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Original Article

Association between interleukin-4 C-590T and C+33T genetic polymorphisms and risk of preeclampsia in pregnant women of Central China

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Abstract: The pathogenesis of preeclampsia involves many environmental and genetic factors. An excessive inflammatory response during pregnancy could cause an imbalance in the immune system, and thus contributes to the development of preeclampsia. Inflammatory cytokines produced by T-helper 2 cells, such as IL-4, contribute to inhibiting cellular immunity and inducing the placental growth. Two common genetic polymorphisms were observed in IL-4, including C-590T and C+33T. We performed a study to investigate the association between IL-4 C-590T and C+33T polymorphisms and development of preeclampsia in a Chinese pregnant women. We conducted a case-control study that included 162 pregnant women with preeclampsia and 266 healthy controls. The genotyping of the IL-4 C-590T and C+33T was carried out by a method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). By logistic regression analysis, the CC genotype of IL-4 C-590T was significant associated with a reduced risk of preeclampsia when compared with the GG genotype, and the adjusted OR (95% CI) was 0.39 (0.15-0.90). In dominant model, we observed that the GC+CC genotype was correlated with a decreased risk of preeclampsia in comparison to the GG genotype (OR=0.59, 95% CI=0.35-0.97). In recessive model, we found that the CC genotype exposed a lower risk of preeclampsia than the GG+GC genotype (OR=0.41, 95% CI=0.16-0.94). However, no significant association was found between L-4 C+33T polymorphism and susceptibility to preeclampsia. In conclusion, our study suggests that IL-4 C-590T polymorphism could be a risk factor for preeclampsia.

Keywords: IL-4 C-590T, IL-4 C+33T, polymorphism, preeclampsia

Introduction

Preeclampsia is a common obstetrical complication characterized by blood pressure ≥140/90 mmHg in previously normotensive women after 20-week gestation with proteinuria (≥300 mg/24 h), which notably leads to perinatal death and cardiovascular diseases of affected babies [1-3]. It is estimated that about 5%-8% pregnant women suffer from preeclampsia [4]. Many studies have investigated the etiology and pathological mechanisms of preeclampsia [5, 6], but the pathogenesis of preeclampsia is still unclear. The pathogenesis of preeclampsia involves many environmental factors, such as preexisting hypertension, advanced maternal age, multiple birth, diabetes and cardiovascular disease as well as obesity [7-9]. However, it is reported that about 35% of pregnant women suffering from preeclampsia are caused by maternal or fetal genetic factors [8]. Previous studies have shown that many genetic factors play an important factor in the development of preeclampsia, such as fibroblast growth factor (FGF) 1, FGF2, transforming growth factor b1, miR-193b-3p, caspase recruitment domain family member 8, vitamin-D receptor and vascular endothelial growth factor genes [10-15].

It is reported that an excessive inflammatory response during pregnancy could cause an imbalance in the immune system, which may contribute to the development of preeclampsia [16, 17]. Inflammatory cytokines produced by T-helper 2 cells, such as IL-4, IL-5 and IL-6 and IL-10, contribute to inhibiting cellular immunity and inducing the placental growth [18]. Interleukin-4 (IL-4) gene is a pleiotropic cytokine, and it is produced by the activate Th2 cells, B lymphocytes, mast cells and basophils. IL-4 is

an important regulation factor for Th2 cellular immune response. IL-4 has an important role in promoting humoral immunity and antagonize Th1 activity of cytokines [19, 20]. IL-4 gene is located at chromosome q531-33, including four exons and three introns. Two common genetic polymorphisms are observed in IL-4, including C-590T and C+33T. The IL-4 C-590T is located at promoter region, and IL-4 C+33T is at exon 1. Currently, several studies have been reported that many interleukin genetic polymorphisms contribute to the development of preeclampsia, such as IL-1, IL-10, IL-17 and IL-27 [21-24]. Currently, only two studies reported the association between IL-4 C-590T polymorphism and risk of preeclampsia [25, 26], but no study reported the correlation of IL-4 C+33T polymorphism with risk of this disease. Therefore, we performed a study to investigate the association between IL-4 C-590T and C+33T polymorphisms and development of preeclampsia in a Chinese pregnant women.

Material and methods

Subjects

We conducted a case-control study that included 162 pregnant women with preeclampsia and 266 healthy controls. These 162 pregnant women with preeclampsia were enrolled from the Department of Obstetrics and Gynecology of the Department of Obsterics, Women and Infants Hospital of Zhengzhou and The First Affiliated Hospital of Xinxiang Medical University between March 2013 and March 2015. All the pregnant women with preeclampsia were confirmed to be preclampsia through clinical findings. The preeclampsia was diagnosed based on the elevated blood pressure (systolic pressure value ≥140 mmHg or diastolic pressure value ≥90 mmHg) and proteinuria (urinary protein ≥0.3 g/24 hours or urine dipstick protein ≥+1 more than 24 hours) after twenty weeks of gestation [1]. The exclusion criteria of pregnant women with preeclampsia were those suffering from diabetes, chronic hypertension, multiple pregnancy and cardiovascular and cerebrovascular diseases. The mean age of women with preeclampsia was 27.8±8.7 years.

The healthy controls were recruited from the Department of Obstetrics and Gynecology of the Department of Obsterics, Women and Infants Hospital of Zhengzhou and The First Affiliated Hospital of Xinxiang Medical University, and the controls were pregnant women receiving prenatal examination. The inclusion criteria were those with gestation >20 weeks and single pregnancy, and without diabetes, chronic hypertension, and cardiovascular and cerebrovascular diseases. The mean age of healthy controls was 26.9±7.7 years.

Details of potential risk factors for preeclampsia and demographic characteristics were obtained using a structured questionnaire or from medical records. The information included age, gestational weeks, tobacco smoking and alcohol drinking prior to pregnancy, body mass index, systolic blood pressure, diastolic blood pressure, delivery weeks, 24-hour urinary protein uric acid, uric acid and newborn birth weight.

Genotyping of IL-4 C-590T and C+33T polymorphisms

Five ml peripheral blood sample was collected from each subject, and the sample was kept in ethylenediaminetetraacetic acid (EDTA)-containing tubes. Then the genomic DNA was extracted using a DNA extraction kit (TaKaRa Bio, Dalian, China) according to manufacturer instructions. The genotyping of the IL-4 C-590T and C+33T was carried out by a method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using a PCR Thermocycle Instrument (MJ Research Inc., St. Bruno, Canada). The primers were provided by ABI Applied Biosystems (Waltham, USA). The forward and reverse primers for IL-4 C-590T were 5'-TAGAGATATCTTTGTCAGCATT-3' and 5'-GACCCATTAATAGGTGTCG-3', respectively. The forward and reverse primers for IL-4 C+33T were 5'-CACTAAACTTGGGAGAACATGGT-3' and 5'-CCTCCTGGGGAAAGATAGAGTAATA-3', respectively. The IL-4 reaction was perform in 50 μl reaction mixture, comprising 2.5 μl each primer, 2 μl DNA, 8 μl 10× PCR Buffer solution (Mg^{2+}), 4 μl dNTP, 0.25 μl Taq polymerase and 30.75 μl ddH_2O. The PCR regimen for IL-4 C-590T was as follows: initiation annealing at 94°C for 5 min; 38 cycles of 94°C for 30 s, 57°C for 40 s, and 72°C for 60 s; and a final extension of 72°C for 7 min. The PCR cycling for IL-4 C+33T was as follows: initiation annealing at 94°C for 5 min; 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 40 s; and a final extension of 72°C for 7 min.

The restriction enzymes for IL-4 C-590T and C+33T were *Ava*II and *Mn*I, respectively. The enzyme digestion reaction for IL-4 C-590T was performed in 20 μl reaction mixture, including

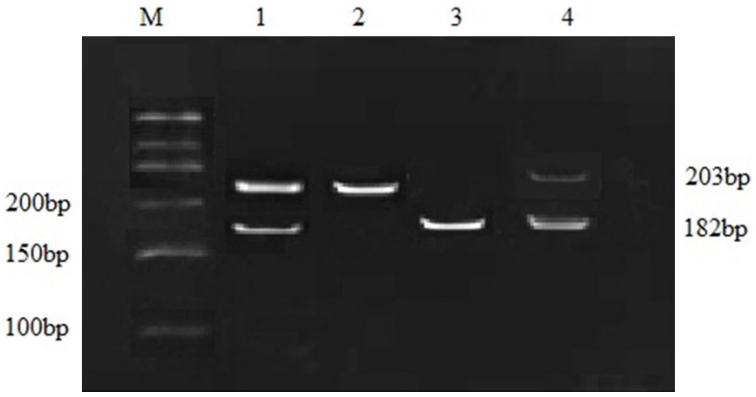

Figure 1. Electrophoregram of PCR products of IL-4 C-590T. M: marker (DNA ladder); lane 1 and 4: TC genotype; lane 2: TT genotype; lane 3: CC genotype.

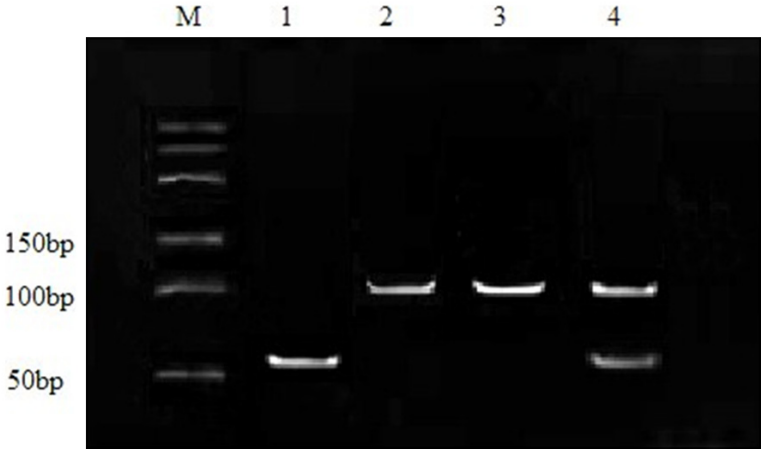

Figure 2. Electrophoregram of PCR products of IL-4 C+33T. M: marker (DNA ladder); lane 1: CC genotype; lane 2 and 3: TT genotype; lane 4: TC genotype.

10 μl PCR products, 2 μl 10× Buffer, 0.5 μl *Ava*II restriction enzymes and 7.5 μl ddH_2O. The digestion reaction for IL-4 C+33T included 10 μl PCR products, 2 μl 10× Buffer, 0.5 μl MnI restriction enzymes and 7.5 μl ddH_2O. The enzyme digestion products of IL-4 C-590T and C+33T were observed on 8% polyacrylamide gel, and they presented in ultraviolet light (**Figures 1** and **2**).

Statistical analysis

The demographic and clinical characteristics were expressed by the frequencies and percentage or mean ± standard deviation. The differences of demographic and clinical characteristics between patients with preeclampsia and controls were compared by Chi-square test or student's *t*-test. Deviation of IL-4 C-590T and C+33T genotype frequencies from the Hardy-Weinberg equilibrium was estimated by Chi-square test. The association of IL-4 C-590T and C+33T genetic polymorphisms with the risk of preeclampsia was analyzed by multiple logistic regression analysis; odds ratios (ORs) and 95% confidence intervals were used to express the results. Three genotype models were used in this study, including dominant, co-dominant and recessive models. Analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA).

Results

The mean ages of preeclampsia pregnant women and controls were 27.80±8.70 and 26.90±7.70 years, respectively (**Table 1**). There were no significant differences between preeclampsia pregnant women and controls in respect to age (t=1.12, P=0.13), gestational weeks (t=1.17, P=0.12), smoking (χ^2=2.55, P=0.11) and drinking (χ^2=0.27, P=0.60) status prior to pregnancy. However, significant differences was found between patients with preeclampsia pregnant women and controls in terms of body mass index (t=25.21, P<0.001), level of systolic blood pressure (t=19.91, P<0.001), diastolic blood pressure (t=14.04, P<0.001) and uric acid (t=29.69, P<0.001), and delivery weeks (t=14.47, P<0.001) as well as newborn birth weight (t=13.55, P<0.001). The 24-hour urinary protein uric acid of preeclampsia pregnant women was 2540.52±486.40 mg.

The electrophoregrams of PCR products of IL-4 C-590T and C+33T were shown in **Figures 1** and **2**. For IL-4 C-590T, the CC genotype was digested into 182 bp fragment, the TC genotype was digested into 203 bp and 182 bp fragments, and the TT genotype was digested into 203 bp fragment (**Figure 1**). For IL-4 C+33T, the CC genotype was digested into 63 bp frag-

Table 1. Demographic and clinical characteristics of patients with preeclampsia and controls

Variables	Patients N=162	%	Controls N=266	%	χ^2 or t test	*P* value
Age, years	27.80±8.70		26.90±7.70		1.12	0.13
Gestational weeks	26.55±4.10		27.05±4.40		1.17	0.12
Smoking status prior to pregnancy						
No	147	90.76	252	94.63		
Yes	15	9.24	14	5.37	2.55	0.11
Drinking status prior to pregnancy						
No	132	81.51	222	83.41		
Yes	30	18.49	44	16.59	0.27	0.60
Body mass index, kg/m^2	28.76±4.53		27.25±3.75		25.21	<0.001
<24	51	42.86	138	67.32		
≥24	68	57.14	67	32.68		
Systolic blood pressure, mmHg	141.50±14.35		116.45±11.45		19.91	<0.001
Diastolic blood pressure, mmHg	103.52±16.75		78.45±18.60		14.04	<0.001
Delivery weeks	33.72±3.52		38.75±3.47		14.47	<0.001
24-hour urinary protein uric acid, mg	2540.52±486.40		-			
Uric acid, umol/L	453.22±71.52		280.53±48.65		29.69	<0.001
Newborn birth weight, g	2615.43±651.30		3515.34±675.75		13.55	<0.001

Table 2. Genotype distributions of IL-4 C-590T and C+33T in patients with preeclampsia and controls

IL-4	Patients N=162	%	Controls N=266	%	χ^2 test	*P* value	χ^2 test for HWE in controls	*P* value for HWE in controls
C-590T								
TT	132	81.48	192	72.18				
TC	22	13.58	44	16.54				
CC	8	4.94	30	11.28	6.28	0.04	59.79	<0.001
C+33T								
TT	120	74.07	187	70.30				
TC	36	22.22	67	25.19				
CC	6	3.71	12	4.51	0.72	0.70	3.33	0.07

ments, the TC genotype was digested into 94 bp and 63 bp fragments, and the TT genotype was digested into 94 bp fragments (**Figure 2**).

The genotype distributions of IL-4 C-590T and C+33T were shown in **Table 2**. Using Chi-square test, we found the TT, TC and CC genotype frequencies of IL-4 C-590T were significantly different between patients with preeclampsia and controls (χ^2=6.28, P=0.04), but the IL-4 C+33T genotype distributions did not (χ^2=0.72, P=0.70). The IL-4 C-590T genotype distributions did not confirm with the HWE in controls (χ^2=59.79, P<0.001).

The association between IL-4 C-590T and C+33T polymorphisms and risk of preeclampsia was shown in **Table 3**. By logistic regression analysis, the CC genotype of IL-4 C-590T was significant associated with a reduced risk of preeclampsia when compared with the GG genotype, and the adjusted OR (95% CI) was 0.39 (0.15-0.90). In dominant model, we observed that the GC+CC genotype was correlated with a decreased risk of preeclampsia in comparison to the GG genotype (OR=0.59, 95% CI=0.35-0.97). In recessive model, we found that the CC genotype exposed a lower risk of preeclampsia than the GG+GC genotype (OR=0.41, 95% CI=0.16-0.94). However, no significant association was found between L-4 C+33T polymorphism and development of preeclampsia in all the genetic models.

Discussion

Single nucleotide polymorphisms (SNPs) refer to DNA sequence polymorphisms caused by a

Table 3. Association of IL-4 C-590T and C+33T polymorphisms with the risk of preeclampsia

IL-6	Patients N=162	%	Controls N=266	%	OR (95% CI)[1]	*P* value
-572G/C						
Co-dominant						
GG	132	81.48	192	72.18	1.0 (Ref.)	-
GC	22	13.58	44	16.54	0.73 (0.40-1.31))	0.26
CC	8	4.94	30	11.28	0.39 (0.15-0.90)	0.02
Dominant						
GG	132	81.48	192	72.18	1.0 (Ref.)	-
GC+CC	30	18.52	74	27.82	0.59 (0.35-0.97)	0.03
Recessive						
GG+GC	154	95.06	236	88.72	1.0 (Ref.)	-
CC	8	4.94	30	11.28	0.41 (0.16-0.94)	0.03
-597G/A						
Co-dominant						
GG	120	74.07	187	70.30	1.0 (Ref.)	-
GA	36	22.22	67	25.19	0.84 (0.51-1.36)	0.45
AA	6	3.71	12	4.51	0.78 (0.23-2.32)	0.63
Dominant						
GG	120	74.07	187	70.30	1.0 (Ref.)	-
GA+AA	42	25.93	79	29.70	0.83 (0.52-1.31)	0.4
Recessive						
GG+GA	156	96.30	254	95.49	1.0 (Ref.)	-
AA	6	3.70	12	4.51	0.81 (0.25-2.40)	0.67

[1]Adjusted for body mass index, systolic blood pressure, diastolic blood pressure, delivery weeks, uric acid and newborn birth weight.

single nucleotide variation, and it is estimated that more than one percentage in a population show genetic polymorphism. The genetic mutation involves the transformation of a single base through transversion, insertion, or deletions, and the SNP is considered to be involved in susceptibility to human diseases [27, 28]. Single nucleotide polymorphisms of IL-4 C-590T and C+33T encoding inflammatory factors may influence an individual's cytokine production level and reaction intensity, and are associated with pathogenesis of many diseases. In our study, we observed that IL-4 C-590T polymorphism was associated with a reduced risk of preeclampsia in co-dominant, dominant and recessive models.

T-helper 2 cells produced many kinds of inflammatory cytokines, including IL-4, IL-5, IL-6 and IL-10, and these inflammatory cytokines could inhibit cellular immunity and cause the placental growth and enhancement [18]. Mansouri et al. performed a study to detect serum levels of cytokines in pregnant women with preeclampsia, and they observed dysregulation of cytokine expression in preeclampsia with increased levels of IL-4 [29]. Vargas-Rojas et al. done a study in Mexico, and they suggested that a shift in the Th1/Th2 could favor skewness towards a proinflammatory status in the umbilical cord blood in preeclampsia [30]. Therefore, the changed expression of IL-4 is associated with the pathogenesis of preeclampsia.

Currently, only two studies reported the correlation between IL-4 C-590T polymorphism and risk of preeclampsia [25, 26]. Fraser et al. performed a study with 78 women with preeclampsia and 125 normal controls in Taiwan, and they did not find a significant correlation between IL-4 C-590T polymorphism and development of preeclampsia in Taiwanese women [26]. Fraser et al. done a study with 117 preeclamptic women and 146 control subjects in the United Kingdom, and they found that the women carrying the TT homozygous of IL-4 C-590T was associated with a higher risk of preeclampsia in comparison to the CC genotype [25]. However, no study has reported the correlation between IL-4 C+33T polymorphism and preeclampsia risk. Currently, previous studies indicated that IL-4 C+33T polymorphism contributed to the development of many kinds of inflammatory related diseases, such as asthma, allergic rhinitis and chronic hepatitis C diseases [31-33]. Our study firstly reported the role of IL-4 C+33T polymorphism in the pathogenesis of preeclampsia, and we did not find a significant correlation between IL-4 C+33T polymorphism and risk of preeclampsia. Further studies are greatly needed to confirm our results.

Two important limitations of our study should be mentioned. First, selection bias should be

considered in this study. All investigated subjects were selected from only one hospital. Second, it is possible that genes apart from IL-4 C-590T and C+33T may also play a role in preeclampsia development, and therefore gene-gene interactions should be considered during future analysis.

In conclusion, our study suggests that the IL-4 C-590T polymorphism contributes to the development of preeclampsia in all genetic models. IL-4 C-590T polymorphism could be a risk factor for preeclampsia. Further studies with more samples and ethnicities are required to verified our findings.

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Disclosure of conflict of interest

None.

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