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Original Article

Association of VEGF +936C/T, -634G/C, -2578C/A and -1154G/A polymorphisms with preeclampsia risk in Chinese pregnant women

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Abstract: Our study aimed to investigate the association between preeclampsia and VEGF +936C/T, -634G/C, -2578C/A and -1154G/A polymorphisms and risk of in the Han Chinese pregnant women. A total of 128 PE cases and 128 normal pregnant women as control were enrolled in a Chinese population. Polymerase chain reaction (PCR) amplification of the genes and sequencing methods were used to genotype VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A. The association between VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A and risk of PE was assessed by the single factor and multiple factors logistic regression. We observed that the TT genotype of VEGF +936C/T was associated with an increased risk of PE when compared with the CC genotype (OR=2.49, 95% CI=1.32-4.68). In recessive model, those carrying the TT genotype of VEGF +936C/T had an strong increased risk of PE in comparison to those with CC+CT genotype (OR=2.43, 95% CI=1.32-4.48). We found the VEGF +936C/T had interaction with smoking and drinking in the risk of PE. In conclusion, the TT genotype of VEGF +936C/T polymorphism have an increased risk of PE, and have an interaction with smoking and drinking. VEGF +936C/T polymorphism could be a susceptibility biomarker for PE.

Keywords: VEGF, preeclampsia, polymorphism

Introduction

Preeclampsia is a common disease unique to pregnant women, and about 6% to 8% of all pregnancies would suffer from this disease [1, 2]. It is estimated that the incidence of PE was 9.4%-10.4% in China [3, 4]. The etiology of preeclampsia has been widely studied, but its real mechanisms are not well understood. Many studies have reported that many risk factors are involved in the onset of the PE, such as primipara, pregnant women over the age of 40, BMI>30 kg/m^2, history of preeclampsia or eclampsia family, multiple pregnancy, preexisting hypertension and diabetes [5]. It is reported that about 40% of the preeclampsia pregnant women can be attributable to maternal genetic factors, and about 25% of them to the fetal hereditary factors, which have showed that the genetic factors directly and indirectly are associated with the development of preeclampsia. Many previous studies have reported the association between genetic factors and risk of PE, such as MMP, MTHFR and interleukin factors [6-8].

Vascular endothelial growth factor has been widely investigated, and considered to have a strong promotion for differentiation, proliferation, migration and invasion of endothelial cell [9]. Previous study also reports that VEGF is involved in maintaining the function of endothelial cells during the formation and regulation of angiogenesis [10]. The biological functions of VEGF can be implemented through the endothelial cell specific receptor (VEGF-R) [11]. Human VEGF gene is located on the chromosome 6p21.3, consisting of 8 exons and 7 introns. The expression of VEGF displays a special role in the function of endothelial and trophoblast cells of placenta, and has a critical role in the formation and maintenance of placenta [12, 13]. Since strong requirement for fetal growth and angiogenesis in pregnant women, VEGF

Table 1. PCR primers used in this study

SNPs	Primers	Sequences (5'-3')
+936C/T	Forward	ACGTTGGATGTGTGTTTCTTCTGACCACGC
	Reverse	ACGTTGGATGTCCAGTCTGGGTCATAGATG
-634G/C	Forward	ACGTTGGATGCAAACAGTGCCTTATGGCAG
	Reverse	ACGTTGGATGTTAAGCCACAGACCTACTGC
-2578C/A	Forward	GTATCCCATACTTTCAGAGTTTT
	Reverse	GATGGGCATGTTCACATCAAGCTC
-1154G/A	Forward	GATGCAAGTTTCCAAAACAGGCCC
	Reverse	GATGATCACGACGGTCACGTGCCT

and its receptor system play a critical role in the growth of embryo. Alteration of subtle expression of VEGF could result in the deformities or embryonic death in the growth of embry [14]. VEGF gene polymorphisms play an important role in regulating and altering the protein expression and function [15], and changing the susceptibility to preeclampsia. Our study aimed to investigate the association between preeclampsia and VEGF +936C/T, -634G/C, -2578C/A and -1154G/A polymorphisms and risk of in the Han Chinese pregnant women.

Materials and methods

Objectives

This study consists of 128 cases of preeclampsia patients, and they were recruited from the Department of Obstetrics of the Affiliated Hospital of Qingdao University between April 2012 and November 2015. The diagnosis of PE was based on the American College of Obstetricians and Gynecologists criteria (ACOG Committee on Practice Bulletins-Obstetrics, 2002) [16]. The exclusion criteria for patients with PE were those with chronic hypertension, pregestational diabetes and end-stage renal and liver diseases as well as serious infectious diseases.

Simultaneously, a total of 128 pregnant women were recruited from the Department of Obstetrics of the same hospital, and served as controls. Each control was selected after selection of one patient, and matched with this patient by age.

Those who had a history of PE, chronic hypertension, cardiovascular disease, end-stage liver or renal diseases were excluded from the control groups.

The demographic characteristics and clinical predictors of preeclampsia of included patients and controls were selected from a instructed questionnaire or medical records. The demographic characteristics included age, maternal age, gestational age at delivery, drinking and smoking habits, and the pre-pregnancy BMI was calculated. The clinical characteristics included gestation weeks, systolic blood pressure, diastolic blood pressure, delivery weeks, 24-hour urinary protein and uric acid.

All the included subjects were unrelated Chinese Han population, and they signed written consents to collect venous blood samples for analysis. The mean age of patients and controls were 30.57±3.62 and 30.79±4.43 years, respectively. The mean BMI of patients and controls were 24.47±3.70 and 23.45±3.58 kg/m^2, respectively.

DNA extraction and genotyping

Each subject was asked to provide 5 ml peripheral venous blood, and the white cell was handled with Blood DNA extraction kit (QIAamp) to extract genomic DNA for genotyping analysis.

We performed DNA sequence analyses and PCR amplification for genotyping the VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A. The primer sequences for VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A were designed with Clone Manager Professor 8.0 and synthesized by the Shanghai Sangon Production Company (**Table 1**). PCR reaction system was 50 uL including: 75 mM dNTPs, 20 ng genomic DNA, 0.5 uM of each primer, 3.5 mM $MgCl_2$, Hotstar Taq 0.5 U enzyme and HPLC grade water. The PCR amplification was performed as follows 94°C for 15 min; 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 60 s; and a final amplification of 72°C for 7 min. The PCR products were sequenced and analyzed by DNAStar software, and compared by MEGA 5.0 software (**Figures 1** and **2**).

Statistical analysis

Comparisons of the general data between the patients and controls were performed by t test or Chi-square (X^2) test. Whether the genotypes of VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A were line with the Hardy-Weinberg equilibrium (HWE) was calculated by the X^2 goodness-of-fit. The association between

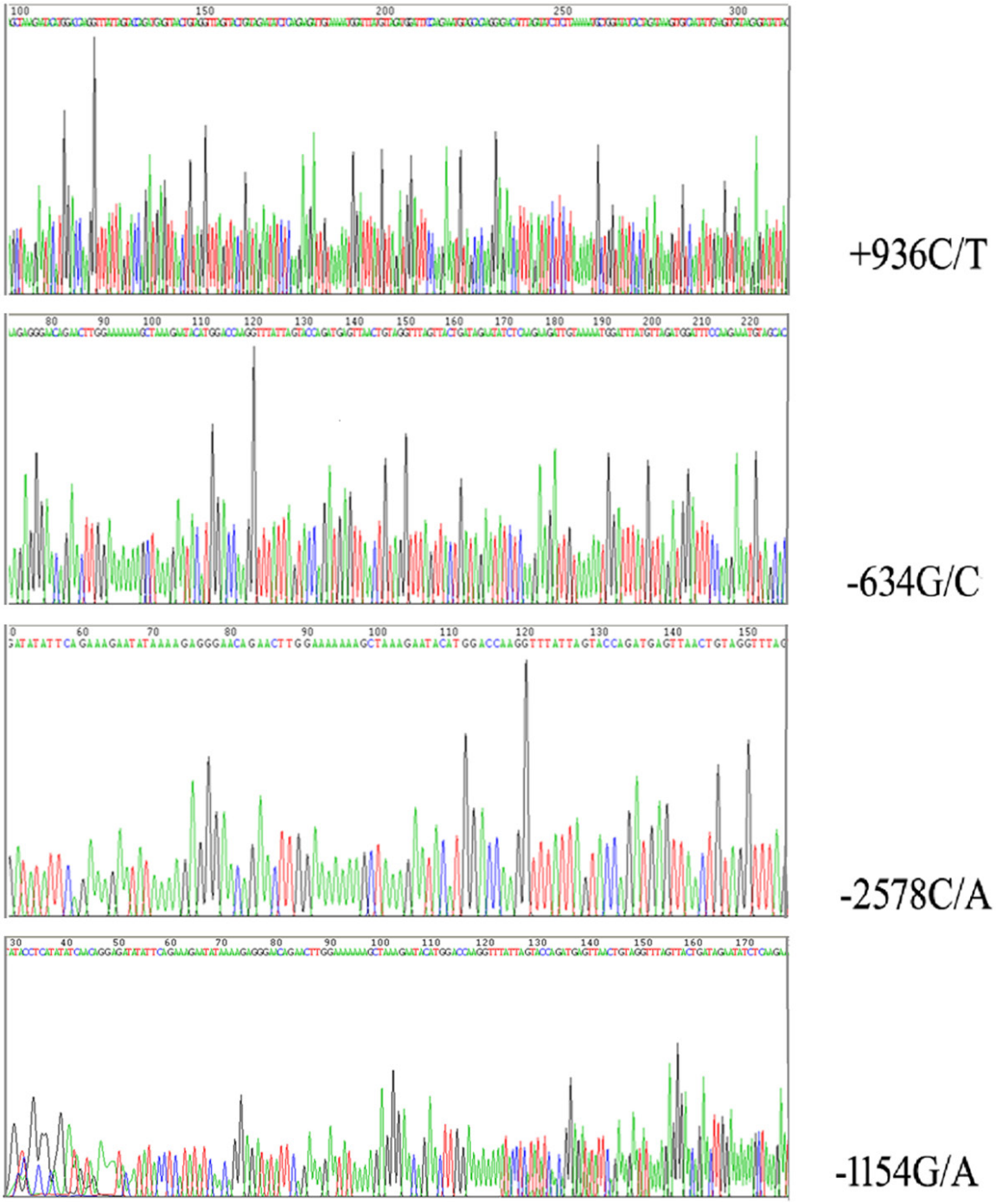

Figure 1. Sequencing peaks of VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A.

VEGF SNPs +936C/T, -634G/C, -2578C/A and -1154G/A and risk of PE was assessed by the single factor and multiple factors logistic regression, and the results were displayed by OR values and 95% CI values. Logistic regression analysis was also performed for the gene-environmental interaction. SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and a two sided $P<0.05$ was recognized as statistically significant.

Results

We observed significant differences in SBP ($t=15.84$, $P<0.001$), DBP ($t=11.99$, $P<0.001$), BMI before pregnancy ($t=2.26$, $P=0.03$) and

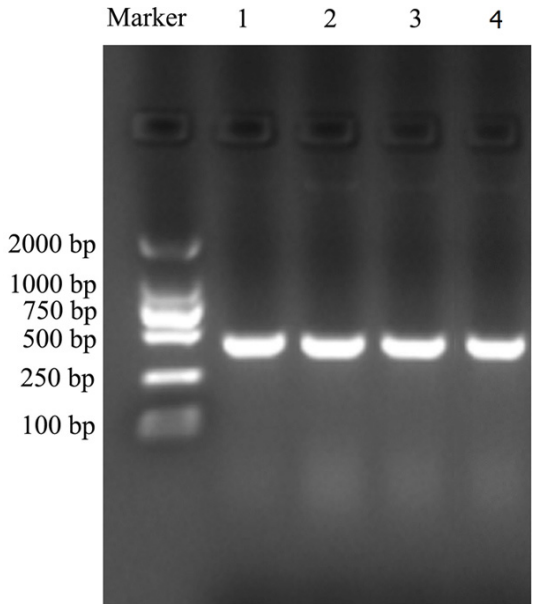

Figure 2. Electrophoretogram of PCR amplification. Lane 1: +936C/T; Lane 2: -634G/C; Lane 3: -2578C/A; Lane 4: -1154G/A.

gestational age at delivery (t=-21.31, P<0.001) (**Table 2**). However, no significant differences were found in maternal age, smoking and drinking during pregnancy.

Distributions of VEGF +936C/T, -634G/C, -2578C/A and -1154G/A loci in controls were in line with Hardy Weinberg equilibrium (P> 0.05). We observed that the TT genotype of VEGF +936C/T was associated with an increased risk of PE when compared with the CC genotype (OR=2.49, 95% CI=1.32-4.68), after adjusting for age, medical history, smoking and drinking. In recessive model, those carrying the TT genotype of VEGF +936C/T had an strong increased risk of PE in comparison to those with CC+CT genotype (OR=2.43, 95% CI=1.32-4.48) (**Table 3**).

Gene-environmental interaction for VEGF +936C/T and susceptibility to PE was also calculated by logistic regression analysis (**Table 4**). We found the VEGF +936C/T had interaction with smoking and drinking in the risk of PE.

Discussion

In this study, we observed that the TT genotype of VEGF +936C/T was associated with an increased risk of PE in co-dominant and recessive models, and we found a gene-environmental interaction between VEGF +936C/T polymorphism and smoking and drinking status. These results suggested that VEGF +936C/T contributed to the pathogenesis of PE among Chinese population, and this SNP could be a susceptibility biomarker for PE.

The etiology of preeclampsia is still not well understood, and improper placentation is reported to be a possible risk factor for this disease [17]. The process of PE is related to the abnormal infiltration of the cytotrophoblast into the decidual endometrium and development of the spiral arteries derived from the myometrium [18]. VEGF was considered to be an essential factor for maintaining the integrity of the maternal endothelial cells [19]. The expression of VEGF has been implied as a hypoxia-inducible factor, and may be correlated to the development of PE [20, 21]. Zhao et al. reported that placental expression of VEGF was elevated in pregnancies with hydatidiform mole, and is possible related with early onset of preeclampsia [22]. Andraweera PH et al. reported that VEGF family of angiogenic growth factor mRNA expression in the palcenta is associated with reduction of gestational hypertensive disorders, small for gestational age infants and preterm birth [23]. Xu X et al. reported that placental VEGF was expressed at lower levels in pregnant women with PE, suggesting that VEGF may contribute to endothelial damage [24]. The +936C/T is located in the 39-untranslated region of VEGF, and +936C/T polymorphism could change the expression and production of protein, and alter the susceptibility to diseases related to the deranged angiogenesis [25]. Several previous studies have reported that VEGF +936C/T polymorphism was associated with several kinds of diseases, such as prematurity, endometriosis, breast cancer and glioma [26-29].

VEGF is reported to be associated with the pathogenesis of PE. Several previous studies have reported the association between VEGF polymorphisms and risk of PE in many populations. Papazoglou D et al. performed a study with 42 PE and 73 healthy pregnant women, and reported a statistically significance in the allelic frequencies of VEGF +936C/T between pregnant women with PE and controls [25]. Shim et al. carried a case-control study with 110 patients with PE and 209 controls in Korea,

Table 2. The general situation comparison between patients and control groups

Groups	PE patients N=128	Controls N=128	t test or X^2	*P* value
Maternal age, years	30.57±3.62	30.79±4.43	-0.42	0.67
SBP, mmHg	140.84±13.55	115.78±11.69	15.84	<0.001
DBP, mmHg	104.88±17.17	77.93±18.76	11.99	<0.001
BMI before pregnancy (kg/m^2)	24.47±3.70	23.45±3.58	2.26	0.03
<24	68 (53.13)	62 (48.44)		
≥24	60 (46.88)	66 (51.56)	0.56	0.53
Smoking during pregnancy (%)				
No	119 (92.97)	121 (94.53)		
Yes	9 (7.03)	7 (5.47)	0.27	0.61
Drinking during pregnancy (%)				
No	120 (93.75)	111 (86.72)		
Yes	8 (6.25)	17 (13.28)	3.59	0.06
24-hour urinary protein uric acid, mg	2465.65±473.27	-	-	-
Gestational age at delivery (weeks)	33.53±3.07	39.99±1.52	-21.31	<0.001

SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 3. Distribution and frequency of four loci with susceptibility of PE in this study

SNP		PE patients	%	Controls	%	HWE	OR (95% CI)[1]	*P* value
+936C/T	CC	70	54.69	84	65.63		1.0 (Ref.)	-
Co-dominant	CT	18	14.06	22	17.19		1.01 (0.50-1.32)	0.98
	TT	40	31.25	22	17.19	>0.05	2.49 (1.32-4.68)	0.005
Dominant	CC	86	67.19	84	65.63		1.0 (Ref.)	-
	CT+TT	58	45.31	44	34.38		1.64 (0.96 -2.75)	0.06
Recessive	CC+CT	88	68.75	106	82.82		1.0 (Ref.)	-
	TT	20	15.63	22	17.19		2.43 (1.32-4.48)	<0.001
-634G/C	GG	39	30.47	34	26.56		1.0 (Ref.)	-
Co-dominant	GC	55	42.97	60	46.88		1.25 (0.69-2.29)	0.46
	CC	34	26.56	34	26.56	>0.05	1.07 (0.54-2.12)	0.85
Dominant	GG	39	30.47	34	26.56		1.0 (Ref.)	-
	GC+CC	89	69.53	94	73.44		1.19 (0.69-2.07)	0.54
Recessive	GG+GC	94	73.44	94	73.44		1.0 (Ref.)	-
	CC	34	26.56	34	26.56		0.96 (0.55-1.70)	0.90
-2578C/A	CC	56	43.75	60	46.88		1.0 (Ref.)	-
Co-dominant	CA	51	39.84	43	33.59		0.77 (0.44-1.35)	0.37
	AA	21	16.41	25	19.53	>0.05	1.03 (0.51-2.10)	0.93
Dominant	CC	56	43.75	60	46.88		1.0 (Ref.)	-
	CA+AA	72	56.25	68	53.12		0.90 (0.55-1.49)	0.69
Recessive	CC+CA	107	83.59	103	80.47		1.0 (Ref.)	-
	AA	21	16.41	25	19.53		1.15 (0.60-2.22)	0.67
-1154G/A	GG	39	30.47	37	28.91		1.0 (Ref.)	-
Co-dominant	GA	57	44.53	62	48.44		1.13 (0.62-2.05)	0.68
	AA	32	25	29	22.66	>0.05	1.06 (0.53-2.12)	0.87
Dominant	GG	39	30.47	37	28.91		1.0 (Ref.)	-
	GA+AA	89	69.53	91	71.1		1.06 (0.62-1.83)	0.83
Recessive	GG+GA	96	75	99	77.35		1.0 (Ref.)	-
	AA	32	25	29	22.66		0.97 (0.54-1.75)	0.93

[1]Adjusted for age, medical history, smoking and drinking.

Table 4. Gene-environmental factors interaction analysis of SNPs +936C/T with susceptibility to PE

Groups	CC		TT		OR value	*P* value	CC+CT		T		OR value	*P* value
	Patients	Control	Patients	Control			Patients	Control	Patients	Control		
Smoking												
No	67	79	37	18	2.42 (1.27-4.65)	0.01	84	101	37	18	2.47 (1.31-14.65)	0.005
Yes	3	7	3	2	3.50 (0.37-32.97)	0.27	4	7	3	2	2.63 (0.30-23.00)	0.38
Drinking												
No	63	80	35	20	2.22 (1.17-4.22)	0.02	76	100	35	20	2.30 (1.23-4.30)	0.009
Yes	7	6	5	0	-	-	12	8	5	0	-	-
BMI before pregnancy (kg/m^2)												
<24	36	48	62	68	2.42 (1.03-5.69)	0.04	42	57	62	68	2.47 (1.07-5.70)	0.03
≥24	34	38	66	60	1.64 (0.82-2.02)	0.09	46	51	66	60	2.46 (1.02-5.95)	0.04

and reported that carriage of the +936C/T allele of the VEGF gene may be related to an increased risk of developing PE in Korean women [30]. Procopciuc et al. reported that the maternal and fetal VEGF +936C/T polymorphism could be used as a modulating factor in PE, and it plays an important role in maintaining the angiogenic balance in PE mothers and their pregnancy outcome [31]. However, some studies reported inconsistent results. Salimi S et al. found the serum VEGF levels were significantly lower in individuals with the VEGF -634 CC genotype than the GG genotype, and VEGF -634 GC and CC genotypes were significantly higher in pregnant women with severe PE when compared with mild PE and controls [32]. In addition, Cheng et al. performed a meta-analysis with 11 case-control studies, they found an association between VEGF +936C/T and -634G/C polymorphisms and risk of PE [33]. The differences among the above studies may refer to differences in populations, sample sizes and study design.

We observed an gene-environmental interaction between VEGF +936C/T and smoking and drinking in the risk of PE. Chen et al. found that VEGF-A gene is associated with serum VEGF-A levels and rheumatoid arthritis activity in patients who have never smoked [34]. Gonçalves FT also reported an interaction between smoking and +936C/T in the risk of age-related macular degeneration [35]. Lu et al. showed that alcohol could promote mammary tumor growth through stimulating VEGF-dependent angiogenesis [36].

Two limitations should be considered in this study. First, all the study participants were recruited from only one hospital located in a single city in China, and thus, they may not represent the PE patients and healthy controls in other places. Second, since the low incidence of PE resulted in small sample size, which may reduce the statistical power to find differences between groups. Therefore, future studies incorporating larger samples sizes and more populations are required to confirm our findings.

In conclusion, we suggest that the TT genotype of VEGF +936C/T polymorphism have an increased risk of PE, and have an interaction with smoking and drinking. VEGF +936C/T polymorphism could be a susceptibility biomarker for PE.

Disclosure of conflict of interest

None.

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