Int J Clin Exp Pathol 2015;8(9):11659-11664
www.ijcep.com /ISSN:1936-2625/IJCEP0012041

Original Article

Association between Interleukin-10 gene polymorphisms and risk of early-onset preeclampsia

Limeng Song[1,2], Mei Zhong[1]

[1]*Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China;* [2]*Department of Obstetrics and Gynecology, Inner Mongolia Maternal and Child Health Hospital, Hohhot 010010, China*

Received June 28, 2015; Accepted July 29, 2015; Epub September 1, 2015; Published September 15, 2015

Abstract: We conducted a case-control study to investigate the role of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms in the development of early-onset preeclampsia. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the polymorphisms of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872). The genotype distributions of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) confirmed with HWE in the controls, and the *P* value for HWE was 0.41, 0.38 and 0.26, respectively. The results of the multivariate logistic regression analysis revealed that the association of individuals expressing the CC genotype and AC+CC of *IL-10* -592A/C (rs1800872) with a significantly increased risk of early-onset preeclampsia in co-dominant and dominant models, compared to the AA genotype; the OR (95% CI) for these individuals was determined to be 2.09 (1.12-3.90) and 1.66 (1.03-2.71), respectively. In the recessive model, we found that CC genotype of *IL-10* -592A/C (rs1800872) was associated with the increased risk of early-onset preeclampsia when compared with AA+AC genotype (OR = 1.67; 95% CI = 1.01-2.92). In conclusion, our study has indicated that *IL-10* -592A/C (rs1800872) polymorphism was associated with an increased risk of early-onset preeclampsia in a Chinese population.

Keywords: Interleukin-10, polymorphism, early-onset preeclampsia

Introduction

Preeclampsia is a multi system disorder unique to pregnancy, which occurs in 3% to 5% of all pregnancies. Preeclampsia is the main cause of preterm birth, intrauterine growth restriction, and could increase the risk of eclampsia and cause renal failure, pulmonary edema, stroke and death. The etiology of preeclampsia is not well understood, and the process of preeclampsia is caused by many environmental factors, such as preexisting hypertension, diabetes, obesity or smoking [1, 2]. However, individuals would not development preeclampsia even though they expose to same environmental risk factors. Therefore, genetic factors may play an important role in the development of preeclampsia.

Previous studies reported that changed concentrations of many cytokines may play an important role in defective placental invasion and endothelial damage in preeclampsia [3]. It is reported that T helper type 2 (Th2) are responsible for successful pregnancy, but the Th1 responses could cause the death of the fetus. Therefore, a balance for Th1 and Th2 is a requirement for placentation. Th1 cells produce proinflammatory cytokines such as interleukin (IL)-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α and are play a key role in cell-mediated responses and delayed type hypersensitivity reactions [4]. It is well known that Th2 cells cause anti-inflammatory cytokines such as *IL-4*, *IL-5*, *IL-10* and *IL-13* and evoke humoral immunity [4]. IL-10 is involved in Th2 immunity, and the several single nucleotide polymorphisms (SNPs) of IL-10 gene locate regions of the promoter region and regulate the levels of circulating *IL-10*, including IL-10 -1082A/G, -819T/C, and -592A/C [5-7]. Only several previous studies reported the association between IL-10 gene polymorphisms and risk of early-onset preeclampsia, but the results

are inconsistent [8-11]. Therefore, we conducted a case-control study to investigate the role of IL-10 -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms in the development of early-onset preeclampsia.

Patients and methods

Patients

A case-control study was taken in our study. A total of 177 women who suffered from early-onset preeclampsia after 20 weeks of gestation were included from Inner Mongolia Maternal and Child Health Hospital between January 2013 and December 2014. The criteria of early-onset preeclampsia were presence of blood pressure values ≥ 140/90 mmHg, and proteinuria (24-hour urinary protein ≥ 300 mg or urine dipstick protein ≥ ++) after 20 weeks of gestation. Patients who had a previous history of intrauterine fetal deaths were excluded from our study. 182 controls with more than 20 weeks of gestation were randomly collected from the individuals who went to accept prenatal in our hospital during the same period. Controls that had chronic hypertension, a history of renal, autoimmune, metabolic or cardiovascular disease were excluded from our study.

The demographic and clinical data of patients with early-onset preeclampsia and controls were collected from a self-designed questionnaire, including age, gender, body mass index, and systolic blood pressure, diastolic blood pressure, smoking status, gestational age, intrauterine growth restriction and intrauterine death. A written informed consent was gained from each included subject before entering the study group. The study was previously approved by Institute Research Ethics Committee of the Inner Mongolia Maternal and Child Health Hospital.

DNA extraction and genotyping

Before the patients began their treatment, 2 ml peripheral blood was collected with EDTA-anticoagulant tubes. Genomic DNA was extracted from the blood, using the QIAamp DNA MAX Kit (Qiagen, Hilden, Germany). Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the polymorphisms of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872). The forward and reverse primers for *IL-10* -1082A/G (rs1800896) were 5'-TCATTCTATGTGCTGGAGATGG-3' and 5'-TGGGGGAAGTGGGTAAGAGT-3', respectively. The forward and reverse primers for IL-10 819T/C (rs1800871) were 5'-GGTGAGCACTACCTGACTAGC-3' and 5'-CCTAGGTCACAGTGACGTGG-3', respectively. The forward and reverse primers for IL-10 -592A/C (rs1800872) were 5'-CCTGAGCACTAGGTGACTAGC-3' and 5'-GGTACCTCACAGTGACGTCC-3', respectively. The restriction enzyme for PCR products were *Bse*RI, *Ms*II and *Rsa*I for *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872), respectively. PCR reactions were carried out with an initial denaturation step of 8 minutes at 94°C, followed by 30 cycles at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. The DNA fragments were confirmed through electrophoresing on 3.5% agarose gel and visualizing under UV light after ethidum staining.

Statistical analysis

Continuous variables were expressed by mean ± standard deviation, and categorical variables were expressed as number (N) and percentage (%). The distributions of continuous and categorical variables between patients with early-onset preeclampsia and controls were compared by Pearson χ^2 test or student t test. Concordance with Hardy-Weinberg equilibrium (HWE) was tested using standard χ^2 test or Fisher's exact test. Unconditional logistic regression analysis was used to evaluate the association between *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms and risk of early-onset preeclampsia after adjustment for confounding factors, and the results were expressed as odds ratio (ORs) and corresponding 95% confidence interval (95% CI). A two-tailed *P* value of < 0.05 was considered to be statistically significant. All of these statistical tests were done using Stata (version 10.0; StataCorp, College Station, TX) software programs.

Results

The demographic and clinical characteristics of patients with early-onset preeclampsia and controls are summarized in **Table 1**. The mean

Table 1. Baseline characteristics of patients with early-onset preeclampsia and controls

Variables	Patients N = 155	%	Controls N = 201	%	*t* or χ^2 test	*P* value
Age, years	27.54 ± 4.65		26.15 ± 4.70		0.78	0.22
Gestational age, weeks	26.70 ± 4.20		26.65 ± 4.31		0.11	0.46
BMI, kg/m^2	30.16 ± 4.12		28.45 ± 3.74		4.09	< 0.001
Systolic blood pressure, mmHg	151.45 ± 10.50		110.62 ± 12.56		32.62	< 0.001
Diastolic blood pressure, mmHg	102.15 ± 21.52		73.25 ± 12.52		15.88	< 0.001
Delivery week, weeks	33.61 ± 3.75		39.25 ± 1.26		19.92	< 0.001
Infant birthweight, g	2306.65 ± 653.60		3410.50 ± 317.40		20.96	< 0.001
Placenta weight, g	363.65 ± 126.40		607.35 ± 119.53		18.6	< 0.001
Mode of delivery						
Normal	64	41.29	126	62.69		
Caesarean	91	58.71	75	37.31	16.1	< 0.001

Table 2. Genotype frequencies of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms in patients with early-onset preeclampsia and controls

IL-10 gene	Patients	%	Controls	%	χ^2 test	*P value*	*P value for HWE*	Minor allele frequency (MAF)	
								In controls	In database
-1082A/G rs1800896									
AA	72	46.45	103	51.24					
AG	69	44.52	85	42.29					
GG	14	9.03	13	6.47	1.27	0.53	0.41	0.2761	0.2722
-819T/C rs1800871									
TT	52	33.55	77	38.31					
TC	72	46.45	90	44.78					
CC	31	20.00	34	16.92	1.06	0.59	0.38	0.3930	0.4347
-592A/C rs1800872									
AA	40	25.81	74	36.82					
AC	72	46.45	90	44.78					
CC	43	27.74	38	18.91	6.37	0.04	0.26	0.4129	0.4349

age of patients with early-onset preeclampsia and controls were 27.54 ± 4.65 and 26.15 ± 4.70 years, respectively. No significant difference was observed in age and gestational age between patients with early-onset preeclampsia and controls ($P > 0.05$). By *t* or χ^2 test, patients with early-onset preeclampsia were more likely to have higher BMI, systolic blood pressure and diastolic blood pressure, and have lower delivery week, infant birth weight and placenta weight, and receive more caesarean delivery, compared to the control subjects ($P < 0.05$) .

The genotype frequencies of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms are summarized in **Table 2**. We found that the observed genotype frequencies of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) in the control samples agreed with the Hardy-Weinberg equilibrium, and the *P*-values were 0.41, 0.38 and 0.26, respectively. The genotype distributions of *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) confirmed with HWE in the controls, and the *P* value for HWE was 0.41, 0.38 and 0.26, respectively (**Table 2**). By χ^2-test, there was significant difference in the observed genotype frequencies of *IL-10* -592A/C (rs1800872) between patients with early-onset preeclampsia and controls ($\chi^2 = 6.37$, $P = 0.04$). Moreover, we found that Minor allele frequencies (MAF) of *IL-10* -1082A/G (rs1800-

Table 3. Association between *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms and risk of early-onset preeclampsia

Models	*IL-10* gene	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
-1082A/G rs1800896							
Co-dominant	AA	72	46.45	103	51.24	Ref.	-
	AG	69	44.52	85	42.29	1.26 (0.82-1.89)	0.56
	GG	14	9.03	13	6.47	1.66 (0.72-4.15)	0.21
Dominant	AA	72	46.45	103	51.24	Ref.	-
	AG+GG	83	53.55	98	48.76	1.21 (0.78-1.88)	0.37
Recessive	AA+AG	141	90.97	188	93.53	Ref.	-
	GG	14	9.03	13	6.47	1.44 (0.60-3.43)	0.36
-819T/C rs1800871							
Co-dominant	TT	52	33.55	77	38.31	Ref.	-
	TC	72	46.45	90	44.78	1.18 (0.72-1.95)	0.48
	CC	31	20.00	34	16.92	1.35 (0.71-2.57)	0.33
Dominant	TT	52	33.55	77	38.31	Ref.	-
	TC+CC	103	66.45	124	61.69	1.23 (0.78-1.96)	0.35
Recessive	TT+TC	124	80.00	167	83.08	Ref.	-
	CC	31	20.00	34	16.92	1.23 (0.68-2.18)	0.46
-592A/C rs1800872							
Co-dominant	AA	40	25.81	74	36.82	Ref.	-
	AC	72	46.45	90	44.78	1.48 (0.88-2.50)	0.12
	CC	43	27.74	38	18.91	2.09 (1.12-3.90)	0.01
Dominant	AA	40	25.81	74	36.82	Ref.	-
	AC+CC	115	74.19	128	63.68	1.66 (1.03-2.71)	0.03
Recessive	AA+AC	112	72.26	164	81.59	Ref.	-
	CC	43	27.74	38	18.91	1.67 (1.01-2.92)	0.04

[1]Adjusted for age, BMI, systolic blood pressure, diastolic blood pressure, delivery weeks, infant birthweight, placenta weight and mode of delivery.

896), -819T/C (rs1800871), and -592A/C (rs1800872) were similar to MAF in dbSNP (http://www.ncbi.nlm.nih.gov/snp/).

The results of the multivariate logistic regression analysis revealed that the association of individuals expressing the CC genotype and AC+CC of *IL-10* -592A/C (rs1800872) with a significantly increased risk of early-onset preeclampsia in co-dominant and dominant models, compared to the AA genotype; the OR (95% CI) for these individuals was determined to be 2.09 (1.12-3.90) and 1.66 (1.03-2.71), respectively. In the recessive model, we found that CC genotype of *IL-10* -592A/C (rs1800872) was associated with the increased risk of early-onset preeclampsia when compared with AA+AC genotype (OR = 1.67; 95% CI = 1.01-2.92) (**Table 3**). However, we indicated that *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871) have no significant association with the risk of early-onset preeclampsia.

We conducted the stratified analysis between the *IL-10* -592A/C rs1800872 polymorphism and risk of early-onset preeclampsia based on demographic and clinical variables, such as BMI, systolic blood pressure, diastolic blood pressure, delivery weeks, infant birth weight, placenta weight and mode of delivery. However, we did not find any association between the *IL-10* -592A/C rs1800872 polymorphism and demographic and clinical variables in the risk of early-onset preeclampsia.

Discussion

In our study, we investigated the association between gene polymorphisms and risk of early-onset preeclampsia in a Chinese population. It is well known that *IL-10* gene is an important cytokine for successful pregnancy, and this gene has an inhibitory effect on Th1-type immune responses [12]. It is reported that promoter polymorphisms of genes could influence

the transcriptional, phenotypic and functional characteristics [13-14], and *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) are located in the *IL-10* gene promoter, and the three gene polymorphisms are considered to influence the dysregulated *IL-10* production and the onset and severity of preeclampsia. In our study, we found that the *IL-10* -592A/C (rs1800872) polymorphism was associated with an increased risk of early-onset preeclampsia in a Chinese population.

Numerous epidemiological studies have investigated the association between *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) gene polymorphisms and development of preeclampsia [9, 15-19]. Mirahmadian et al. reported that CC genotype of *IL-10* -819T/C (rs1800871) and -592A/C (rs1800872) polymorphisms contributed to the pathogenesis of pre-eclampsia in an Iranian population [16]. Vural et al. reported that AA genotype of IL-10 -1082A/G (rs1800896) was associated with increased risk of preeclampsia when compared with GG genotype [18]. Sowmya et al. reported that *IL-10* -819T/C (rs1800871) gene polymorphism can be a major genetic risk factor in the development of preeclampsia. Pissetti et al. suggested that G allele of *IL-10* -1082A/G (rs1800896) gene polymorphism was associated with the development of preeclampsia [9]. However, some studies reported no association between IL-10 gene polymorphism and risk of preeclampsia. Stonek et al. reported that no significant association between *IL-10* gene polymorphisms and development of women with preeclampsia [15]. de Lima et al. did not find significant association between *IL-10* -1082A/G (rs1800896), -819T/C (rs1800871), and -592A/C (rs1800872) polymorphisms and development of preeclampsia [17]. Sowmya et al. suggested that *IL-10* -1082A/G (rs1800896) polymorphism is not a major genetic regulator in the etiology of preeclampsia [19]. In our study, we found an association between the expression of the CC genotype and AC+CC genotype of *IL-10* -592A/C (rs1800872) and a significantly increased risk of early-onset preeclampsia compared to the AA genotype. The discrepancies between these studies may be caused by differences in tumor types, study design and populations.

Three limitations should be considered in our study. First, patients with early-onset preeclampsia and controls were selected from a single hospital, and these subjects may not well representative of other populations. Selection bias might exist in this study. Second, other functional SNPs in the interleukin genes may influence the development of early-onset preeclampsia, and further studies should considered other functional SNPs in the interleukin genes in their studies. Third, the sample size of our study is relatively small, which may influence the statistical power to find differences between groups. Therefore, further large sample size studies are greatly needed to confirm our findings.

In conclusion, our study has indicated that *IL-10* -592A/C (rs1800872) polymorphism was associated with an increased risk of early-onset preeclampsia in a Chinese population. Further studies with large sample size may help to elucidate the impact of *IL-10* gene polymorphisms on the development of early-onset preeclampsia.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Mei Zhong, Department of Obstetrics and Gynecology, Nanfang Hospital, Southern Medical University, 1838 Guangzhou Road, Guangzhou 510515, China. Tel: +86-471-6637640; Fax: +86-471-6965931; E-mail: zhongmei@fimmu.com

References

[1] Wang JX, Knottnerus AM, Schuit G, Norman RJ, Chan A, Dekker GA. Surgically obtained sperm, and risk of gestational hypertension and preeclampsia. Lancet 2002; 359: 673-4.

[2] Chelbi ST, Mondon F, Jammes H, Buffat C, Mignot TM, Tost J, Busato F, Gut I, Rebourcet R, Laissue P, Tsatsaris V, Goffinet F, Rigourd V, Carbonne B, Ferré F, Vaiman D. Expressional and epigenetic alterations of placental serine protease inhibitors: SERPINA3 is a potential marker of preeclampsia. Hypertension 2007; 49: 76-83.

[3] Udenze I, Amadi C, Awolola N, Makwe CC. The role of cytokines as inflammatory mediators in preeclampsia. Pan Afr Med J 2015; 20: 219.

[4] Mosmann TR and Sad S. The expanding universe of T-cell subsets. Immunol Today 1996; 7: 138-46.

[5] D'Alfonso S, Rampi M, Rolando V, Giordano M, Momigliano-Richiardi P. New polymorphisms in the IL-10 promoter region. Genes Immun 2000; 1: 231-3.
[6] Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998; 95: 9465-70.
[7] Mörmann M, Rieth H, Hua TD, Assohou C, Roupelieva M, Hu SL, Kremsner PG, Luty AJ, Kube D. Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. Genes Immun 2004; 5: 246-55.
[8] Sowmya S, Ramaiah A, Sunitha T, Nallari P, Jyothy A, Venkateshwari A. Role of IL-10 -819(t/c) promoter polymorphism in preeclampsia. Inflammation 2014; 37: 1022-7.
[9] Sowmya S, Sri Manjari K, Ramaiah A, Sunitha T, Nallari P, Jyothy A, Venkateshwari A. Interleukin 10 gene promoter polymorphisms in women with early-onset pre-eclampsia. Clin Exp Immunol 2014; 178: 334-41.
[10] Pissetti CW, Bianco TM, Tanaka SC, Nascentes GA, Grecco RL, da Silva SR, Balarin MA. Protective role of the G allele of the polymorphism in the Interleukin 10 gene (-1082G/A) against the development of preeclampsia. Rev Bras Ginecol Obstet 2014; 36: 456-60.
[11] Yang W, Zhu Z, Wang J, Ye W, Ding Y. Evaluation of association of maternal IL-10 polymorphisms with risk of preeclampsia by A meta-analysis. J Cell Mol Med 2014; 18: 2466-77.
[12] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001; 19: 683-765.
[13] Howell WM, Rose-Zerilli MJ. Cytokine gene polymorphisms, cancer susceptibility, and prognosis. J Nutr 2007; 137: 194-9.
[14] Schneider BG, Camargo MC, Ryckman KK, Sicinschi LA, Piazuelo MB, Zabaleta J, Correa P, Williams SM. Cytokine polymorphisms and gastric cancer risk: an evolving view. Cancer Biol Ther 2008; 7: 157-62.
[15] Stonek F, Hafner E, Metzenbauer M, Katharina S, Stümpflen I, Schneeberger C, Zeisler H, Husslein P, Philipp K. Absence of an association of tumor necrosis factor (TNF)-alpha G308A, interleukin-6 (IL-6) G174C and interleukin-10 (IL-10) G1082A polymorphism in women with preeclampsia. J Reprod Immunol 2008; 77: 85-90.
[16] Mirahmadian M, Kalantar F, Heidari G, Safdarian L, Mansouri R, Amirzargar AA. Association of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in Iranian patients with pre-eclampsia. Am J Reprod Immunol 2008; 60: 179-85.
[17] de Lima TH, Sass N, Mattar R, Moron AF, Torloni MR, Franchim CS, Daher S. Cytokine gene polymorphisms in preeclampsia and eclampsia. Hypertens Res 2009; 32: 565-9.
[18] Vural P, Degirmencioglu S, Saral NY, Demirkan A, Akgul C, Yildirim G, Issever H, Eroglu H. Tumor necrosis factor alpha, interleukin-6 and interleukin-10 polymorphisms in preeclampsia. J Obstet Gynaecol Res 2010; 36: 64-71.
[19] Sowmya S, Ramaiah A, Sunitha T, Nallari P, Jyothy A, Venkateshwari A. Evaluation of Interleukin-10 (G-1082A) Promoter Polymorphism in Preeclampsia. J Reprod Infertil 2013; 14: 62-6.