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Polymorphisms in interleukin-6 and interleukin-10 may be associated with risk of preeclampsia

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ABSTRACT. Preeclampsia is a common condition unique to pregnant women. Previous studies have suggested that several cytokines may contribute to defective placental invasion and endothelial damage in this condition. We investigated the influence of four single nucleotide polymorphisms (SNPs) in the promoters of *IL-6* (-572G/C, -597G/A, and -174G/C) and *IL-10* (-592A/C) on susceptibility to preeclampsia in a Chinese population. This study included 142 newly diagnosed preeclampsia patients and 260 controls recruited from Qingdao Women and Children's Hospital between January 2013 and May 2015. Genotyping of *IL-6* and *IL-10* SNPs was performed using the polymerase chain reaction-restriction fragment length polymorphism method. Logistic regression analysis was then performed to determine the association between these variants and preeclampsia risk. Our findings indicated that compared to the AA genotype, the CC and AC+CC genotypes of *IL-10* -592A/C correlate with elevated risk of developing preeclampsia, with adjusted odds ratios (and 95% confidence intervals) of 2.45 (1.26-4.72) and 1.71 (1.09-2.68), respectively. However, the *IL-6* -572G/C, -597G/A, and -174G/C polymorphisms were not found

to play a critical role in susceptibility to this disorder. In conclusion, the *IL-10* -592A/C genetic variant was observed to be associated with preeclampsia risk in pregnant women.

Key words: Interleukin-6; Interleukin-10; Polymorphism; Preeclampsia

INTRODUCTION

Preeclampsia is a common condition unique to pregnant women, and is estimated to occur in approximately 3 to 5% of all pregnancies (Dekker and Sibai, 1998; Dekker et al., 2011; Sibai, 2012). The clinical manifestations of preeclampsia are high blood pressure and proteinuria after 20 weeks of gestation. This condition is associated with serious complications for both the mother and fetus, including acute renal failure, liver injury, intracranial hemorrhage, pulmonary edema, death, preterm birth, or fetal intrauterine growth restriction (Cnattingius et al., 2004; Saftlas et al., 2005; Stella and Sibai, 2006). The etiology of preeclampsia has been widely studied, but the actual mechanisms responsible are not clear. Many previous investigations have shown that several environmental factors play a key role in the pathogenesis of preeclampsia, such as preexisting hypertension, family history of the disease, lack of vitamin C, and vascular endothelial injury (Dekker et al., 2011). Previous studies have shown that polymorphisms in many genes are associated with preeclampsia risk, notably in those encoding nitric oxide synthase 3, dopamine receptor D1, DRD4, lymphotoxin-α, matrix metalloprotease, transforming growth factor-β1 (TGF-β1), cyclooxygenase 2, and platelet-activating factor acetylhydrolase (Deepthi et al., 2015; Leonardo et al., 2015; Pissetti et al., 2015; Ren et al., 2015; Su et al., 2015; Wolski et al., 2015; Zeng et al., 2016).

Th2/Th1 cytokine balance correlates with pregnancy outcome. Production of Th1 cytokines is inhibited during normal pregnancy, and their excessive expression is likely to induce preeclampsia (Vargas-Rojas et al., 2016). Th1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α, whereas Th2 cells produce IL-6, IL-10, IL-4, and TGF-β (Kiyomi et al., 2015; Talaat et al., 2015; Yuan et al., 2015). Altered plasma concentrations of many cytokines, including TNF-α, IL-1β, IL-6, IL-10, and IL-18, have been implicated in defective placental invasion and endothelial damage in preeclampsia (Roland et al., 2010; Sarojini et al., 2013; Wang et al., 2014).

IL-6 is an important mediator of the acute phase response, and trophoblastic proliferation, invasion, and differentiation involve modification of IL-6 levels (Santhanam et al., 1991; Erzen et al., 2007). IL-10 is thought to promote the resistance of trophoblastic cells to Fas-mediated apoptosis (Aschkenazi et al., 2002). A deficiency in placental IL-10 has been reported in pregnant women with preeclampsia in comparison to control subjects, and serum IL-10 level is associated with genetic variations in the *IL-10* promoter (Makris et al., 2006). Several previous studies have analyzed the association between *IL-10* gene polymorphisms and development of preeclampsia, but their results conflict (Vural et al., 2010; Xie et al., 2011; Valencia Villalvazo et al., 2012; Liu et al., 2015). Moreover, none has investigated the relationship between *IL-6* genetic variation and risk of this developing this condition in pregnant Chinese women. Therefore, we carried out a study to assess the effect of four promoter single nucleotide polymorphisms (SNPs) in *IL-6* (-572G/C, -597G/A, and -174G/C) and *IL-10* (-592A/C) on preeclampsia susceptibility among expectant Chinese women.

MATERIAL AND METHODS

Patients

One hundred and forty-two preeclampsia patients were enrolled in our study between January 2013 and May 2015. All patients were recruited from the Department of Obstetrics at Qingdao Women and Children's Hospital, and had their diagnoses newly confirmed within 1 week.

Preeclampsia was diagnosed according to the following criteria: blood pressure ≥140/90 mmHg and 24-h urinary protein ≥300 mg after 20 weeks of gestation, in addition to complications comprising at least one of the following: blood pressure ≥160/110 mmHg, 24-h urinary protein ≥2000 mg, serum transaminase ≥two times the normal level, thrombocyte count <100 x 10^9/L, elevated serum lactate dehydrogenase, serum creatinine >106 μM, fetal growth restriction, oligohydramnios, persistent headache, or vision disorder.

Between January 2013 and May 2015, 260 women in at least their 20th week of pregnancy were enrolled in our study as control subjects. These women were recruited during regular prenatal examination at the Department of Obstetrics at Qingdao Women and Children's Hospital. Individuals with a history of chronic hypertension, cardiovascular disease, end-stage liver or renal diseases, or diabetes were excluded from this study.

Baseline demographic and clinical measurements, including age, weeks of gestation when enrollment, body mass index, blood pressure, delivery week, 24-h urinary protein level, and birth weight, were collected from medical records of all participants. All patients and control subjects gave their signed informed consent before enrollment, and the performance of this study was approved by the Ethics Committee of the Qingdao Women and Children's Hospital.

Genotyping methods

A venous blood sample (5 mL) was obtained from each subject after enrollment, from which DNA was extracted using a TIANGEN Blood DNA Kit (TIANGEN, Beijing, China). A polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was carried out to genotype the *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C SNPs. Details of the corresponding primers and PCR products are shown in Table 1. PCRs were conducted on a thermocycler (PerkinElmer, Inc., Waltham, MA, USA), as follows: initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s; then a final extension at 72°C for 5 min. The *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C amplification products were digested by *Bsr*BI, *Fok*I, *Nla*III, and *Rsa*I, respectively. The reproducibility of the results was verified by repeating the genotyping process for 5% of the samples in a blind manner.

Table 1. Primers and polymerase chain reaction (PCR) products used to genotype *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C polymorphisms.

Polymorphism	Primers (5'-3')	PCR product (bp)
-572G/C	GAGACGCCTTGAAGTAACTG (forward) AACCAAAGATGTTCTGAACTGA (reverse)	182
-597G/A	GAGACGCCTTGAAGTAACTG (forward) AACCAAAGATGTTCTGAACTGA (reverse)	182
-174G/C	AGCCTCAATGACGACCTA (forward) GAGCCTCAGACATCTCCAGT (reverse)	223
-592A/C	GGTGAGCACTACCTGACTAGC (forward) CCTAGGTCACAGTGACGTGG (reverse)	412

Following amplification, 2% agarose gel electrophoresis was used to detect PCR products, which were observed under ultraviolet light using a Syngene Gel Imaging System (Syngene, Cambridge, UK).

Statistical analysis

Comparisons of categorical and continuous variables were carried out using the chi-square test and the Student *t*-test. Conformity of genotype frequencies to Hardy-Weinberg equilibrium was assessed by the chi-square test. Associations between the polymorphisms of interest and risk of preeclampsia were determined by unconditional logistic regression analysis, and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated regarding these relationships. These calculations were performed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA), and P values < 0.05 were considered to indicate statistical significance.

RESULTS

The mean ages of patients and controls were 28.12 ± 5.86 and 27.85 ± 5.56 years, respectively. In comparison to healthy controls, preeclampsia patients showed higher body mass index values (28.76 ± 4.53 *vs* 27.25 ± 3.75 kg/m^2, t = 3.58, P < 0.001) and systolic (153.60 ± 12.52 *vs* 110.58 ± 10.54 mmHg, t = 36.55, P < 0.001) and diastolic blood pressure (100.65 ± 17.56 *vs* 76.75 ± 14.60 mmHg, t = 14.58, P < 0.001), a lower number of weeks at delivery (33.57 ± 4.10 *vs* 39.30 ± 2.55 weeks, t = 17.25, P < 0.001), and reduced birth weights (2564.60 ± 674.40 *vs* 2853.50 ± 637.55 g, t = 4.25, P < 0.00; Table 2). No significant difference was determined between patients and controls with respect to age (t = 0.46, P = 0.32) or number of gestation weeks (t = 0.96, P = 0.17).

Table 2. Baseline characteristics of preeclampsia patients and control subjects.

Variable	Patients (N = 142)	Controls (N = 260)	*t*-test	P value
Age, years	28.12 ± 5.86	27.85 ± 5.56	0.46	0.32
Weeks of gestation	25.64 ± 4.52	26.10 ± 4.63	0.96	0.17
Body mass index, kg/m^2	28.76 ± 4.53	27.25 ± 3.75	3.58	<0.001
Systolic blood pressure, mmHg	153.60 ± 12.52	110.58 ± 10.54	36.55	<0.001
Diastolic blood pressure, mmHg	100.65 ± 17.56	76.75 ± 14.60	14.58	<0.001
Weeks at delivery	33.57 ± 4.10	39.30 ± 2.55	17.25	<0.001
24-h urinary protein	2426.54 ± 512.44	-		
Newborn birth weight, g	2564.60 ± 674.40	2853.50 ± 637.55	4.25	<0.001

We then analyzed the distributions of *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C genotypes within the two study groups (Table 3). A chi-square test indicated a significant difference in *IL-10* -592 AA, AC, and CC genotype frequencies between the preeclampsia patients and controls (chi-square = 8.81, P = 0.01). However, no significant variation in the frequencies of *IL-6* -572G/C, -597G/A, and -174G/C genotypes was observed between the two investigated groups. Moreover, genotype distributions of the *IL-6* -572G/C (chi-square = 0.80, P = 0.37), -597G/A (chi-square = 0.51, P = 0.48), and -174G/C (chi-square = 0.17, P = 0.68) and *IL-10* -592A/C (chi-square = 0.28, P = 0.60) SNPs in the control group were in agreement with Hardy-Weinberg equilibrium.

Table 3. *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C genotype distributions.

SNP	Patients	%	Controls	%	Chi-square	P value	Chi-square (HWE)	P value (HWE)
-572G/C								
GG	66	46.48	136	52.31				
GC	58	40.84	100	38.46				
CC	18	12.68	24	9.23	1.65	0.44	0.80	0.37
-597G/A								
GG	136	95.77	238	91.54				
GA	6	4.23	22	8.46				
AA	0	0.00	0	0.00	2.54	0.111	0.51	0.48
-174G/C								
GG	137	96.48	247	95.00				
GC	5	3.52	13	5.00				
CC	0	0.00	0	0.00	0.47	0.49	0.17	0.68
-592A/C								
AA	47	33.10	119	45.77				
AC	66	46.48	111	42.69				
CC	29	20.42	30	11.54	8.81	0.01	0.28	0.60

SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium.

We subsequently examined the relationship between the polymorphisms under investigation and preeclampsia risk (Table 4). Unconditional logistic regression analysis revealed that *IL-10* -592 CC and AC+CC genotype carriers exhibited a higher susceptibility to preeclampsia than individuals with the wild-type genotype did, with ORs (and 95%CIs) of 2.45 (1.26-4.72) and 1.71 (1.09-2.68), respectively. However, we did not establish significant associations between preeclampsia and the *IL-6* -572G/C, -597G/A, and -174G/C polymorphisms.

Table 4. Relationship between preeclampsia risk and *IL-6* -572G/C, -597G/A, and -174G/C and *IL-10* -592A/C polymorphisms.

SNP	Patients	%	Controls	%	OR (95%CI)[1]	P value
-572G/C						
GG	66	46.48	136	52.31	1.0 (Ref.)	
GC	58	40.84	100	38.46	1.18 (0.74-1.86)	0.46
CC	18	12.68	24	9.23	1.52 (0.72-3.15)	0.22
GC+CC	76	53.52	124	47.69	1.24 (0.81-1.91)	0.29
-597G/A						
GG	136	95.77	238	91.54	1.0 (Ref.)	
GA	6	4.23	22	8.46	0.47 (0.15-1.25)	0.11
AA	0	0	0	0	-	-
GA+AA	6	4.29	22	8.46	0.47 (0.15-1.25)	0.11
-174G/C						
GG	137	96.48	247	95	1.0 (Ref.)	
GC	5	3.52	13	5	0.69 (0.19-2.13)	0.49
CC	0	0	0	0	-	-
GC+CC	5	3.52	13	5	0.69 (0.19-2.13)	0.49
-592A/C						
AA	47	33.10	119	45.77	1.0 (Ref.)	
AC	66	46.48	111	42.69	1.51 (0.93-2.44)	0.08
CC	29	20.42	30	11.54	2.45 (1.26-4.72)	0.004
AC+CC	95	66.90	141	54.23	1.71 (1.09-2.68)	0.01

[1]Adjusted for body mass index, systolic and diastolic blood pressure, weeks at delivery, and newborn birth weight.
SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval; Ref. = reference.

DISCUSSION

Decidual cells producing Th1 and Th2 cytokines maintain a specific immune microenvironment equilibrium during normal pregnancy. If this balance is compromised,

habitual abortion or complications of pregnancy may result (Kamali-Sarvestani et al., 2005; Choi and Kwak-Kim, 2008). IL-10 is a Th2 cytokine able to promote placental cell growth and differentiation, inhibit lymphocyte proliferation and IFN-γ production, and prevent the detrimental effects of killer lymphocytes on trophoblastic cells (Fan et al., 2006). IL-6 is also a Th2 cytokine, and similarly promotes immune tolerance. It can regulate the production of human chorionic gonadotropin from the syncytiotrophoblast, and exert an immunosuppressive effect to maintain pregnancy, although IL-6 expression is elevated for delivery (Aris et al., 2008). In the present study, we observed that the *IL-10* -592 CC and AC+CC genotypes contribute to the development of preeclampsia in the Chinese population.

IL-10 can inhibit production of Th1 cytokines, balance the Th1/Th2 cytokine ratio, upregulate members of the human leukocyte antigen system, promote placental hormone secretion, and maintain pregnancy together with estradiol and progesterone (Wang et al., 2015). Previous *in vitro* and *in vivo* experimental studies have indicated that IL-10 expression may influence preeclampsia pathogenesis (Medeiros et al., 2014; Cornelius et al., 2015; Wang et al., 2015).

Previous investigations have tested the association between *IL-10* gene polymorphisms and preeclampsia development, but with conflicting results (Stonek et al., 2008; Vural et al., 2010; Valencia Villalvazo et al., 2012; Pissetti et al., 2014; Sowmya et al., 2014; Song and Zhong, 2015; Liu et al., 2015). Liu et al. (2015) and Sowmya et al. (2014) reported the *IL-10* -819T/C genetic variant to be associated with increased preeclampsia risk in pregnant women in China and India. Similarly, in a study of 177 preeclamptic pregnant women and 182 controls, Song and Zhong (2015) found that the *IL-10* -592A/C SNP elevated risk of early-onset preeclampsia in a Chinese population. In addition, Pissetti et al. (2014) and Vural et al. (2010) carried out an investigation involving expectant women in Turkey and Brazil, revealing that the *IL-10* -1082A/G sequence variant is associated with preeclampsia susceptibility. However, Valencia Villalvazo et al. (2012) and Stonek et al. (2008) failed to detect a correlation between this condition and polymorphisms in *IL-6* and *IL-10*. In the present study, we identified a significant relationship between the *IL-10* -592A/C variant and risk of developing preeclampsia among pregnant women in China. Our findings are thus consistent with those of Song and Zhong (2015). The discrepancies between the abovementioned studies may be attributed to differences in populations, selection of patients and controls, and sample size.

Two limitations to our study should be considered. First, the included patients and controls were recruited from only one hospital in a single Chinese city, and therefore may not be adequately representative of pregnant women in other regions and countries. Second, the sample size in this analysis was not large, potentially restricting its statistical power to distinguish differences between groups. Therefore, further studies incorporating bigger samples of the population are greatly needed to confirm our findings.

In conclusion, we observed that the *IL-10* -592A/C polymorphism was associated with the development of preeclampsia. Further large-scale studies should be conducted to gain better insight into the impact of *IL-6* and *IL-10* sequence variations on preeclampsia risk.

Conflicts of interest

The authors declare no conflict of interest.

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