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Association between interleukin-4 polymorphisms and risk of pre-eclampsia in a population of Chinese pregnant women

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ABSTRACT. Preeclampsia is a common disease unique to pregnant women, and its development involves many genetics l factors. IL-4 is an important regulatory factor of the Th2 cellular immune response, and plays an important role in the induction of placental growth. In this study, we investigated the relationship between *IL-4* C-590T, C+33T and G-1098T polymorphisms and risk of pre-eclampsia in a population of pregnant women. A case-control study of 196 pregnant women with pre-eclampsia and 257 healthy controls was conducted. Genotyping of *IL-4* C-590T, C+33T and G-1098T was performed by polymerase chain reaction–restriction fragment length polymorphism. We observed that the TT genotype, compared to the CC genotype, of *IL-4* C-590T harbored a lower risk of pre-eclampsia; adjusted OR (95%CI) was 0.29 (0.11-0.81). The CT+TT genotype, compared to the CC genotype, harbored a lower risk of pre-eclampsia (adjusted OR = 0.50, 95%CI = 0.30-0.84) in the dominant model. In the recessive model, the TT genotype, compared to the CC+CT genotype, harbored a lower risk of pre-eclampsia (adjusted OR = 0.31, 95%CI = 0.11-0.86). However,

no significant correlation was observed between the *IL-4* C+33T and G-1098T polymorphisms and risk of pre-eclampsia in three genetic models. In conclusion, *IL-4* C-590T polymorphism could be used as a predictive risk factor for pre-eclampsia.

Key words: *IL-4* C-590T; *IL-4* C+33T; *IL-4* G-1098T; Polymorphism; Pre-eclampsia

INTRODUCTION

Preeclampsia is a common disease unique to pregnant women, and an estimated about 5% of pregnant women were be affected with pre-eclampsia (Dekker and Sibai, 1998; Sibai et al., 2005; Aggarwal et al., 2010). There have been many studies on the etiology of pre-eclampsia (Lwaleed et al., 2011); however, the pathogenesis of pre-eclampsia remains unclear. Many factors play important roles in the development of pre-eclampsia, such as preexisting hypertension, diabetes, high body mass index and obesity (Das, 2015; Hod et al., 2015; Mol et al., 2016). Pre-eclampsia in 35% of pregnant women is reportedly resulted from genetic factors (Hod et al., 2015). Many genetic factors influence the pathogenesis of pre-eclampsia, such as glutathione S-transferase pi 1, glutathione peroxidase 1, matrix metalloproteinase-9, interleukin-8, tumor necrosis factor-α, interleukin-1-β, insulin receptor genes (Gao et al., 2016; Sun et al., 2016a,b; Tavakkol Afshari et al., 2016; Andraweera et al., 2017).

Interleukin-4 (IL-4), IL-6, and IL-10 produced by T-helper 2 (Th2) cells plays an important role in the inhibition of cellular immunity and induction of placental growth (Gratacs et al., 1998; Yoneyama et al., 2002). IL-4 is an important regulatory factor of the Th2 cellular immune response and has a key role in the promotion of humoral immunity and antagonization of Th1 activity of cytokines (Paul, 2015; Redpath et al., 2015). *IL-4* is located on chromosome q531-33. Three common polymorphisms of *IL-4* were observed, including C-590T, C+33T and G-1098T. Although several recent studies have indicated that many polymorphisms of *IL-1β*, *IL-1RL1*, *IL-8*, *IL-10, IL-12* and *IL-33* are involved in the pathogenesis of pre-eclampsia (Liu et al., 2015; Ren et al., 2016; Sun et al., 2016b; Tavakkol Afshari et al., 2016; Wang et al., 2016). However, only two studies have reported the association of *IL-4* polymorphism with the development of pre-eclampsia so far (Fraser et al., 2008; Kang et al., 2014). To our knowledge, no study has shown on the correlation of *IL-4* C+33T and G-1098T polymorphisms with the development of pre-eclampsia in the Chinese population. In this study, we investigated the relationship between *IL-4* C-590T, C+33T and G-1098T polymorphisms and risk of pre-eclampsia in a population of pregnant women in West China.

MATERIAL AND METHODS

Subjects

A case-control study of 196 pregnant women with pre-eclampsia and 257 controls was conducted between January 2013 and December 2015. The patients were recruited from the Department of Obstetrics and Gynecology of Nanfang Hospital of Southern Medical University. Pre-eclampsia was confirmed in the patients through clinical findings. Diagnosis was according to the elevated blood pressure and presence of proteinuria after 20 weeks of

gestation (ACOG Committee on Practice Bulletins-Obstetrics, 2002). The exclusive criteria for patients included patients with both pre-eclampsia and diabetes, chronic hypertension, multiple pregnancies, or cardiovascular diseases. The mean age of patients was 28.23 ± 4.93 years, and the gestational weeks when enrolled were 27.36 ± 3.93 weeks.

Healthy controls were recruited from pregnant women who came for childbirth and maternal health care in the Nanfang Hospital of Southern Medical University. The inclusive criteria for the control group included women at more than 20 weeks' gestation with a single pregnancy and without pre-eclampsia, diabetes, chronic hypertension, or cardiovascular diseases. The mean age of this group was 27.42 ± 4.22 years, and the gestational weeks when enrolled were 26.80 ± 4.40 weeks.

Details of potential risk factors for pre-eclampsia were collected from medical records, including primiparity, tobacco smoking and alcohol consumption status prior to pregnancy, body mass index, family history of pre-eclampsia, systolic blood pressure (SBP), diastolic blood pressure (DBP), 24-h urinary protein content uric acid, and newborn birth weight. Informed consent was obtained from all investigated pregnant women prior to study enrollment. Approval for the study was received from the ethical committee of Nanfang Hospital, Southern Medical University.

Genotyping of *IL-4* C-590T, C+33T and G-1098T polymorphisms

Peripheral blood sample (5 mL from each study participant) was obtained and saved in ethylenediaminetetraacetic acid-containing tubes. Genomic DNA of samples was extracted using a DNA extraction kit (TaKaRa Bio Inc., Dalian, China) according to the manufacturer's instructions. *IL-4* C-590T, C+33T and G-1098T genotyping was performed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. Primers were supplied by Applied Biosystems (Foster City, CA, USA). The PCR regimen for *IL-4* C-590T was as follows: initial denaturation at 94°C for 5 min; then 38 cycles of 94°C for 30 s, 57°C for 40 s, and 72°C for 60 s; and a final extension at 72°C for 7 min. The PCR regimen for *IL-4* C+33T was as follows: initial denaturation at 94°C for 5 min; then 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 PCR regimen for G-1098T was as follows: initial denaturation at 94°C for 5 min; then 35 cycles of 94°C for 30 s, 60.7°C for 45 s, and 72°C for 45 s; and a final extension of 72°C for 7 min. The amplification reaction was performed in 25-µL PCR reaction mixture, and each 25-µL PCR included 18.3 µL H_2O, 2.5 µL 10X PCR buffer, 2 µL deoxynucleotides, 0.5 µL forward primer, 0.5 µL reverse primer, 0.2 µL r*Taq* DNA polymerase, and DNA template. Successful amplification of samples was verified by electrophoresis on a 2% agarose gel. Finally, the digested product was electrophoresed on a 4% agarose gel. The electropherograms of PCR products of *IL-4* C-590T, C+33T and G-1098T are shown in Figures 1-3.

Statistical analysis

The collected demographic and clinical characteristics are reported as their frequencies and percentages or means ± standard deviation in the patient and control groups. Comparison of these data between groups was conducted using the Chi-square (χ^2) test or student *t*-test. *IL-4* C-590T, C+33T and G-1098T genotype frequency deviations from the Hardy-Weinberg equilibrium were estimated by the Chi-square test. The correlation between of *IL-4* C-590T,

C+33T and G-1098T polymorphisms and the risk of pre-eclampsia was determined by multiple logistic regression analysis; results were expressed in OR and 95%CI. Three genetic models were performed in analysis, namely dominant, co-dominant, and recessive models. IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA) was used for statistical analysis.
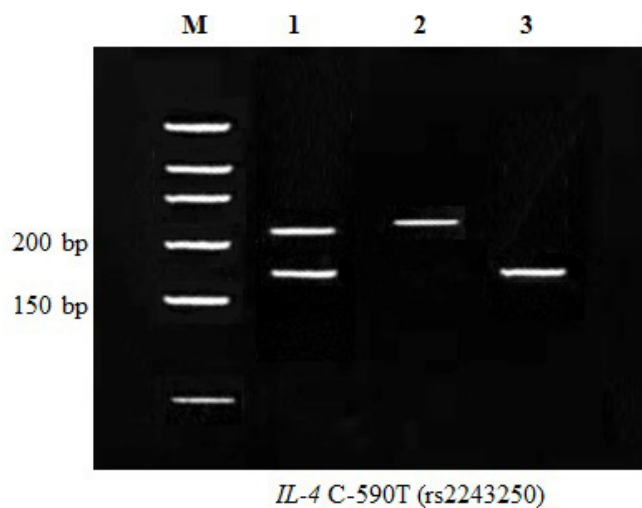
Figure 1. Electropherogram of the PCR products of *IL-4* C-590T. *Lane M* = marker (DNA ladder); *lane 1* = TC genotype; *lane 2* = TT genotype; *lane 3* = CC genotype.
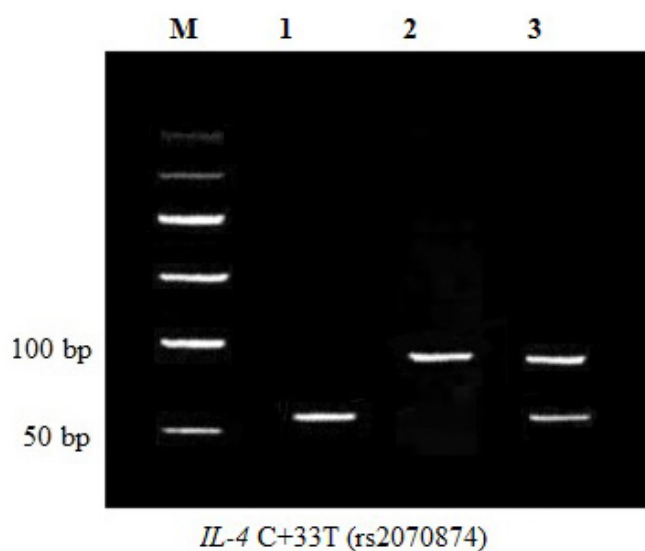
Figure 2. Electropherogram of the PCR products of *IL-4* C+33T. *Lane M* = marker (DNA ladder); *lane 1* = CC genotype; *lanes 2* and *3* = TT genotype; *lane 4* = TC genotype.
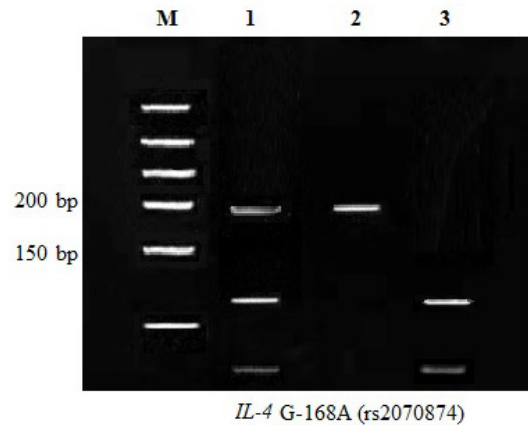
Figure 3. Electropherogram of the PCR products of *IL-4* G-1098T. *Lane M* = markers (DNA ladder); *lane 1* = GT genotype; *lane 2* = TT genotype; *lane 3* = GG genotype.

RESULTS

No significant differences were observed between the pregnant women with pre-eclampsia and controls based on age, gestational weeks, the smoking and drinking status prior to pregnancy, and the family history of pre-eclampsia. However, there were significant differences between patients and controls in terms of body mass index ($\chi^2 = 17.16$, P < 0.001), SBP (t = 53.05, P < 0.001), DBP (t = 5.41, P = 0.02), and serum uric acid (t = 16.65, P < 0.001). The 24-h urinary protein content of pregnant women with pre-eclampsia was 2453.06 ± 487.16 mg (Table 1).

Table 1. Demographic and clinical characteristics of patients with pre-eclampsia and controls.

Variables	Controls (N = 257)	%	Patients (N = 196)	%	χ^2 or *t* value	P value
Age (years)	27.42 ± 4.22		28.23 ± 4.93		-1.89	0.08
Gestational weeks when enrolled	26.80 ± 4.40		27.36 ± 3.93		1.04	0.31
Primiparity						
No	126	49.03	94	47.96		
Yes	131	50.97	102	52.04	0.51	0.82
Tobacco smoking prior to pregnancy						
No	241	93.77	179	91.33		
Yes	16	6.23	17	8.67	0.99	0.32
Alcohol drinking prior to pregnancy						
No	215	83.66	166	84.69		
Yes	42	16.34	30	15.31	0.09	0.77
BMI (kg/m²)						
<24	163	63.42	86	43.88		
≥24	94	36.58	110	56.12	17.16	<0.001
Family history of pre-eclampsia						
No	181	70.43	126	64.29		
Yes	76	29.57	70	35.71	1.92	0.17
SBP (mmHg)	113.89 ± 11.79		141.36 ± 19.93		53.05	<0.001
DBP (mmHg)	76.63 ± 13.16		95.06 ± 15.75		5.41	0.02
24-h urinary protein content (mg)	2453.06 ± 487.16		-			
Serum uric acid (µmol/L)	333.93 ± 43.90		406.74 ± 51.94		8.09	0.005

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure.

The genotype frequencies of *IL-4* C-590T, C+33T and G-1098T are shown in Table 2. We observed that the TT, TC, and CC genotype frequencies of *IL-4* C-590T showed significantly different between patients with pre-eclampsia and controls (χ^2 = 8.04, P = 0.02). The *IL-4* C+33T (χ^2 = 1.64, P = 0.44) and G-1098T (χ^2 = 0.59, P = 0.74) genotype distributions were not statistically significant between the two groups. However, the *IL-4* C-590T genotype frequencies in the controls were not in line with the HWE (χ^2 = 12.39, P = 0.004).

Table 2. Genotype distributions of *IL-4* C-590T, C+33T and G-1098T in patients with pre-eclampsia and controls.

IL-4		Controls (N = 257)	%	Patients (N = 196)	%	χ^2 value	P	χ^2 for HWE in controls	P
C-590T	TT	199	77.43	169	86.22				
	TC	37	14.40	22	11.22				
	CC	21	8.17	5	2.55	8.04	0.02	12.39	0.004
C+33T	TT	180	70.04	142	72.45				
	TC	61	23.74	47	23.98				
	CC	16	6.23	7	3.57	1.64	0.44	1.50	0.22
G-1098T	GG	109	42.41	78	39.80				
	GT	110	42.80	91	46.43				
	TT	38	14.79	27	13.78	0.59	0.74	0.003	0.96

By binary logistic regression models, we observed that the TT genotype of *IL-4* C-590T, compared to the CC genotype, was significantly related to a reduced risk of pre-eclampsia; crude and adjusted ORs (95%CI) were 0.28 (0.10-0.75) and 0.29 (0.11-0.81) (Table 3). In

Table 3. Association of *IL-4* C-590T, C+33T and G-1098T polymorphisms with the risk of pre-eclampsia.

IL-4	Controls (N = 257)	%	Patients (N = 196)	%	Crude OR	P	OR (95%CI)[1]	P
C-590T								
Co-dominant								
CC	199	77.43	169	86.22	1.0 (Ref.)	-	1.0 (Ref.)	-
CT	37	14.40	22	11.22	0.69 (0.39-1.22)	0.20	0.61 (0.34-1.10)	0.10
TT	21	8.17	5	2.55	0.28 (0.10-0.75)	0.01	0.29 (0.11-0.81)	0.02
Dominant								
CC	199	77.43	169	86.22	1.0 (Ref.)	-	1.0 (Ref.)	-
CT+TT	58	22.57	27	13.78	0.54 (0.33-0.89)	0.01	0.50 (0.30-0.84)	0.01
Recessive								
CC+CT	236	91.83	191	97.45	1.0 (Ref.)	-	1.0 (Ref.)	-
TT	21	8.17	5	2.55	0.29 (0.11-0.79)	0.02	0.31 (0.11-0.86)	0.03
C+33T								
Co-dominant								
CC	180	70.04	142	72.45	1.0 (Ref.)	-	1.0 (Ref.)	-
CT	61	23.74	47	23.98	0.90 (0.58-1.41)	0.65	0.89 (0.56-1.40)	0.60
TT	16	6.23	7	3.57	0.54 (0.21-1.36)	0.19	0.57 (0.22-1.48)	0.25
Dominant								
CC	180	70.04	142	72.45	1.0 (Ref.)	-	1.0 (Ref.)	-
CT+TT	77	29.96	54	27.55	0.86 (0.56-1.30)	0.46	0.84 (0.55-1.28)	0.42
Recessive								
CC+CT	241	93.77	189	96.43	1.0 (Ref.)	-	1.0 (Ref.)	-
TT	16	6.23	7	3.57	0.54 (0.22-1.36)	0.19	0.59 (0.23-1.51)	0.27
G-1098T								
Co-dominant								
GG	109	42.41	78	39.80	1.0 (Ref.)	-	1.0 (Ref.)	-
GT	110	42.80	91	46.43	1.12 (0.74-1.68)	0.59	1.14 (0.75-1.73)	0.55
TT	38	14.79	27	13.78	1.03 (0.57-1.85)	0.92	1.12 (0.61-2.05)	0.71
Dominant								
GG	109	42.41	78	39.80	1.0 (Ref.)	-	1.0 (Ref.)	-
GT+TT	148	57.59	118	60.20	1.13 (0.77-1.66)	0.52	1.16 (0.78-1.72)	0.56
Recessive								
GG+GT	219	85.21	169	86.22	1.0 (Ref.)	-	1.0 (Ref.)	-
TT	38	14.79	27	13.78	0.93 (0.54-1.59)	0.79	0.98 (0.57-1.70)	0.95

[1]Adjusted for age, BMI, SBP, DBP and uric acid.

the dominant model, we observed that the CT+TT genotype, compared to the CC genotype, showed a lower risk of pre-eclampsia (crude OR = 0.54, 95%CI = 0.33-0.89; adjusted OR = 0.50, 95%CI = 0.30-0.84). In the recessive model, we found that the TT genotype harbored a 0.31-fold risk of pre-eclampsia than that by the CC+CT genotype (adjusted OR = 0.31, 95%CI = 0.11-0.86). However, no significant correlation was observed between the *IL-4* C+33T and G-1098T polymorphisms and risk of pre-eclampsia in the co-dominant, dominant and recessive genetic models.

DISCUSSION

IL-4 C-590T, C+33T and G-1098T polymorphisms, encoding inflammatory factors, may affect an individual's levels of cytokine produced and reaction intensities and are, therefore, correlated with the development of many types of diseases. In the current study, we found that the *IL-4* C-590T polymorphism harbored a decreased risk of pre-eclampsia in the Chinese population.

Th2 cells produce many types of cytokines, such as IL-4, that are capable of inhibiting cellular immunity and promoting placental growth (Gratacs et al., 1998; Yoneyama et al., 2002). A previous study conducted a study in Iranian patients with pre-eclampsia, and reported that dysregulation of cytokine expression occurred in preeclampsia with a raised levels of IL-4 and other cytokines (Mansouri et al., 2007). Another study observed that the cytokine balance in the Th1/Th2 was broken in pregnant women with pre-eclampsia, encouraging the development of an excessive inflammatory reaction (Vargas-Rojas et al., 2016). Gharesi-Fard et al. (2016) reported that the Th1/Th2/Th17/Treg balance within placenta played an important role in the fate of a normal pregnancy. Therefore, the altered expression of IL-4 is involved in risk of pre-eclampsia.

Two studies have reported the relationship between *IL-4* polymorphism and the pathogenesis of pre-eclampsia (Fraser et al., 2008; Kang et al., 2014). Kang et al. (2014) carried out a study on 78 pregnant women with pre-eclampsia and 125 healthy controls in Taiwan and reported the *IL-4* C-590T polymorphism did not affect the development of pre-eclampsia. Fraser et al. (2008) have conducted a study on 117 women with pre-eclampsia and 146 normal control subjects in English, which indicated that pregnant women harboring the TT genotype of *IL-4* C-590T was associated with an increased risk of developing pre-eclampsia compared to those carrying the CC genotype. However, to our knowledge, we firstly reported the association between *IL-4* C+33T and G-1098Tpolymorphisms and risk of pre-eclampsia. The contribution of *IL-4* C+33T and G-1098T polymorphisms to the pathogenesis of many types of inflammation-related diseases, such as asthma, cerebral palsy, wheezing, allergic rhinitis, and chronic hepatitis C diseases, has been well established by previous studies (Smith et al., 2008; Djukic et al., 2009; Yang, 2013; Zhenzhen et al., 2013). The present study is the first to consider the role of the *IL-4* C+33T and G-1098T polymorphisms in the development of pre-eclampsia and report no significant relationship between the polymorphism and risk of pre-eclampsia.

The present study has two important limitations. First, since all the study participants were recruited from only one hospital, a sample selection bias exists in this study. Second, only 196 pregnant women with pre-eclampsia and 257 controls were recruited in this study, and the results could be undervalued due to the limitation of sample size. Therefore, further large sample size studies should be taken into consideration in future analyses.

In conclusion, our study suggests that the *IL-4* C-590T polymorphism is independently associated with the risk of pre-eclampsia in all genetic models and could be considered as a risk factor for the disease. Further investigation of larger samples sizes and other ethnicities are needed to validate our findings.

Conflicts of interest

The authors declare no conflict of interest.

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