RESEARCH ARTICLE

Association of vascular endothelial growth factor expression and polymorphisms with the risk of gestational diabetes mellitus

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Objective: To study the associations of vascular endothelial growth factor (VEGF) expression and its gene polymorphisms with the risk of gestational diabetes mellitus (GDM).

Methods: A total of 239 GDM patients (GDM group) and 275 healthy pregnant women (Control group) were included in this study. VEGF genotypes (including rs2146323, rs2010963, rs3025039, rs3025010, and rs833069) were analyzed by TaqMan assay. ELISA was used to determine the serum VEGF levels. The software SHEsis was performed to analyze haplotypes.

Results: The carrier with the rs2146323 AA, CA+AA genotypes, and A allele, as well as the rs3025039 CT, TT, CT+TT genotypes, and T allele showed the increased risk of GDM (all $P < 0.05$), but the distributions of genotype and allele at rs2010963, rs3025010, and rs833069 were not significantly different between GDM patients and controls (all $P > 0.05$). Notably, the frequency of rs2010963-rs833069-rs2146323-rs3025010 haplotypes CAAC, CAAT, CACC, CACT, GACT, and GGCT was found statistically different between GDM patients and controls (all $P < 0.05$). The patients with rs3025039 CT+TT genotype had higher VEGF levels than those with CC genotype (all $P < 0.05$). Besides, age, family histories of diabetes, previous GDM, hypertension, pre-pregnancy body mass index, fasting plasma glucose, fasting insulin, homeostasis model assessment (HOMA)-IR, rs2146323 CA+AA, rs3025039 CT+TT, and VEGF expression level were independent risk factors, while HOMA- β was an independent protective factor for GDM (all $P < 0.05$).

Conclusion: VEGF rs2146323 and rs3025039 polymorphisms and its expression were significantly correlated with the risk of GDM, providing a great clinical value for GDM assessment and diagnosis.

KEYWORDS

gestational diabetes mellitus, risk, single nucleotide polymorphism, VEGF

1 | INTRODUCTION

Gestational diabetes mellitus (GDM), as one of the common complications during pregnancy, is a complex metabolic disorder closely related to the increased risk of adverse outcomes for both mothers and offspring,^{1,2} such as excessive amniotic fluid, macrosomia,

gestational hypertension, and neonatal respiratory distress syndrome.^{3,4} Besides, the incidence of GDM is increasing rapidly, especially in pregnant women with GDM history, who had an approximate 17%-63% chance of progressing into type 2 diabetes mellitus (T2DM) within 5-16 years after childbirth.^{5,6} Mechanically, GDM is believed as a clinical syndrome caused by the interaction

of genetic and environmental factors, which has a similar genetic basis with T2DM.⁷ In recent years, genomewide association study (GWAS) has revealed a close relation of the gene single nucleotide polymorphism (SNP) in the genetic susceptibility to T2DM, including TCF7L2, SLC30A8, HHEX, FTO, PPARG, and KCNJ11.^{8,9} GWAS was also applied for candidate genes of pregnant women and found that some T2DM-susceptibility genes were also associated with the increased risk of GDM.¹⁰

Recent studies have reported that the gene polymorphism and expression level of vascular endothelial growth factor (VEGF) are closely related to the pathogenesis of T2DM, as well as its corresponding complications.^{11,12} VEGF gene is located at the p21.3 of chromosome 6, consisting of seven introns and eight exons with a full molecular length of 14 kb.¹³ As an endothelial cell-specific mitogen, VEGF has identified to have various functions, such as promoting endothelial cell proliferation, increasing vascular permeability, and inducing angiogenesis.^{14,15} Actually, a number of factors, including oxidative stress, hypoxia, and inflammatory response, could induce the increase in VEGF expression in the presence of high glucose.¹⁶ In the study by Zhou *et al*,¹⁷ the serum VEGF levels measured by ELISA were found to be up-regulated in T2DM patients. The significant elevation of serum VEGF expression also constituted a risk factor for the progression of diabetic retinopathy (DR), as indicated by Ozturk *et al*.¹⁸ Additionally, VEGF +936C/T polymorphism contributed to the risk of developing T2DM according to the study of Porojan *et al*.¹⁹ The polymorphism in the promoter region of VEGF gene was significantly linked to the risk of retinopathy in T2DM patients.²⁰ More importantly, this genetic variation could dysregulate the levels of VEGF protein expression.^{21,22} Nevertheless, there are little data related to the association of VEGF polymorphisms and its expression with GDM.

Therefore, by screening Tag SNPs with the software Haploview (Broad Institute, Cambridge, MA, USA), we selected five polymorphic loci of VEGF gene, to explore the correlation of VEGF polymorphism and its expression with GDM, which would provide a scientific support and theoretical basis for the early diagnosis, intervention, and individualized treatment of GDM.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

This study was approved by the Ethics Committee of Yantai Shan Hospital and conformed to the Helsinki Declaration,²³ and all patients signed the informed consent form prior to the study.

2.2 | Subjects

From October 2015 to October 2017, GDM patients admitted to our hospital were recruited for this study and they were given 75 g oral glucose for oral glucose tolerance test (OGTT) at the 24th–28th week of pregnancy. The OGTT results were evaluated by using the diagnostic criteria published by American Diabetes

Society (ADA) in 2011,²⁴ with the following threshold plasma glucose levels: 5.3 mmol/L at 0 hour, 10 mmol/L at 1 hour, and 8.6 mmol/L at 2 hours. To be specific, if the value at each time point is lower than corresponding threshold level, patients have normal glucose tolerance (NGT), and if the value at two or more time points is higher than corresponding threshold level, patients would be confirmed as GDM. At last, 239 GDM patients constituted the GDM group, while 275 healthy pregnant women with normal glucose tolerance were in the Control group. The exclusion criteria were as follows: (a) patients with DM before pregnancy; (b) patients with severe system diseases (connective tissue disease, endocrine disease, chronic liver disease, etc); and (c) patients received any hormone drugs.

2.3 | Detection of VEGF polymorphism and expression

Through the human genome databases, like <https://browser.1000genomes.org/index.html> and the software Haploview, Tag SNPs were selected according to the criteria of SNP selection. In this study, five polymorphic loci of the VEGF gene were selected, namely rs2146323, rs2010963, rs3025039, rs3025010, and rs833069. Fasting peripheral blood of 2 mL was taken from all subjects, anticoagulated by EDTA, and centrifugated for 10 minutes at the rate of 1000 g before the extraction of total DNA with a DNA Extraction Kit (Takara Company, Tokyo, Japan). Then, total RNA was determined for purity with a DNA concentration detector (NanoDrop 2000; Thermo Fisher Scientific, San Jose, CA, USA), with ratios of absorbance A260 nm/A280 nm between 1.6 and 1.8. The DNA extracted was cryopreserved at -80°C in a refrigerator. Besides, the genotyping was performed by using the TaqMan assay and the ABI 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). The expression levels of VEGF in peripheral blood of patients were detected by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the detection kit (R&D Systems, Abingdon, UK). The experiment was repeated three times.

2.4 | Collection and determination of clinical indicators

General information of subjects was enquired and recorded, including age, recent illness, past medical history, personal history, pregnancy history, and pre-pregnancy weight, and height, blood pressure, and other indicators were also determined. The pre-pregnancy body mass index (BMI) was calculated according to the formula: pre-pregnancy BMI = pre-pregnancy weight (kg)/height² (m²). Besides, an automatic biochemical analyzer (HITACHI, Tokyo, Japan) was used to detect the levels of triglyceride (TG), serum total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Glucose oxidase method was used to detect the fasting blood glucose, and chemiluminescence method was used to determine the fasting plasma insulin. Homeostasis model assessment (HOMA) was

performed to assess insulin resistance and β -cell function. The following formulas were used for corresponding calculation: Insulin resistance index (HOMA-IR) = fasting plasma insulin $\times$ fasting blood glucose/22.5, and β -cell function (HOMA- β) = $20 \times$ fasting plasma insulin/(fasting blood glucose - 3.5).²⁵

2.5 | Statistical methods

The software SPSS 19.0 (SPSS Inc, Chicago, IL, USA) was applied for statistical analysis. The genotype frequencies of VEGF were checked for the Hardy-Weinberg equilibrium using chi-square test (χ^2). Univariate and multivariate logistic regression analysis models were used to calculate the odds ratio (OR) and its 95% confidence interval (CI), so as to evaluate the influence of genotypes on the risk of GDM. The haplotype of VEGF polymorphisms was analyzed by using SHEsis. The linkage disequilibrium of genotype and allele pairs was calculated via the Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). In addition, enumeration data were presented by ratio or percentage and compared by chi-square test, while measurement data were presented by mean $\pm$ standard deviation ($\bar{x} \pm s$) and tested by Student's *t* test. Power analysis with an α error of 5% was performed using the QUANTO program (Gauderman & Morrison, 2006)²⁶. Statistical significance was set at $P < 0.05$.

TABLE 1 Comparison of the general clinical data of the subjects between two groups

	Control group (n = 275)	GDM group (n = 239)	<i>t</i> / χ^2	<i>P</i>
Age (y)	30.64 $\pm$ 4.22	32.83 $\pm$ 5.56	5.066	<0.001
Mean gestational weeks (W)	26.39 $\pm$ 2.38	26.15 $\pm$ 2.46	1.123	0.262
Number of pregnancies	1.55 $\pm$ 0.50	1.64 $\pm$ 0.48	1.141	0.253
Conception (spontaneous/ART)	253/22	219/20	0.023	0.879
Smoking (Yes/No)	5/270	9/230	1.830	0.176
Family history of diabetes (Yes/No)	72/203	155/84	77.551	<0.001
Previous GDM (Yes/No)	2/273	24/215	23.101	<0.001
Hypertension (Yes/No)	13/262	90/149	86.531	<0.001
Pregnancy-induced hypertension (Yes/No)	30/245	40/199	5.264	0.022
Pre-pregnancy BMI (kg/m ²)	21.42 $\pm$ 3.14	22.35 $\pm$ 3.27	3.285	0.001
FPG (mmol/L)	4.75 $\pm$ 0.51	5.68 $\pm$ 0.84	15.39	<0.001
FINS (mU/L)	2.41 $\pm$ 0.26	2.56 $\pm$ 0.31	5.965	<0.001
TG (mmol/L)	1.12 $\pm$ 0.31	1.15 $\pm$ 0.26	1.179	0.239
TC (mmol/L)	5.56 $\pm$ 1.04	5.62 $\pm$ 1.15	0.621	0.535
SBP (mm Hg)	121.26 $\pm$ 11.45	126.15 $\pm$ 12.25	4.675	<0.001
DBP (mm Hg)	68.25 $\pm$ 6.52	74.52 $\pm$ 8.36	9.540	<0.001
HDL-C (mmol/L)	1.28 $\pm$ 0.52	1.51 $\pm$ 0.41	5.509	<0.001
LDL-C (mmol/L)	2.96 $\pm$ 0.82	3.63 $\pm$ 0.98	8.438	<0.001
HOMA- β	273.28 $\pm$ 112.36	215.34 $\pm$ 101.35	6.101	<0.001
HOMA-IR	2.06 $\pm$ 1.06	3.31 $\pm$ 1.42	11.400	<0.001

ART, assisted reproductive technique; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; FINS, fasting insulin; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

3 | RESULTS

3.1 | General clinical information of subjects

As shown in Table 1, the subjects in two groups were not statistically different from each other in mean gestational weeks, number of pregnancies, mode of conception, smoking, TG, and TC levels (all $P > 0.05$). Compared with the Control group, the patients in GDM group presented significant increases in age, pre-pregnancy BMI, FBG, fasting insulin (FINS), SBP, DBP, LDL-C, HDL-C, and HOMA-IR levels, but had a statistical reduction in HOMA- β level (all $P < 0.05$). Family histories of diabetes and previous GDM patients, as well as hypertension identified at initial obstetric visit and pregnancy-induced hypertension patients, were significantly different between the groups (all $P < 0.05$).

3.2 | Distribution of genotype and allele frequencies of VEGF polymorphisms

The genotype and allele frequency distribution of the selected population reached the Hardy-Weinberg equilibrium ($P > 0.05$), suggesting the representative of the population. Post hoc analysis revealed that our sample size had a power more than 0.8. As displayed in Table 2, compared with CC genotype, the patients carried with the AA genotype, CA+AA genotype, and A allele of rs2146323 had a significantly higher

risk of GDM (AA vs CC, OR = 1.999, 95% CI = 1.036-3.857, $P = 0.046$; CA+AA vs CC, OR = 1.494, 95% CI = 1.049-2.127, $P = 0.031$; C vs A, OR = 1.456, 95% CI = 1.095-1.935, $P = 0.011$). In comparison with the rs3025039 CC genotype carriers, the carriers with CT genotype, TT genotype, CT+TT genotype, and T allele of rs3025039 were obviously increased in GDM risk (CT vs CC, OR = 1.951, 95% CI = 1.358-2.803, $P < 0.001$; TT vs CC, OR = 6.034, 95% CI = 1.952-18.65, $P = 0.001$; CT+TT vs CC, OR = 2.118, 95% CI = 1.486-3.019, $P < 0.001$; T vs C, OR = 1.894, 95% CI = 1.416-2.532, $P < 0.001$). However, the genotype and allele frequency distribution of rs2010963, rs3025010,

and rs833069 in GDM group was not apparently different from the Control group (all $P > 0.05$).

3.3 | Haplotype analysis of VEGF gene polymorphisms

The linkage disequilibrium analysis revealed one SNP block that includes four SNPs: rs2010963, rs833069, rs2146323, and rs3025010 (Figure 1). Six major haplotypes (frequencies more than 0.03) were estimated, and each was compared between the Control group and

TABLE 2 The distribution of genotype and allele frequencies within the VEGF gene polymorphisms in subjects from two groups

	Control group, n = 275 (%)	GDM group, n = 239 (%)	OR (95% CI)	P	Power
rs2146323					
CC	174 (63.3)	128 (53.6)	Reference		0.825
CA	84 (30.5)	86 (36.0)	1.392 (0.954-2.030)	0.102	
AA	17 (6.2)	25 (10.4)	1.999 (1.036-3.857)	0.046	
CA+AA	101 (36.7)	111 (46.4)	1.494 (1.049-2.127)	0.031	
C allele	432 (78.5)	342 (71.5)	Reference		
A allele	118 (21.5)	136 (28.5)	1.456 (1.095-1.935)	0.011	
rs2010963					
GG	80 (29.1)	68 (28.4)	Reference		0.861
GC	145 (52.7)	135 (56.5)	1.095 (0.735-1.633)	0.685	
CC	50 (18.2)	36 (15.1)	0.847 (0.495-1.449)	0.587	
GC+CC	195 (70.9)	171 (71.5)	1.032 (0.703-1.513)	0.922	
G allele	305 (55.5)	271 (56.7)	Reference		
C allele	245 (44.5)	207 (43.3)	0.951 (0.743-1.217)	0.706	
rs3025039					
CC	177 (64.4)	110 (46.0)	Reference		0.991
CT	94 (34.2)	114 (47.7)	1.951 (1.358-2.803)	<0.001	
TT	4 (1.4)	15 (6.3)	6.034 (1.952-18.65)	0.001	
CT+TT	98 (35.6)	129 (54.0)	2.118 (1.486-3.019)	<0.001	
C allele	448 (81.5)	334 (69.9)	Reference		
T allele	102 (18.5)	144 (30.1)	1.894 (1.416-2.532)	<0.001	
rs3025010					
TT	153 (55.6)	123 (51.5)	Reference		0.879
TC	106 (38.5)	105 (43.9)	1.232 (0.860-1.765)	0.272	
CC	16 (5.8)	11 (4.6)	0.855 (0.383-1.910)	0.84	
TC+CC	122 (44.4)	116 (48.5)	1.183 (0.835-1.675)	0.375	
T	412 (74.9)	351 (73.4)	Reference		
C	138 (25.1)	127 (26.6)	1.080 (0.816-1.429)	0.617	
rs833069					
GG	54 (19.6)	43 (18.0)	Reference		0.844
GA	141 (51.3)	131 (54.8)	1.167 (0.732-1.859)	0.555	
AA	80 (29.1)	65 (27.2)	1.020 (0.608-1.712)	1	
GA+AA	221 (80.4)	196 (82.0)	1.114 (0.714-1.737)	0.653	
G	249 (45.3)	217 (45.4)	Reference		
A	301 (54.7)	261 (54.6)	0.995 (0.7778-1.273)	1	

OR, odds ratio; 95% CI, 95% confidence interval.

FIGURE 1 Linkage disequilibrium plot generated by Haploview 4.2

GDM group, as analyzed by the online software SHEsis (Table 3). As a result, the distribution of haplotype CAAC, CAAT, CACC, CACT, GACT, and GGCT was statistically different between the GDM group and the Control group (all $P < 0.05$), among which CAAC, CACT, and GGCT reduced the risk of GDM, while CAAT, CACC, and GACT increased the risk of GDM (all $P < 0.05$).

3.4 | Comparison of VEGF expression levels

As illustrated by Figure 2, the expression levels of VEGF in GDM patients were significantly higher than controls ($P < 0.05$). Besides, the subjects with rs3025039 CT+TT genotype had higher VEGF expression levels than those with corresponding CC genotype (all $P < 0.05$). However, the different genotypes and alleles of rs2146323, rs2010963, rs3025010, and rs833069 in patients had no observable influence on the expression levels of VEGF (all $P > 0.05$).

3.5 | Logistic regression analysis

The logistic regression analysis was conducted with GDM as the dependent variable, and with general clinical indicators, VEGF

SNPs, and VEGF expression level as independent variables, which was demonstrated in Table 4. Finally, the mean gestational weeks, number of pregnancies, mode of conception, smoking, pregnancy-induced hypertension, TG, TC, SBP, DBP, HDL-C, and LDL-C, as well as rs2010963, rs3025010, and rs833069 polymorphisms, were not significantly associated with the risk of GDM (all $P > 0.05$). Nevertheless, age, family histories of diabetes, previous GDM, hypertension, pre-pregnancy BMI, fasting plasma glucose (FPG), FINS, HOMA-IR, CA+AA genotype of rs2146323, CT+TT genotype of rs3025039, and VEGF expression levels were turned out to be the independent risk factors for GDM, and meanwhile, HOMA- β was suggested as the independent protective factor for GDM (all $P < 0.05$).

4 | DISCUSSION

Through detecting five SNPs within VEGF gene, we found that subjects carried with AA, CA+AA genotypes, and A allele of rs2146323, and those who carrying CT, TT, CT+TT genotypes, and T allele of rs3025039 had the dramatically increased risk of GDM, which suggested that these two genetic polymorphisms (rs2146323 and rs3025039) in the VEGF gene would be recognized as the susceptible factor for GDM. In agreement, VEGF rs2146323 A allele has been suggested by Ying Zeng *et al*²⁷ to increase the risk of DR via a meta-analysis. Besides, the TT genotype of 936C>T (rs3025039) in the VEGF gene was the risk factor for the pathogenesis of metabolic syndrome.²⁸ However, the resting three polymorphisms (rs2010963, rs3025010, and rs833069) were found unrelated to the risk of GDM in this study. In diabetic patients, previous studies also confirmed that the VEGF rs3025010 and rs833069 polymorphisms had no significant difference in the risk of DR.^{20,29} Interestingly, in South Korean and Japanese populations, rs2010963 was found to be closely associated with the incidence of DR,^{21,30} but did not show any associations with DR in the meta-analysis by Lu *et al*,³¹ which could possibly attributed to the interactions of different ethnic genetic and environmental factors between VEGF gene polymorphisms and diseases, leading to the great difference among different populations.^{32,33}

On the other hand, VEGF gene polymorphisms could induce the abnormal expression of VEGF and then aggravate the disease.²² After detecting the expression level of VEGF, the dramatically

TABLE 3 Haplotype analysis of VEGF gene polymorphisms rs2010963, rs833069, rs2146323, and rs3025010

Haplotype	GDM (freq)	Control (freq)	P	Odds ratio (95% CI)
CAAC	14.33 (0.030)	116.92 (0.213)	4.73E-16	0.129 (0.073-0.227)
CAAT	22.67 (0.047)	1.06 (0.002)	2.51E-07	29.139 (4.135-205.338)
CACC	40.97 (0.086)	21.06 (0.038)	0.000237	2.674 (1.556-4.597)
CACT	38.03 (0.080)	105.96 (0.193)	6.43E-06	0.411 (0.277-0.610)
GACT	94.66 (0.198)	55.98 (0.102)	2.42E-07	2.522 (1.762-3.610)
GGCT	54.21 (0.113)	249.00 (0.453)	1.33E-27	0.176 (0.127-0.245)

OR, odds ratio; 95% CI, 95% confidence interval.

FIGURE 2 The expression levels of vascular endothelial growth factor (VEGF) in different loci of subjects from two groups. A, Comparison of the VEGF expression levels between the gestational diabetes mellitus and Control groups; B-G, comparison of the VEGF expression levels in different genotypes of rs3025039 (B), rs2146323 (C), rs2010963 (D), rs3025010 (E), and rs833069 (F)

Variables	B	SE	Wald	P	OR	95% CI
Age	1.625	0.184	110.382	0.001	2.152	1.221-3.742
Pre-pregnancy body mass index	0.517	0.258	9.258	0.002	2.047	1.516-3.385
Family history of diabetes	0.403	0.154	6.338	0.018	2.348	1.524-3.778
Previous gestational diabetes mellitus	0.781	0.445	12.178	0.008	2.789	1.152-5.486
Hypertension	0.712	0.351	7.195	0.019	2.143	1.124-3.247
Fasting plasma glucose	0.498	0.412	6.263	<0.001	2.536	1.458-3.561
Fasting insulin	0.442	0.406	9.126	<0.001	1.325	1.031-2.124
HOMA-β	-0.384	0.589	3.856	0.002	0.511	0.317-0.916
HOMA-IR	0.469	0.268	4.269	0.016	1.247	1.016-1.832
rs2146323 (CA+AA vs AA)	0.681	0.415	6.526	0.032	1.547	1.264-2.632
rs3025039 (CT+TT vs CC)	1.256	0.232	11.257	<0.001	2.685	1.167-5.894
Vascular endothelial growth factor expression	0.365	0.246	7.281	0.006	2.166	1.437-3.978

HOMA, homeostasis model assessment.

TABLE 4 Multiple logistic regression analysis

increased serum levels of VEGF were exhibited in GDM patients. Possibly, the up-regulated VEGF might be directly or indirectly by protein kinase (PKC), advanced glycation end products (AGEs), transforming growth factor-β (TGF-β), and intracellular insulin-like growth factor (IGF) under the condition of high glucose.^{34,35}

Similarly, the serum VEGF expression level was apparently elevated and it was correlated to the severity of diabetic polyneuropathy.³⁶ Further, we also found that the rs3025039 CT+TT genotype carriers had apparently higher VEGF expression levels than rs3025039 CC genotype carriers, while no observable difference was found

among subjects with other SNPs. As reported by Kim *et al.*,¹¹ 936C>T (rs3025039) within VEGF gene may constitute a crucial factor affecting the serum level of VEGF. As we know, rs3025039 was located at the 3'untranslated region (UTR), where SNPs may affect the stability of the mRNA and the gene expression via alteration of the miRNA and mRNA interaction.^{37,38} On the contrary, rs833069 (Intron 2), rs2146323 (Intron 2), and rs3025010 (Intron 5) were all located at introns,³⁹ and their mutations may have no impact on the VEGF protein translation. However, rs2010963, located at the 5'UTR, did not find its association with the VEGF expression level, which was consistent with the study by Bleda *et al.*⁴⁰ Thus, we hypothesized that rs3025039 may affect the diseases by regulating the transcriptional level of VEGF, and other SNPs may be through the combined interaction of each other. To further analyze the interaction between different SNPs, we used the software Haploview and found the linkage disequilibrium among rs2010963, rs833069, rs2146323, and rs3025010. Besides, haplotypes CAAC, CACT, and GGCT had the reduced risk of GDM, while CAAT, CACC, and GACT increased the occurrence of GDM, which was consistent with the findings of previous studies.^{41,42} Therefore, we may conclude that these four SNPs (rs2010963, rs833069, rs2146323, and rs3025010) may exert functions through the interaction with each other.

Finally, we found age, pre-pregnancy BMI, FPG, FINS, HOMA-IR, rs2146323 CA+AA genotype, rs3025039 CT+TT genotype, and VEGF expression level to be the independent risk factors for GDM, while HOMA- β to be an independent protective factor for GDM after performing logistic regression analysis. Of note, evidence has pointed out that the incidence rate of GDM would increase with the age of pregnant women,⁴³ and obesity (especially BMI > 30) or obesity-related insulin resistance plays an important role in the pathogenesis of GDM,^{44,45} showing that the occurrence of GDM is caused by the interaction of many factors, and early screening from many aspects is of great value for the diagnosis and treatment of GDM. The limitations of the current study include a relatively smaller number of enrolled subjects than previous studies. We will collect more data from a larger sample size in the future and hope to obtain more exact results therefrom.

In summary, we found VEGF rs2146323 and rs3025039 polymorphisms and its expression levels were associated with the risk of GDM. Besides, multiple VEGF loci may affect the pathogenesis of GDM through their interaction with each other, which is of great significance for the clinical assessment and diagnosis of GDM.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

- DeSisto CL, Kim SY, Sharma AJ. Prevalence estimates of gestational diabetes mellitus in the United States, Pregnancy Risk Assessment Monitoring System (PRAMS), 2007–2010. *Prev Chronic Dis.* 2014;11:E104.
- Ao D, Wang HJ, Wang LF, Song JY, Yang HX, Wang Y. The rs2237892 polymorphism in KCNQ1 influences gestational diabetes mellitus and glucose levels: a case-control study and meta-analysis. *PLoS One.* 2015;10(6):e0128901.
- de Wilde MA, Veltman-Verhulst SM, Goverde AJ, et al. Preconception predictors of gestational diabetes: a multi-centre prospective cohort study on the predominant complication of pregnancy in polycystic ovary syndrome. *Hum Reprod.* 2014;29(6):1327–1336.
- Archambault C, Arel R, Filion KB. Gestational diabetes and risk of cardiovascular disease: a scoping review. *Open Med.* 2014;8(1):e1–e9.
- Poomalar GK. Changing trends in management of gestational diabetes mellitus. *World J Diabetes.* 2015;6(2):284–295.
- Li JY, Wu GM, Hou Z, Cao YM. Expression of C1q/TNF-related protein-3 (CTRP3) in serum of patients with gestational diabetes mellitus and its relationship with insulin resistance. *Eur Rev Med Pharmacol Sci.* 2017;21(24):5702–5710.
- Noctor E, Dunne FP. Type 2 diabetes after gestational diabetes: The influence of changing diagnostic criteria. *World J Diabetes.* 2015;6(2):234–244.
- Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature.* 2007;445(7130):881–885.
- Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science.* 2007;316(5829):1341–1345.
- Lauenborg J, Grarup N, Damm P, et al. Common type 2 diabetes risk gene variants associate with gestational diabetes. *J Clin Endocrinol Metab.* 2009;94(1):145–150.
- Kim HW, Ko GJ, Kang YS, et al. Role of the VEGF 936 C/T polymorphism in diabetic microvascular complications in type 2 diabetic patients. *Nephrology.* 2009;14(7):681–688.
- Wirostko B, Wong TY, Simo R. Vascular endothelial growth factor and diabetic complications. *Prog Retin Eye Res.* 2008;27(6):608–621.
- Al-Habboubi HH, Sater MS, Almawi AW, Al-Khateeb GM, Almawi WY. Contribution of VEGF polymorphisms to variation in VEGF serum levels in a healthy population. *Eur Cytokine Netw.* 2011;22(3):154–158.
- Zhai R, Liu G, Zhou W, et al. Vascular endothelial growth factor genotypes, haplotypes, gender, and the risk of non-small cell lung cancer. *Clin Cancer Res.* 2008;14(2):612–617.
- Dai J, Rabie AB. VEGF: an essential mediator of both angiogenesis and endochondral ossification. *J Dent Res.* 2007;86(10):937–950.
- Katavetin P, Miyata T, Inagi R, et al. High glucose blunts vascular endothelial growth factor response to hypoxia via the oxidative stress-regulated hypoxia-inducible factor/hypoxia-responsible element pathway. *J Am Soc Nephrol.* 2006;17(5):1405–1413.
- Zhou Z, Ju H, Sun M, et al. Serum fetuin-A concentrations are positively associated with serum VEGF levels in patients with newly diagnosed type 2 diabetes. *Endocr J.* 2015;62(10):879–885.
- Ozturk BT, Bozkurt B, Kerimoglu H, Okka M, Kamis U, Gunduz K. Effect of serum cytokines and VEGF levels on diabetic retinopathy and macular thickness. *Mol Vis.* 2009;15:1906–1914.

19. Porojan MD, Catana A, Popp RA, Dumitrascu DL, Bala C. The role of NOS2A -954G/C and vascular endothelial growth factor +936C/T polymorphisms in type 2 diabetes mellitus and diabetic nonproliferative retinopathy risk management. *Ther Clin Risk Manag.* 2015;11:1743-1748.
20. Yang X, Deng Y, Gu H, et al. Polymorphisms in the vascular endothelial growth factor gene and the risk of diabetic retinopathy in Chinese patients with type 2 diabetes. *Mol Vis.* 2011;17:3088-3096.
21. Awata T, Inoue K, Kurihara S, et al. A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. *Diabetes.* 2002;51(5):1635-1639.
22. Watson CJ, Webb NJ, Bottomley MJ, Brenchley PE. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine.* 2000;12(8):1232-1235.
23. Muthuswamy V. The new 2013 seventh version of the Declaration of Helsinki—more old wine in a new bottle? *Indian J Med Ethics.* 2014;11(1):2-4.
24. Shirazian N, Mahboubi M, Emdadi R, et al. Comparison of different diagnostic criteria for gestational diabetes mellitus based on the 75-g oral glucose tolerance test: a cohort study. *Endocr Pract.* 2008;14(3):312-317.
25. Lai A, Chen W, Helm K. Effects of visfatin gene polymorphism RS4730153 on exercise-induced weight loss of obese children and adolescents of Han Chinese. *Int J Biol Sci.* 2013;9(1):16-21.
26. Zeng Y, Dai F, Yang K, Tang Y, Xu M, Zhou Y. Association between a vascular endothelial growth factor gene polymorphism (rs2146323) and diabetic retinopathy: a meta-analysis. *BMC Ophthalmol.* 2015;15:163.
27. Gauderman W, Morrison J. QUANTO 1.2: A computer program for power and sample size calculation for genetic-epidemiology studies. 2006. <https://www.scienceopen.com/document?vid=2944f68a-3b3d-4e86-90a3-3d3c1fab3ffa>. Accessed October 12, 2018.
28. Kim YR, Hong SH. Association between the polymorphisms of the vascular endothelial growth factor gene and metabolic syndrome. *Biomed Rep.* 2015;3(3):319-326.
29. Al-Kateb H, Mirea L, Xie X, et al. Multiple variants in vascular endothelial growth factor (VEGFA) are risk factors for time to severe retinopathy in type 1 diabetes: the DCCT/EDIC genetics study. *Diabetes.* 2007;56(8):2161-2168.
30. Awata T, Kurihara S, Takata N, et al. Functional VEGF C-634G polymorphism is associated with development of diabetic macular edema and correlated with macular retinal thickness in type 2 diabetes. *Biochem Biophys Res Commun.* 2005;333(3):679-685.
31. Lu Y, Ge Y, Shi Y, Yin J, Huang Z. Two polymorphisms (rs699947, rs2010963) in the VEGFA gene and diabetic retinopathy: an updated meta-analysis. *BMC Ophthalmol.* 2013;13:56.
32. Liew G, Klein R, Wong TY. The role of genetics in susceptibility to diabetic retinopathy. *Int Ophthalmol Clin.* 2009;49(2):35-52.
33. Schwartz SG, Brantley MA Jr, Flynn HW Jr. Genetics and diabetic retinopathy. *Curr Diabetes Rev.* 2013;9(1):86-92.
34. Flyvbjerg A, Khatir DS, Jensen LJ, Dagnaes-Hansen F, Gronbaek H, Rasch R. The involvement of growth hormone (GH), insulin-like growth factors (IGFs) and vascular endothelial growth factor (VEGF) in diabetic kidney disease. *Curr Pharm Des.* 2004;10(27):3385-3394.
35. Lee EY, Chung CH, Kim JH, Joung HJ, Hong SY. Antioxidants ameliorate the expression of vascular endothelial growth factor mediated by protein kinase C in diabetic podocytes. *Nephrol Dial Transplant.* 2006;21(6):1496-1503.
36. Deguchi T, Hashiguchi T, Horinouchi S, et al. Serum VEGF increases in diabetic polyneuropathy, particularly in the neurologically active symptomatic stage. *Diabet Med.* 2009;26(3):247-252.
37. Wei R, Yang F, Urban TJ, et al. Impact of the Interaction between 3'-UTR SNPs and microRNA on the expression of human xenobiotic metabolism enzyme and transporter genes. *Front Genet.* 2012;3:248.
38. Haas U, Szczakiel G, Laufer SD. MicroRNA-mediated regulation of gene expression is affected by disease-associated SNPs within the 3'-UTR via altered RNA structure. *RNA Biol.* 2012;9(6):924-937.
39. Kang M, Ding X, Xu M, et al. Genetic variation rs10484761 on 6p21.1 derived from a genome-wide association study is associated with gastric cancer survival in a Chinese population. *Gene.* 2014;536(1):59-64.
40. Bleda S, De Haro J, Varela C, Esparza L, Ferruelo A, Acin F. Vascular endothelial growth factor polymorphisms are involved in the late vascular complications in Type II diabetic patients. *Diab Vasc Dis Res.* 2012;9(1):68-74.
41. Langsenlehner U, Hofmann G, Renner W, et al. Association of vascular endothelial growth factor—a gene polymorphisms and haplotypes with breast cancer metastases. *Acta Oncol.* 2015;54(3):368-376.
42. Su MT, Lin SH, Lee IW, Chen YC, Kuo PL. Association of polymorphisms/haplotypes of the genes encoding vascular endothelial growth factor and its KDR receptor with recurrent pregnancy loss. *Hum Reprod.* 2011;26(4):758-764.
43. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. *Nat Rev Endocrinol.* 2012;8(11):639-649.
44. Holemans K, Caluwaerts S, Poston L, Van Assche FA. Diet-induced obesity in the rat: a model for gestational diabetes mellitus. *Am J Obstet Gynecol.* 2004;190(3):858-865.
45. Holliman RE, Stevens PJ, Duffy KT, Johnson JD. Serological investigation of ocular toxoplasmosis. *Br J Ophthalmol.* 1991;75(6):353-355.

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