold was set to 0.5, and the prior probability was set to 0.1. The FPRP value was calculated using the statistical power to detect an OR of 1.5 (or its reciprocal, 0.67). The association between SNPs and FPG levels was analyzed using independent samples *t*-test and multivariate linear regression in dominant genetic models with the above described confounder factors adjusted and the regression coefficients (*b*) with 95% CI presented. We further carried out stratified analysis of the susceptible variant and GDM risk based on pre-BMI. We subdivided women into two groups: underweight/normal weight and overweight/obese. We selected the BMI cut-off point of 23.0 kg/m^2 for overweight participants, according to a reported optimal cut-off value of BMI for urban Chinese female adults.[34] Bonferroni adjustment was applied to correct for multiple comparisons in the stratification analysis.

RESULTS

Clinical 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. GDM patients showed significantly higher pre-pregnancy BMI (22.15 vs 21.69 kg/m^2) and fasting plasma glucose levels (5.18 vs 4.31 mmol/L) than healthy controls. No statistically significant differences were observed in all other listed clinical characteristics between the two groups (Table 1).

Candidate eQTL SNPs

A total of 23 *TRPM5* eQTL SNPs in pancreas tissue were retrieved from the GTEx database (Table S3). We found that 16 *TRPM5* eQTL SNPs were within the same LD block (Table S3), and any of them could serve as the tag-SNP for this

Table 1 | Clinical and biomedical characteristics of the study population

Characteristics	All participants (n = 380)	Cases (n = 241)	Controls (n = 139)	P-value
Age (years)	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*
FPG (mmol/L)	4.86 ± 0.80	5.18 ± 0.72	4.31 ± 0.62	<10^{-6}*
Parity				
0	178 (46.84%)	110 (45.64%)	68 (48.92%)	0.594
≥1	202 (53.16%)	131 (54.36%)	71 (51.08%)	
Educational level				
High school or lower	344 (90.53%)	221 (91.70%)	123 (88.49%)	0.363
College or higher	36 (9.47%)	20 (8.30%)	16 (11.51%)	

Data were presented as n (%) and mean ± standard deviation. *Significant P-values ($P < 0.05$). BMI, body mass index; FPG, fasting plasma glucose.

LD block. We further examined the chromatin features of these 16 *TRPM5* eQTL SNPs using the UCSC Genome Browser, and found that the chromatin features of rs35197079 were particularly interesting. As shown in Figure S1, rs35197079 locates within DNaseI hypersensitive sites that mark open chromatin regions, indicating that it likely resides in a regulatory element. Furthermore, multiple transcription factors bind to this site, implicating its possible role in gene expression regulation. Thus, we finally selected rs35197079 as the tag-eQTL for this LD block in the subsequent genotyping experiment. The rest of the *TRPM5* eQTL SNPs were not in close linkage disequilibrium with each other and should all be selected for genotyping, so eventually we chose a total of eight representative *TRPM5* eQTL SNPs for genotyping (Table S3).

TRPM5 eQTL SNPs and GDM susceptibility

The observed genotype distribution of all the genotyped eQTL SNPs in both cases and controls was consistent with the Hardy–Weinberg equilibrium. To enhance the statistical power, we combined the rare homozygous genotype with the heterozygous genotype to compare with the wild-type genotype in the dominant genetic model. According to the results of logistic regression analysis shown in Table 2, GDM risk in AC and CC carriers of rs35197079 was 45.6% lower compared with the wild-type AA genotype carriers (OR 0.544, 95% CI 0.317–0.934, $P = 0.027$), and CG and GG carriers of rs74848824 had a significantly lower risk compared with the CC genotype participants (OR 0.619, 95% CI 0.404–0.948), $P = 0.027$), suggesting that the minor alleles of these two SNPs were protective

Table 2 | Genotype or allele distributions and association with gestational diabetes mellitus in Chinese women

SNP ID	Genetic model	Genotypes/minor allele	GDM n	Controls n	OR (95% CI)	P-value
rs11022827	Dominant	C/C	97	58	1	
		C/T + T/T	143	81	1.007 (0.655–1.549)	0.975
rs7105456	Dominant	A/A	108	65	1	
		A/G + G/G	125	69	1.055 (0.685–1.624)	0.808
rs11602629	Dominant	T/T	184	100	1	
		T/C + C/C	56	39	0.790 (0.489–1.278)	0.338
rs12277647	Dominant	C/C	84	42	1	
		C/A + A/A	155	97	0.795 (0.505–1.251)	0.321
rs7106016	Dominant	C/C	79	39	1	
		C/T + T/T	162	100	0.851 (0.535–1.354)	0.496
rs74848824	Dominant	C/C	127	57	1	
		C/G + G/G	113	82	0.619 (0.404–0.948)	0.027*
rs80194575	Dominant	C/C	169	89	1	
		C/G + G/G	71	50	0.735 (0.469–1.153)	0.181
rs35197079	Dominant	A/A	207	106	1	
		A/C + C/C	34	33	0.544 (0.317–0.934)	0.027*

P-values were adjusted for age, pre-pregnancy body mass index, parity and educational levels. *Significant P-values ($P < 0.05$). CI, confidence interval; GDM, gestational diabetes mellitus; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 3 | False positive report probability values of associations between rs35197079/rs74848824 and gestational diabetes mellitus susceptibility

SNP	Genotype (cases/controls)		Statistical power	OR (95% CI)	*P*-value	FPRP value
	Common†	Heterozygous + rare‡				
rs35197079	207/106	34/33	0.195	0.528 (0.310–0.899)	0.018	0.4625
rs74848824	127/57	113/82	0.363	0.618 (0.405–0.944)	0.025	0.3918

CI, confidence interval; FPRP, false positive report probability; OR, odds ratio; SNP, single-nucleotide polymorphism. †AA for rs35197079, CC for rs74848824. ‡AC + CC for rs35197079, CG + GG for rs74848824.

Table 4 | Correlations of rs35197079 and rs74848824 with fasting plasma glucose levels

SNP ID	Minor allele		Fasting plasma glucose
rs35197079	C	b^{dom} (95% CI)	−0.145 (−0.272, −0.018)
		P^{dom} value	0.026*
rs74848824	G	b^{dom} (95% CI)	−0.110 (−0.207, −0.013)
		P^{dom} value	0.026*

P-values were adjusted for age, pre-pregnancy body mass index, parity and educational levels. *Significant *P*-values ($P < 0.05$). CI, confidence interval; SNP, single-nucleotide polymorphism.

Table 5 | Relationships of rs35197079 and rs74848824 with fasting plasma glucose levels

Genotypes	Fasting plasma glucose	
	Mean ± SD	*P*-value
rs35197079		
AA	4.91 ± 0.80	
AC/CC	4.63 ± 0.74	0.007*
rs74848824		
CC	4.93 ± 0.86	
CG/GG	4.81 ± 0.74	0.151

*Significant *P*-values ($P < 0.05$). SD, standard deviation.

against GDM. No significant association was detected between GDM susceptibility and other genotyped SNPs. Then we calculated FPRP values for rs35197079 and rs74848824, and found that the estimated FPRP of both rs35197079 and rs74848824 were below the prespecified FPRP value of 0.5 (Table 3), suggesting that this finding is noteworthy.

Correlations between rs35197079/rs74848824 and FPG levels

We further examined the effect of rs35197079 and rs74848824 on FPG levels using multivariate linear regression analyses, and found that both rs35197079 and rs74848824 were significantly associated with this trait after adjusting confounder factors in dominant models (Table 4). However, we did not observe any significant association between rs74848824 and FPG levels when using independent samples *t*-test (Table 5).

Stratification analyses

We further carried out stratification analysis to evaluate the potential association between rs35197079 and GDM risk in subgroups based on pre-BMI. In the 'underweight/normal weight' group (pre-BMI <23.0), rs35197079 was strongly associated with GDM susceptibility (OR 0.412, 95% CI 0.217–0.783; $P = 0.007$), and the AC and CC genotypes decreased the GDM risk by ~58.8% (Table 6). After Bonferroni correction, the significance of this association still existed. No significant association was observed between rs35197079 and GDM susceptibility in the 'overweight/obese' group. No significant association was observed between rs74848824 and GDM susceptibility in both subgroups after Bonferroni correction.

Comparison of transcriptional activity between variants of rs35197079

To investigate the functional impact of rs35197079 on gene transcription, we constructed luciferase plasmids containing a 685 bp segment surrounding rs35197079 and generated plasmids with the minor allele through site-directed mutagenesis. As shown in Figure 1, the C allele of rs35197079 showed significantly lower luciferase expression than the A allele, indicating that rs35197079 was functional and could regulate gene expression in pancreatic β-cells.

Minor C allele of rs35197079 increased binding affinity of specific nuclear proteins

We carried out an EMSA experiment to further explore the mechanism of rs35197079 in regulating gene expression. We found that the minor allele of rs35197079 had an enhanced binding with nuclear proteins extracted from the mouse insulinoma β-TC-6 cells (Figure 2).

DISCUSSION

In the present study, for the first time, we examined the association between eQTL SNPs of *TRPM5* and GDM susceptibility, and found that rs35197079 was significantly associated with reduced GDM risk in Chinese pregnant women, and it could modulate gene transcription activity. We observed a significant association of both rs35197079 and rs74848824 with GDM susceptibility in logistic regression analysis, and results of the FPRP analysis suggested that the association between rs35197079/rs74848824 and GDM risk is authentic. Further statistical

Table 6 | Stratified analysis of the association between rs35197079/rs74848824 and gestational diabetes mellitus risk based on pre-pregnancy body mass index

Stratifying factors	Underweight/normal weight (n = 273)			Overweight/obese (n = 107)		
	Cases/controls	OR (95% CI)	*P*-value	Cases/controls	OR (95% CI)	*P*-value
rs35197079						
AA	154/72	1		53/34	1	
AC + CC	22/25	0.412 (0.217, 0.783)	0.007*	12/8	0.994 (0.364, 2.716)	0.991
rs74848824						
CC	89/41	1		38/16	1	
CG + GG	86/56	0.692 (0.418, 1.146)	0.153	27/26	0.440 (0.198, 0.978)	0.044

P-values were adjusted for age, parity and educational levels. *Significant *P*-values ($P < 0.05$).

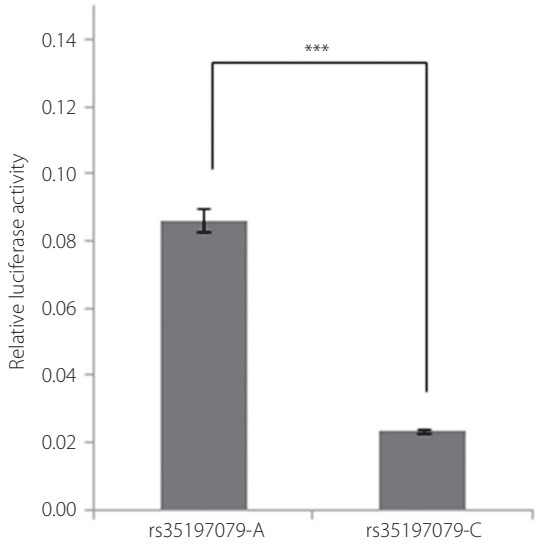

Figure 1 | The transcriptional activity of different rs35197079 alleles in mouse insulinoma cells (β-TC-6). All data are normalized by the read from pGL4.23 [luc2/minP] vector and expressed as the mean ± standard deviation (***$P < 0.001$).

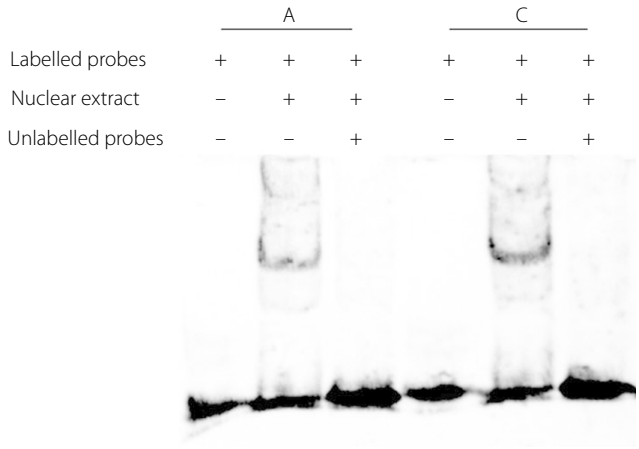

Figure 2 | Analysis of deoxyribonucleic acid binding affinity with the wild-type (A) and mutant (C) alleles of rs35197079 by electrophoretic mobility shift assay carried out with β-TC-6 nuclear extracts.

analysis assessing the association between rs35197079/rs74848824 and FPG levels validated the relationship of rs35197079 and gestational glucose metabolism-related trait, and stratification analysis showed that rs35197079 was strongly associated with decreased GDM risk in women with lower bodyweight (pre-BMI <23.0). Additionally, luciferase assays and EMSA experiments provided evidence that rs35197079 was functional in pancreatic β-cells.

Nowadays, the most effective strategy for identifying causative variants for multifactorial diseases is large-scale GWA studies. However, due to the difficulties in enrolling a great number of pregnant women with GDM and suitable healthy controls, GWA studies on GDM are rarely carried out, and knowledge about genetic components of this disease is relatively scarce, underlining the importance of identifying new disease-causing variants for this disease.[11] Recently, in genetic research on multifactorial diseases, growing attention has been attracted to eQTLs, which modulate levels of gene expression and therefore could have a great chance of being pathogenic. In the present study we reported a newly identified GDM susceptible SNP rs35197079, which was associated with a reduced risk in the present study participants, adding to the current knowledge about the genetics of GDM and corroborating the notion that examining eQTLs of potential pathogenic genes is an effective approach when searching for susceptible variants of complex diseases.

Despite the extensive knowledge about the regulatory function of TRPM5 in glucose metabolism in diverse tissues, the relationship between TRPM5 and GDM has not been investigated so far, and none of these *TRPM5* eQTL SNPs have been previously explored in any human diseases. The present results once again showed the important role of TRPM5 in regulation of glucose homeostasis, and provided evidence that its expression level in the pancreas likely affects glucose tolerance during pregnancy. The present study and the previous Ketterer's study

collectively suggest that *TRPM5* is an important candidate pathogenic gene in glucose metabolic disorders.

Although both rs35197079 and rs74848824 were significantly associated with GDM susceptibility in the logistic regression models (Table 2), we noticed that the OR for CG+GG genotypes of rs74848824 relative to CC (OR 0.619, 95% CI 0.404–0.948) is higher than the OR for AC+CC genotypes of rs35197079 relative to AA (OR 0.544, 95% CI 0.317–0.934), suggesting that the protective effect of rs74848824 is weaker than rs35197079. We further carried out stratification analysis based on pre-BMI, and we were also unable to detect any significant association between rs74848824 and GDM risk in both subgroups after Bonferroni correction. In addition, information from the UCSC Genome Browser (genome.ucsc.edu; Assembly GRCh37/hg19) showed that rs74848824 is not located within open chromatin regions, nor does it bind to any regulatory transcription factor (Figure S1), indicating that rs74848824 is less likely a functional variant. Of note, luciferase assays and EMSA experiments further provided evidence that rs35197079 was functional in pancreatic β-cells. Taken together, we assume that rs35197079 was a more potent pathogenic factor, whereas the relevance of rs74848824 to GDM is not very affirmative.

In the stratification analysis we found that rs35197079 was strongly associated with reduced GDM risk in women with normal or lower bodyweight, but not in the overweight/obese women (Table 6). It is possible that in the overweight/obese group, the impact of high bodyweight is robust, so that it prevails over the protective effect of rs35197079. If future studies with larger sample sizes could validate the results, then rs35197079 might be used as a genetic marker in the diagnosis of GDM patients with normal or lower bodyweight.

Information from the GTEx database shows that rs35197079 regulates TRPM5 transcription in human pancreas tissue (Figure S2), and luciferase and electrophoretic mobility shift assays in rat pancreatic β-cells validated that rs35197079 could modulate gene transcription. We speculate that rs35197079 likely represses TRPM5 expression. However, it should be noted that the genotype distribution of rs35197079 represented that of all 16 *TRPM5* eQTL SNPs in the same LD block (Table S3), of which rs35197079 might not be the only functional variant. It has been suggested that variants in close LD might cooperatively or reciprocally regulate transcriptional activity[35–37]. Future experiments examining functions of other *TRPM5* eQTL SNPs in the same LD block would reveal the combinatorial effects of those SNPs on *TRPM5* expression.

Glucose-stimulated insulin secretion in β-cells shows a distinct oscillatory pattern accompanied by parallel rhythmic changes of the cell membrane potential and cytosolic Ca^{2+} level[38,39]. The rhythmic activities in β-cells reflect a complex interplay among a variety of ion channels, intracellular Ca^{2+} levels and the cellular metabolism[40,41]. It has been suggested that this oscillatory pattern plays a critical role in the regulation of insulin secretion. Disruption of this oscillation in *Trpm5*$^{-/-}$ mice resulted in impaired glucose-stimulated insulin secretion. Activation of TRPM5 has been proposed to lead to membrane depolarization required for glucose-induced insulin secretion[42]. In addition, using arginine to sufficiently depolarize β-cell membrane in *Trpm5*$^{-/-}$ mice still led to a significant reduction of insulin secretion, indicating an additional role of TRPM5 in the secretion process aside from membrane depolarization[19]. Given its essential function and involvement in an intricate regulatory network in the process of glucose-induced insulin secretion and the fact that its transcription level in human islets is relatively low[18,42–45], it is highly likely that its transcription is under stringent control. The present results showed that the alternative C allele of the tag-SNP rs35197079 was associated with reduced GDM risk. It is possible that the 16 *TRPM5* eQTL SNPs in close LD with rs35197079 function collaboratively to protect against the disease through increasing TRPM5 expression and activity.

The sample size in the present study was modest and might not have the optimal statistical power. Multicentered research with larger sample sizes in geologically diverse populations would better evaluate the contribution of these SNPs to GDM susceptibility.

For the first time, we systematically examined the association between *TRPM5* eQTL SNPs and GDM susceptibility, and reported a novel GDM susceptible SNP rs35197079 in Chinese pregnant women that was protective against the disease, possibly by modulating levels of *TRPM5* transcription. Further studies with larger sample sizes are required to verify these findings, and functional experiments are needed to elucidate the detailed mechanism for its protective effects.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Information of primers for Sequenom MassARRAY iPLEX assays.

Table S2 | Primers in plasmid construction and mutagenesis.

Table S3 | *TRPM5* expression quantitative trait loci single-nucleotide polymorphisms downloaded from the GTEx database.

Figure S1 | ENCODE data of histone modifications, TF binding and open chromatin measured by ChIP-seq and DNase-seq of the genomic regions surrounding (a) rs35197079 and (b) rs74848824.

Figure S2 | The box plot of TRPM5 expression as a function of rs35197079 genotypes in pancreas tissue downloaded from the GTEx eQTL Browser.