Vascular endothelial growth factor +936C/T and -634G/C polymorphisms exhibited significantly different allelic distributions and no associations with the risk of preeclampsia, gestational hypertension or gestational diabetes mellitus in the Xinjiang Uygur Autonomous Region of China

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Research article

Keywords: Vascular endothelial growth factor (VEGF), polymorphism, preeclampsia, gestational hypertension, gestational diabetes mellitus

Posted Date: June 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-32022/v1

Abstract

Background:

The relationships between vascular endothelial growth factor (VEGF) + 936C/T and − 634C/G gene polymorphisms and certain diseases have been widely reported. According to reported data, there may be geographical and/or ethnic differences in VEGF + 936C/T and − 634C/G allele frequencies. Xinjiang is a multi-ethnic region in China in which the distributions of the + 936C/T and − 634C/G allele frequencies in various ethnic groups and their correlations with common complications during pregnancy (preeclampsia (PE), gestational hypertension (GH) and gestational diabetes mellitus (GDM)) have not been studied.

Methods:

A total of 2161 women (1551 controls with normal pregnancy, 232 patients with PE, 59 patients with GH and 319 patients with GDM) were recruited for this study. Sanger sequencing was used to screen VEGF + 936C/T and − 634C/G genotypes. The Pearson chi-square test was used to test the associations between diseases and + 936C/T and − 634C/G polymorphisms.

Results:

We investigated the distributions of VEGF + 936C/T and − 634G/C allele frequencies in healthy pregnant women in different regions of the world and found significant differences. Our data showed that the distributions of + 936C/T and − 634G/C allele frequencies between healthy Han and Uygur women in the Xinjiang area were significantly different. We found that VEGF + 936C/T and − 634G/C gene polymorphisms were not correlated with mild PE (mPE), severe PE (sPE), GH or GDM. Ethnic subgroup analysis revealed that the + 936C/T and − 634G/C gene polymorphisms in the Han, Uygur and Hui populations were also not related to PE, GH or GDM.

Conclusions:

The results showed that VEGF + 936C/T and − 634G/C gene polymorphisms had significant regional and ethnic distribution differences. In Xinjiang, VEGF + 936C/T and − 634G/C gene polymorphisms do not confer susceptibility to PE, GH or GDM.

Background

Vascular endothelial growth factor (VEGF) is a mitogen and survival factor specific to endothelial cells and is a crucial promoter of angiogenesis in physiological and pathological conditions[1, 2]. The human VEGF gene is located on chromosome 6p21.3; it is a single gene with a total length of 14 kb that consists of 8 exons and 7 introns[3]. Alternative exon splicing was initially shown to result in the generation of six

different isoforms (VEGF121, VEGF145, VEGF165, VEGF183, VEGF189 and VEGF206), having 121, 145, 165, 183, 189 and 206 amino acids, respectively, after signal sequence cleavage[4–6]. During pregnancy, VEGF is essential for the proliferation of trophoblasts, the development of embryonic vasculature and the growth of maternal and foetal blood vessels in the uterus[7]. During embryonic development, VEGF expression can be initially detected in the giant cells of the trophoblast within the first few days after implantation[8]. VEGF mRNA expression can be detected in almost all tissues in human foetuses (16–22 weeks)[2].

Preeclampsia (PE) is a disease unique to pregnancy. It is characterized by high blood pressure and proteinuria during pregnancy and affects approximately 3–5% of pregnant women worldwide; it is the leading cause of maternal, foetal, and neonatal death worldwide. Genetic factors, maternal factors and immunological factors may cause placental dysfunction, which in turn can lead to the release of anti-angiogenic factors (such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sENG)). sFlt-1 and sENG act as anti-angiogenic factors to trap circulating VEGF, decreasing its free levels, leading to endothelial dysfunction and the clinical manifestations of PE[9]. There is evidence that levels of free angiogenic and anti-angiogenic factors can be used to predict the onset of PE[9, 10]. Significant changes in serum VEGF levels in PE patients suggest that VEGF may play an important role in the pathogenesis of PE[11, 12]. There are at least 30 single nucleotide polymorphisms (SNPs) in the VEGF gene[13, 14]. The VEGF + 936C/T and − 634C/G mutation sites have been shown to further affect the development of the disease by changing the transcription level and protein expression of VEGF[14–16]. There are many reports on the relationship between VEGF + 936C/T and − 634C/G and PE susceptibility[17–25]. However, the results are inconsistent across regions. Valeria et al found that VEGF genotypes and haplotypes were associated with PE but not with gestational hypertension (GH), thus suggesting that GH and PE may each have a different genetic basis[26]. Indeed, significant differences exist in the pathophysiological bases of these two hypertensive disorders of pregnancy, especially when the role of VEGF is considered[27–29].

Gestational diabetes mellitus (GDM) is a common complication during pregnancy. It is defined as the appearance of diabetes mellitus for the first time during pregnancy and is characterized by impaired glucose tolerance of different severities. GDM is considered a clinical syndrome caused by the interaction of genetic and environmental factors and has a genetic basis similar to that of type 2 diabetes mellitus (T2DM). Recent studies have reported that VEGF gene polymorphisms and expression levels are closely related to the pathogenesis of T2DM and its corresponding complications[30, 31]. Many factors, including oxidative stress, hypoxia, and inflammation, can induce increased VEGF expression under high glycaemic conditions[32]. In fact, plasma VEGF levels are elevated in patients with GDM[33]. Elevated VEGF levels increase the number of placental blood vessels, increase the permeability of the blood vessels, and provide adequate or even excessive nutrients, resulting in the birth of large babies[34]. Dong et al found that the + 936C/T polymorphism and its expression are closely related to the risk of GDM in China (Yantai)[35].

Published data indicate that the distributions of the VEGF + 936C/T and − 634C/G alleles and genotype frequencies vary greatly in different regions. In this study, we investigated the distributions of VEGF +

936C/T and − 634C/G allele frequencies in healthy pregnant women in different parts of the world. Based on the data of this study, genetic variations in + 936C/T and − 634C/G polymorphisms in four main ethnic groups in Xinjiang (Han, Uyghur, Hui and Kazak) was studied. The role of VEGF in normal pregnancy and the abnormalities in VEGF functions possibly associated with PE, GH or GDM support the idea that genetic polymorphisms in VEGF could affect susceptibility to the development of PE, GH or GDM. In the present study, we compared the distributions of genetic variants of the two abovementioned VEGF polymorphisms among healthy pregnant women and PE, GH, and GDM women. In addition, we also examined the associations of the VEGF gene haplotypes with these clinical complications of pregnancy.

Methods

Subjects

A total of 2161 women (1551 controls with normal pregnancy, 232 patients with PE, 59 patients with GH and 319 patients with GDM) were recruited for this study. All participants were examined and treated at the Xinjiang Uygur Autonomous Region Maternal and Child Health Care Hospital from January 2016 to October 2017. The selection of and diagnostic criteria for the PE and GH groups referred to the "Guidelines for the Diagnosis and Treatment of Hypertension in Pregnancy (2015)"[36]. The diagnosis of GDM was based on the results of an oral glucose tolerance test (OGTT) at 24-28 weeks of gestation. The OGTT results were evaluated according to the diagnostic criteria of the "Diagnosis and Treatment Guidelines for Pregnancy with Diabetes (2014)"[37]. The exclusion criteria for the patient group were a history of hypertension, renal disease, proteinuria before the 20th week of pregnancy, multiple pregnancy and diabetes mellitus. The inclusion criteria for the control group were as follows: ①no abnormal blood pressure during pregnancy (blood pressure <140/90 mmHg), premature rupture of membranes, placenta previa or threatened abortion; ②no history of chronic hypertension, PE, diabetes, heart disease, liver disease or kidney disease, and no history of immunotherapy or blood transfusion; and ③auxiliary examination during pregnancy, with normal results for all routine blood/urine, urine protein, and liver and kidney function tests.

Genomic DNA extraction

Genomic DNA samples were extracted from 200 μL of EDTA-anti-coagulated peripheral blood by using the QIAamp DNA Blood Mini Kit (Qiagen, Shanghai, China). The extracted genomic DNA was dissolved in elution buffer (10 mM Tris–HCl, 0.5 mM EDTA, pH 9.0) and stored at -20 °C. The DNA concentration was measured by using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Fitchburg, WI, USA).

Genotyping

Sanger sequencing was used to screen VEGF SNPs (+936C/T and -634C/G) using the primers listed in Table S1. PCR was carried out using the Gene Amp PCR System ABI Veriti™Dx (Applied Biosystems, Foster City, CA, USA) according to the 2X Taq PCR MasterMix (Runde, Xi'an, China) user manual in a total

volume of 20 μl. The sequencing of amplified fragments was carried out on an ABI Prism 3100 Sequencer (Applied Biosystems, Foster City, CA, USA), as described previously[38].

Statistical methods

SPSS 25.0 software (SPSS Inc, Chicago, IL, USA) was used to perform the statistical analyses. The genotype frequencies of VEGF were checked for Hardy-Weinberg equilibrium (HWE) using the chi-square test (χ^2). The Pearson chi-square test was used to compare the differences between the +936C/T and -634C/G genotype and allele frequencies in different regions and different ethnic groups in Xinjiang. The measurement data are expressed as x±s, and the independent samples *t* test was used for comparison between groups. The Pearson chi-square test (or Fisher's exact test for small samples) was used to compare the genotype and allele frequency differences between the case group and the control group, and the odds ratio (OR) and 95% confidence interval (CI) were used to express the relative risk. $P<0.05$ was considered statistically significant. Linkage disequilibrium statistics and haplotype analysis, including haplotype frequency estimation, as well as an analysis of association between haplotypes and cases, were computed and performed using SHEsis[39] software http://analysis.bio-x.cn.

Results

Correlations between VEGF +936C/T and -634C/G gene polymorphisms and PE have been widely studied[17-25], but the conclusions of studies in different regions are inconsistent. Some studies had a small sample size (N≤100)[28, 40-42], which may have affected the accuracy of the results. To clarify the distributions of the +936C/T and -634C/G allele frequencies in different parts of the world, the distributions of +936C/T and -634C/G alleles in published literature control samples were analysed (the number of control samples in the group>100). Analysis of the +936C/T locus included data from 8 studies. Chi-square analysis showed that the distribution of T alleles had significant regional differences among China, Korea, Philippines, Iran, Sri-Lanka and Tunisia (χ^2=30.965, P<0.001); -634G/C locus analysis included data from 4 studies. The chi-square analysis showed that the distribution of C alleles had significant regional differences among Korea, Iran, Tunisia and Brazil (χ^2=63.508, P<0.001). The results are shown in Table 1.

Table 1 The distributions of the VEGF +936C/T and -634G/C allele frequencies in different regions of the world

Region	Published time	VEGF-1	Sample size (controls only)	Allele (N) C	Allele (N) T	T Allele frequency(%)	χ^2	*P*
China(Hunan)[17]	2009	rs3025039 (+936C/T)	231	377	85	18.40%	30.965	<0.001
China(Shenzhen)[18]	2019		286	477	95	16.61%		
Korean[19]	2007		237	374	90	19.40%		
Korean[20]	2007		209	355	63	15.07%		
Philippines(Manila)[21]	2016		190	324	56	14.74%		
Iran(Tehran)[22]	2018		191	332	50	13.09%		
Sri-Lanka(Colombo)[23]	2012		168	306	30	8.93%		
Tunisian(Sousse)[24]	2017		300	534	66	11.00%		
Region	Published time	VEGF-2	Sample size (controls only)	Allele (N) G	Allele (N) C	C Allele frequency(%)	χ^2	*P*
Korean[19]	2007	rs2010963 (-634G/C)	237	255	183	41.78%	63.508	<0.001
Iran(Zahedan)[16]	2015		201	262	140	34.83%		
Tunisian(Sousse)[24]	2017		300	246	354	59.00%		
Brazil(Uberaba)[25]	2014		210	226	194	46.19%		

To verify whether the subjects included in this study came from the same group, the +936C/T and -634G/C genotype distributions were subjected to HWE testing. The results showed that the distributions of genotypes in the Han, Uyghur, Hui, and Kazakh populations and the total sample were all in HWE ($P>0.05$), indicating that the research sample conformed to the genetic balance of the population, and the data were representative of the population. As shown in Table 2, the chi-square test results showed that the frequencies of the +936C/T and -634G/C alleles in healthy women in Xinjiang were significantly different among the Han, Uyghur, Hui and Kazak populations ($\chi^2 = 8.837$, $P = 0.032$; $\chi^2 = 11.585$, $P = 0.009$). Pairwise comparisons between ethnic groups showed that the allele frequencies of the two loci were significantly different between the Han and Uygur populations ($P = 0.011$ and $P = 0.001$) (results are not shown).

Table 2 The allele frequencies of VEGF +936C/T and-634G/C and their comparison among the different populations

Populations		VEGF-1	Genotype distributions(N,%)			T Allele	HWE-*P*	χ^2,*P*	VEGF-2	Genotype distributions(N,%)			G Allele	HWE-*P*	χ^2,*P*
			CC	CT	TT	frequency(%)				GG	GC	CC	frequency(%)		
Han*	977	rs3025039	654(67%)	292(30%)	31(3%)	18.12%	0.28	8.837,	rs2010963	348(36%)	469(48%)	160(16%)	40.38%	0.93	11.585,
Uighur*	323	(+936C/T)	239(74%)	79(24%)	5(2%)	13.78%	0.6	0.032	(-634G/C)	146(45%)	140(43%)	37(11%)	33.13%	0.7	0.009
Hui	144		103(72%)	38(26%)	3(2%)	15.28%	0.82			53(37%)	75(52%)	16(11%)	37.15%	0.17	
Kazakh	64		48(75%)	16(25%)	0(0%)	12.5%	0.25			26(40.63%)	31(48.44%)	7(10.94%)	35.16%	0.62	
Other	35		26(74.29%)	7(20%)	2(5.71%)	15.71%	0.15			13(37.14%)	16(45.71%)	6(17.14%)	40%	0.78	
All samples	1551		1077(69%)	433(28%)	41(3%)	16.6%	0.75			591(38%)	732(47%)	228(15%)	38.3%	0.96	

*The allele frequencies of the two loci are significantly different between the Han and Uygur populations

The comparisons of the general clinical data of the case and control groups are shown in Table 3. Pregnant women in the PE group had higher body mass index (BMI) values before pregnancy, higher systolic and diastolic blood pressure during pregnancy, and lighter newborns (P <0.001) than pregnant women in the control group. The GH group was older, had a higher BMI value before pregnancy, and had higher systolic and diastolic blood pressure during pregnancy (P <0.001) than the control group. The GDM group was older and had a higher BMI value before pregnancy, higher systolic and diastolic blood pressure during pregnancy, and heavier newborns (P <0.001) than the control group.

Table 3 Demographic and clinical characteristics of the patients and healthy controls

Criteria	Controls(n=1551)	PE (n=232)	GH (n=59)	GDM (n=319)	*P-value*†	*P-value*‡	*P-value*§
Age (years)	29.69±4.6	30.33±4.87	32.23±6.15	30.69±4.85	0.057	0.003	0.001
Height(cm)	163.26±5.03	162.35±5.15	162.14±5.77	162.89±4.9	0.011	0.1	0.232
BMI (kg/m^2)	22.11±3.55	24.04±4.16	24.62±4.08	23.62±4.31	<0.001	<0.001	<0.001
Systolic blood pressure(mm Hg)	110.81±8.74	131.6±23.14	130.81±17.59	113.45±9.79	<0.001	<0.001	<0.001
Diastolic blood pressure(mm Hg)	70.21±7.06	84.39±15.32	84.98±14.08	72.09±8.25	<0.001	<0.001	<0.001
Birthweight (g)	3417.79±561.25	3001.18±834.5	3438.79±366.71	3562.72±450.69	<0.001	0.832	0.002

Data are expressed as the mean ± SEM. BMI, body mass index. †indicates comparison between the control and PE groups; ‡indicates comparison between the control and GH groups; §indicates comparison between the control and GDM groups.

Tables 4-6 show the correlation analysis results of the +936C/T and -634G/C polymorphisms with PE, GH and GDM. Table 4 summarizes the distribution of the +936C/T and -634G/C alleles and genotypes in the dominant mode [(GC+CC) vs GG] and recessive mode [CC vs (GC+GG)] in the control group and the PE group (severe PE (sPE) and mild PE (mPE)). In addition, the samples in the control group and PE group were divided into Han, Uyghur and Hui ethnic groups, and the distributions of the two SNP alleles and genotypes among the different ethnic groups were evaluated. After uncorrected chi-square test analysis, the allele frequency distributions of +936C/T and -634G/C showed no significant differences between the case group and the control group. The genotype distribution analysis was conducted using the dominant mode [(GC + CC) vs GG] and recessive mode [CC vs (GC + GG)] genetic patterns, and the results were also not significantly different between the case group and the control group. The results are shown in Table 4. The same method was used to analyse the correlations between the two SNPs and GH and GDM, and the results were not statistically significant (Table 5 and Table 6). Correlations were corrected for age, BMI, and ethnicity (results not shown), although the results were still not statistically significant.

Table 4 Correlations between the VEGF +936C/T and -634G/C genetic polymorphisms and PE risk

Samples(N)/ Different Models		VEGF (+936C/T) rs3025039						VEGF (-634G/C) rs2010963					
		Allele(N)		Dominant(N)		Recessive(N)		Allele(N)		Dominant(N)		Recessive(N)	
Allele/Genotype		T	C	CT+TT	CC	TT	CT+CC	C	G	GC+CC	GG	CC	GC+GG
Control (1551)		515	2587	474	1077	41	1510	1188	1914	960	591	228	1323
PE (232)		65	399	60	172	5	227	183	281	149	83	34	198
OR (95% CIs);*P*		0.818(0.619-1.081);0.158		0.793(0.58-1.084);0.145		0.811(0.317-2.074);0.662		1.049(0.859-1.281);0.637		1.105(0.829-1.473);0.495		0.996(0.675-1.472);0.986	
sPE (163)		46	280	44	119	2	161	131	195	109	54	22	141
OR (95% CIs);*P*		0.825(0.596-1.143);0.247		0.84(0.585-1.207);0.345		0.458(0.11-1.909);0.403		1.082(0.857-1.366);0.506		1.243(0.883-1.749);0.212		0.905(0.565-1.45);0.679	
mPE (69)		19	119	16	53	3	66	52	86	40	29	12	57
OR (95% CIs);*P*		0.802(0.49-1.314);0.38		0.686(0.388-1.212);0.192		1.674(0.505-5.546);0.394		0.974(0.685-1.385);0.884		0.849(0.521-1.385);0.512		1.222(0.645-2.313);0.538	
Han	Control (977)	354	1600	323	654	31	946	789	1165	629	348	160	817
	PE (96)	28	164	26	70	2	94	84	108	69	27	15	81
	OR (95% CIs);*P*	0.772(0.509-1.171);0.222		0.752(0.47-1.203);0.233		0.649(0.153-2.756);0.779		1.148(0.852-1.549);0.364		1.414(0.889-2.248);0.142		0.946(0.531-1.683);0.849	
Uighur	Control (323)	89	557	84	239	5	318	214	432	177	146	37	286
	PE (74)	18	130	17	57	1	73	47	101	40	34	7	67
	OR (95% CIs);*P*	0.867(0.504-1.489);0.604		0.849(0.468-1.54);0.589		0.871(0.1-7.57);0.689*		0.939(0.641-1.378);0.749		0.97(0.584-1.611);0.908		0.808(0.345-1.89);0.622	
Hui	Control (144)	44	244	41	103	3	141	107	181	91	53	16	128
	PE (21)	6	36	6	15	0	21	20	22	14	7	6	15
	OR (95% CIs);*P*	0.924(0.368-2.324);0.867		1.005(0.365-2.769);0.992		1.149(1.083-1.219);0.663*		1.538(0.802-2.949);0.193		1.165(0.442-3.068);0.757		3.2(1.087-9.423);0.064	

*Results of Fisher's exact test

Table 5 Correlations between the VEGF +936C/T and -634G/C genetic polymorphisms and GH risk

Samples (N)/ Different Models		VEGF (+936C/T) rs3025039						VEGF (-634G/C) rs2010963					
		Allele(N)		Dominant(N)		Recessive(N)		Allele(N)		Dominant(N)		Recessive(N)	
Allele/Genotype		T	C	CT+TT	CC	TT	CT+CC	C	G	GC+CC	GG	CC	GC+GG
Control (1551)		515	2587	474	1077	41	1510	1188	1914	960	591	228	1323
GH (58)		24	92	23	35	1	57	47	69	35	23	12	46
OR (95% CIs);*P*		0.978(0.777-1.232);0.852		0.993(0.764-1.29);0.957		0.826(0.367-1.859);0.644		0.965(0.809-1.151);0.692		1.007(0.786-1.291);0.954		0.856(0.599-1.223);0.392	
Han	Control (977)	354	1600	323	654	31	946	789	1165	629	348	160	817
	GH (28)	14	42	13	15	1	27	24	32	17	11	7	21
	OR (95% CIs);*P*	1.507(0.814-2.789);0.189		1.755(0.825-3.732);0.139		1.13(0.149-8.586);1		1.107(0.647-1.894);0.709		0.855(0.396-1.846);0.69		1.702(0.712-4.071);0.342	
Uighur	Control (323)	89	557	84	239	5	318	214	432	177	146	37	286
	GH (15)	6	24	6	9	0	15	14	16	10	5	4	11
	OR (95% CIs);*P*	1.565(0.622-3.935);0.49		1.897(0.656-5.489);0.368		1.047(1.023-1.072);0.796*		1.766(0.846-3.686);0.125		1.65(0.552-4.935);0.366		2.811(0.851-9.281);0.174	
Hui	Control (144)	44	244	41	103	3	141	107	181	91	53	16	128
	GH (8)	1	15	1	7	0	8	5	11	4	4	1	7
	OR (95% CIs);*P*	0.37(0.048-2.87);0.53		0.359(0.043-3.009);0.564		1.057(1.017-1.098);0.849*		0.769(0.26-2.273);0.634		0.582(0.14-2.426);0.708		1.143(0.132-9.898);1	

*Results of Fisher's exact test

Table 6 Correlations between the VEGF +936C/T and -634G/C genetic polymorphisms and GDM risk

Samples (N)/ Different Models		VEGF (+936C/T) rs3025039						VEGF (-634G/C) rs2010963					
		Allele(N)		Dominant(N)		Recessive(N)		Allele(N)		Dominant(N)		Recessive(N)	
Allele/Genotype		T	C	CT+TT	CC	TT	CT+CC	C	G	GC+CC	GG	CC	GC+GG
Control (1551)		515	2587	474	1077	41	1510	1188	1914	960	591	228	1323
GDM (319)		104	534	97	222	7	312	239	399	198	121	41	278
OR (95% CIs);*P*		1.31(0.828-2.074);0.247		1.493(0.873-2.555);0.141		0.646(0.087-4.78);0.991		1.097(0.752-1.601);0.629		0.937(0.548-1.601);0.811		1.514(0.79-2.902);0.209	
Han	Control (977)	354	1600	323	654	31	946	789	1165	629	348	160	817
	GDM (221)	78	364	73	148	5	216	170	272	143	78	27	194
	OR (95% CIs);*P*	0.969(0.739-1.269);0.817		0.999(0.732-1.362);0.993		0.706(0.272-1.838);0.474		0.923(0.747-1.141);0.458		1.014(0.747-1.377);0.927		0.711(0.459-1.1);0.124	
Uighur	Control (323)	89	557	84	239	5	318	214	432	177	146	37	286
	GDM (46)	10	82	9	37	1	45	33	59	24	22	9	37
	OR (95% CIs);*P*	0.763(0.381-1.527);0.444		0.692(0.321-1.494);0.347		1.413(0.161-12.374);1*		1.129(0.715-1.782);0.602		0.9(0.485-1.67);0.738		1.88(0.841-4.205);0.119	
Hui	Control (144)	44	244	41	103	3	141	107	181	91	53	16	128
	GDM (32)	9	55	8	24	1	31	24	40	21	11	3	29
	OR (95% CIs);*P*	0.907(0.418-1.969);0.806		0.837(0.348-2.015);0.692		1.516(0.153-15.067);0.555*		1.015(0.58-1.776);0.959		1.112(0.497-2.485);0.796		0.828(0.226-3.029);1	

*Results of Fisher's exact test

Table S2 shows the distribution frequency of haplotypes among the control, mPE, sPE, GH and GDM groups. The CT haplotypes were identified as severe PE protective type and GH risk type. Since this is not a common haplotype (frequency <0.15), the results are not representative.

Discussion

VEGF is one of the most effective pro-angiogenic factors known. During pregnancy, VEGF plays a vital role in trophoblast infiltration, placental growth and uterine blood flow regulation[9, 43]. At least 30 SNPs have been found in the VEGF gene, and the correlations between the + 936C/T and − 634G/C polymorphisms and PE have been extensively studied in various regions of the world. In the present study, we investigated the distributions of + 936C/T and − 634G/C allele frequencies in healthy pregnant women in different regions of the world and found significant differences (Table 1). However, most of the published literature does not specify the ethnicity of the included population. We studied genetic variations in + 936C/T and − 634G/C polymorphisms in four major ethnic groups (Han, Uyghur, Hui and Kazakh) in Xinjiang, China. Our data showed that the distributions of the + 936C/T and − 634G/C allele frequencies between healthy Han and Uygur women were significantly different (Table 2). The frequency of the + 936C/T allele in the Xinjiang Han population obtained in this study is consistent with the frequency of the previously reported Hunan Han allele (18.12% vs 18.40%)[17]. The results showed that

the + 936C/T and − 634G/C polymorphisms had significant regional and ethnic differences in distribution and morphology.

The pathogenesis of PE has not been elucidated. It is generally believed that maternal-foetal interface imbalance, thrombosis, genetic susceptibility or other factors prevent trophoblast cells from invading the endometrium and remodelling the spiral uterine artery[9]. VEGF is thought to be involved in the pathogenesis of PE vascular injury. In reports of the relationship between the VEGF + 936C/T and − 634G/C polymorphisms and PE susceptibility[17–26, 40–42], some reports indicated that the + 936C/T polymorphism was related to the risk of PE[17, 20–22], while other studies reported that the + 936C/T polymorphism was not associated with PE risk[18, 19, 23, 24]. Similarly, regarding the relationship between the − 634G/C polymorphism and PE susceptibility, most of the results were negative[19, 24, 25], but there are also reports that this polymorphism is associated with severe PE[16]. Analysis of the possible factors affecting the results and speculation that certain clinical factors (such as the differences in the study area and population included in the study, the sample size, and the inclusion and exclusion criteria for the study group) may cause bias, resulting in false negative or false positive results. In addition, sPE and mPE have high clinical heterogeneity, and subgroup analysis is recommended, but only two of the above documents classified PE[16, 21].

In this study, a total of 1551 cases in the control group, 232 cases in the PE group and 59 cases in the GH group were included. To avoid bias caused by heterogeneity of the included samples, the diseases were analysed in subgroups. We found that VEGF + 936C/T and − 634G/C gene polymorphisms were not correlated with mPE, sPE or GH. An ethnic subgroup analysis of the population revealed that + 936C/T and − 634G/C gene polymorphisms in the Han, Uygur and Hui populations were also not related to PE or GH. A total of 319 patients in the GDM group were included in this study. We found that + 936C/T and − 634G/C gene polymorphisms were not associated with GDM in Xinjiang, China. An ethnic subgroup analysis found that in the Han, Uygur and Hui populations, the two SNP sites were not related to GDM.

An interesting result of this study is that although the detected VEGF + 936C/T and − 634G/C allele and genotype distributions were not comparable in case and control groups, there were significant differences in haplotype distributions. The CT haplotypes were identified as severe PE protective type and GH risk type. Since this is not a common haplotype (frequency < 0.15), the results are not representative. The identification of haplotypes in VEGF loci may explain the changes in VEGF secretion, highlighting the role of haplotype analysis in genetic association studies.

Conclusions

In conclusion, this study is the first case-control study to assess the possible link between VEGF genotype/haplotype and pregnancy complications in the Xinjiang population. The results showed that VEGF + 936C/T and − 634G/C gene polymorphisms had significant regional and ethnic distribution differences. In Xinjiang, VEGF + 936C/T and − 634G/C gene polymorphisms do not confer susceptibility

to PE, GH or GDM. The conclusions of this study still need further verification in a study with a larger sample size.

Abbreviations

VEGF: Vascular endothelial growth factor; PE: preeclampsia; GH: gestational hypertension; GDM: gestational diabetes mellitus; mPE: mild preeclampsia; sPE: severe preeclampsia; sFlt-1: soluble fms-like tyrosine kinase 1; sENG: soluble endoglin; SNPs: single nucleotide polymorphisms; T2DM: type 2 diabetes mellitus; OGTT: oral glucose tolerance test; HWE: Hardy-Weinberg equilibrium; OR: odds ratio; CI: confidence interval; BMI: body mass index

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Xinjiang Uygur Autonomous Region Maternal and Child Health Care Hospital. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The original data set is available on individual request by emailing the corresponding author, yanhua_xida@163.com.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Shaanxi Natural Science Basic Research Project, China (NO.2020JQ-958); and National Natural Science Foundation of China (NSFC) (81960285). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

HC, JM, GD, SX, XZ and HY were involved in the concept and design. HC, JM and SX performed the analyses and HC contributed to the interpretation of the data. HC drafted the manuscript, and CC, GD, XZ and HY provided critical revision. HC, CC, GD, XZ and HY were involved in the final approval. All authors read and approved the final manuscript.

Acknowledgements

This study was financially supported by the Tianshan Innovation Team Plan.

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References

1. Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev. 1997;18(1):4-25.
2. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr Rev. 1992;13(1):18-32.
3. Vincenti V, Cassano C, Rocchi M, Persico G. Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. Circulation. 1996;93(8):1493-1495.
4. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol. 1991;5(12):1806-1814.
5. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, *et al*. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. J Biol Chem. 1991;266(18):11947-11954.
6. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9(6):669-676.
7. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem. 2001;47(4):617-623.
8. Breier G, Albrecht U, Sterrer S, Risau W. Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development. 1992;114(2):521-532.
9. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol. 2019;15(5):275-289.
10. Nabiel Y, Mosbah A. Maternal Serum sEndoglin and Cell-Free Fetal DNA as Probable Markers of Preeclampsia: A Study in Single Center, Egypt. Immunol Invest. 2019;48(6):608-617.
11. Liberis A, Stanulov G, Ali EC, Hassan A, Pagalos A, Kontomanolis EN. Pre-eclampsia and the vascular endothelial growth factor: a new aspect. Clin Exp Obstet Gynecol. 2016;43(1):9-13.
12. Odibo AO, Rada CC, Cahill AG, Goetzinger KR, Tuuli MG, Odibo L, *et al*. First-trimester serum soluble fms-like tyrosine kinase-1, free vascular endothelial growth factor, placental growth factor and

uterine artery Doppler in preeclampsia. J Perinatol. 2013;33(9):670-674.

13. Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene. Hum Immunol. 1999;60(12):1245-1249.
14. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res. 2000;37(6):443-448.
15. 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.
16. Salimi S, Yaghmaei M, Tabatabaei E, Mokhtari M, Naghavi A. Vascular endothelial growth factor (VEGF)-634G/C polymorphism was associated with severe pre-eclampsia and lower serum VEGF level. J Obstet Gynaecol Res. 2015;41(12):1877-1883.
17. Huang YL, Liu J. Analysis on gene polymorphism of vascular endothelial growth factor and susceptibility of pre-eclampsia patient**.** Maternal Child Health Care China**.** 2019;24:2712–14.
18. Zhou L, Huang Y, Hong XP, Liu YL, Xu Y, Xie JS. Association study between vascular endothelial related gene polymorphism and pre-eclampsia susceptibility. Chin J Birth Health & Heredity. 2019;27:139–41.
19. Kim YJ, Park BH, Park H, Jung SC, Pang MG, Ryu HM, *et al*. No association of the genetic polymorphisms of endothelial nitric oxide synthase, dimethylarginine dimethylaminohydrolase, and vascular endothelial growth factor with preeclampsia in Korean populations. Twin Res Hum Genet. 2008;11(1):77-83.
20. Shim JY, Jun JK, Jung BK, Kim SH, Won HS, Lee PR, *et al*. Vascular endothelial growth factor gene +936 C/T polymorphism is associated with preeclampsia in Korean women. Am J Obstet Gynecol. 2007;197(3):271 e271-274.
21. Amosco MD, Villar VA, Naniong JM, David-Bustamante LM, Jose PA, Palmes-Saloma CP. VEGF-A and VEGFR1 SNPs associate with preeclampsia in a Philippine population. Clin Exp Hypertens. 2016;38(7):578-585.
22. Amin-Beidokhti M, Gholami M, Abedin-Do A, Pirjani R, Sadeghi H, Karamoddin F, *et al*. An intron variant in the FLT1 gene increases the risk of preeclampsia in Iranian women. Clin Exp Hypertens. 2018:1-5.
23. Andraweera PH, Dekker GA, Dissanayake VH, Bianco-Miotto T, Jayasekara RW, Roberts CT. Vascular endothelial growth factor family gene polymorphisms in preeclampsia in Sinhalese women in Sri-Lanka. J Matern Fetal Med. 2013;26(5):532-536.
24. Ben Ali Gannoun M, Al-Madhi SA, Zitouni H, Raguema N, Meddeb S, Hachena Ben Ali F, *et al*. Vascular endothelial growth factor single nucleotide polymorphisms and haplotypes in pre-eclampsia: A case-control study. Cytokine. 2017;97:175-180.

25. Silva VR, Soardi FC, Tanaka SC, da Silva-Grecco RL, Paschoini MC, Balarin MA. Investigation of polymorphisms in pre-eclampsia related genes VEGF and IL1A. Arch Gynecol Obstet. 2015;291(5):1029-1035.
26. Sandrim VC, Palei AC, Cavalli RC, Araújo FM, Ramos ES, Duarte G, *et al*. Vascular endothelial growth factor genotypes and haplotypes are associated with pre-eclampsia but not with gestational hypertension. Mol Hum Reprod. 2009;15(2):115-120.
27. Simmons LA, Hennessy A, Gillin AG, Jeremy RW. Uteroplacental blood flow and placental vascular endothelial growth factor in normotensive and pre-eclamptic pregnancy. BJOG. 2000;107(5):678-685.
28. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Invest. 1997;99(9):2152-2164.
29. Sgambati E, Marini M, Zappoli Thyrion GD, Parretti E, Mello G, Orlando C, *et al*. VEGF expression in the placenta from pregnancies complicated by hypertensive disorders. BJOG. 2004;111(6):564-570.
30. Kim HW, Ko GJ, Kang YS, Lee MH, Song HK, Kim HK, *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.
31. Wirostko B, Wong TY, Simó R. Vascular endothelial growth factor and diabetic complications. Prog Retin Eye Res. 2008;27(6):608-621.
32. Katavetin P, Miyata T, Inagi R, Tanaka T, Sassa R, Ingelfinger JR, *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.
33. Lygnos MC, Pappa KI, Papadaki HA, Relakis C, Koumantakis E, Anagnou NP, *et al*. Changes in maternal plasma levels of VEGF, bFGF, TGF-beta1, ET-1 and sKL during uncomplicated pregnancy, hypertensive pregnancy and gestational diabetes. In Vivo. 2006;20(1):157-163.
34. CHEN Zhi-Fang. Characteristics and significance of changes of serum VEGF and sFlt-1 levels during pregnancy and postpartum in patients with GDM. Maternal & Child Health Care of China. 2015;030(001):42-44.
35. Dong PP. Association of vascular endothelial growth factor expression and polymorphisms with the risk of gestational diabetes mellitus. J Clin Lab Anal. 2019;33(2):e22686.
36. Yang Zi, Zhang Weiyuan. Guidelines for the Diagnosis and Treatment of Hypertension in Pregnancy (2015). Chinese Journal of Perinatal Medicine. 2016;19(03):161-169.
37. Yang Huixia. Guidelines for diagnosis and treatment of pregnancy with diabetes mellitus. Chinese Journal of Obstetrics and Gynecology. 2014;49(8):561-569.
38. Chen L, Qin S, Xie J, Tang J, Yang L, Shen W, *et al*. Genetic polymorphism analysis of CYP2C19 in Chinese Han populations from different geographic areas of mainland China. Pharmacogenomics. 2008;9(6):691-702.
39. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, *et al*. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res. 2009;19(4):519-523.

40. Chedraui P, Solis EJ, Bocci G, Gopal S, Russo E, Escobar GS, *et al*. Feto-placental nitric oxide, asymmetric dimethylarginine and vascular endothelial growth factor (VEGF) levels and VEGF gene polymorphisms in severe preeclampsia. J Matern-Fetal Neo M. 2013;26(3):226-232.
41. Papazoglou D, Galazios G, Koukourakis MI, Panagopoulos I, Kontomanolis EN, Papatheodorou K, *et al*. Vascular endothelial growth factor gene polymorphisms and pre-eclampsia. Mol Hum Reprod. 2004;10(5):321-324.
42. Procopciuc LM, Caracostea G, Zaharie G, Stamatian F. Maternal/newborn VEGF-C936T interaction and its influence on the risk, severity and prognosis of preeclampsia, as well as on the maternal angiogenic profile. J Matern-Fetal Neo M. 2014;27(17):1754-1760.
43. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. Lancet. 2016;387(10022):999-1011.

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