Polymorphisms in Inflammatory Mediator Genes and Risk of Preeclampsia in Taiyuan, China

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Weiwei Wu, MD¹, Hailan Yang, MD, PhD²,
Yongliang Feng, MD, PhD¹, Ping Zhang, MD¹,
Shuzhen Li, MD, PhD³, Xin Wang, MD⁴, Tingting Peng, MD¹,
Fang Wang, MD¹, Bingjie Xie, MD¹, Pengge Guo, MD¹, Mei Li, MD¹,
Ying Wang, MD¹, Nan Zhao, MD⁵, Suping Wang, MD, PhD¹,
and Yawei Zhang, MD, PhD^{1,5}

Abstract

Excessive maternal inflammatory response is involved in the pathogenesis of preeclampsia. Few epidemiologic studies have investigated the associations between genetic variations in the inflammatory mediator genes and preeclampsia risk, and these studies have reached inconsistent results. We examined 31 single-nucleotide polymorphisms in *IL-1A*, *IL-1B*, *IL-1RI*, *IL-2RA*, *IL-5RA*, *IL-6*, *IL-6R*, *TNFSF11*, *TNFRSF11A*, *IL-28RA*, *IRAK4*, and *KIT* genes and the risk of preeclampsia and its clinical subtypes in a nested case-control study including 203 preeclampsia cases and 233 controls. We found that *IL-1RI*, *IL-5RA*, *IL-6R*, and *TNFSF11* were associated with the risk of preeclampsia. Although the significant associations observed for preeclampsia overall were mainly seen for late-onset preeclampsia and severe preeclampsia, *IL-6R* (rs2229238) and *TNFSF11* (rs9525643) polymorphisms were associated with the risk of early-onset preeclampsia. *TNFSF11* (rs2200287 and rs2148072) polymorphisms were associated with risk of mild preeclampsia. Our study provided the first evidence that genetic variations in inflammatory mediator genes *IL-1RI*, *IL-6R*, *TNFSF11*, and *IL-5RA* were associated with preeclampsia risk, and the risk varied by preeclampsia subtypes.

Keywords

preeclampsia, inflammatory mediator gene, genetic polymorphisms, *IL-5RA*, *IL-1RI*

Introduction

Preeclampsia, a major pregnancy complication characterized by gestational hypertension and proteinuria, affects about 5% to 8% of all pregnancies worldwide.¹ It is among the leading causes of maternal and neonatal morbidity and mortality.² Although significant effort has been made, the etiology of preeclampsia is poorly understood.

Preeclampsia has been linked to reduced perfusion of placenta, maternal vascular endothelia activation, and abnormal remodeling of uterine spiral arteries that induced oxidative stress.³ Emerging evidence suggests that excessive maternal inflammatory response with cytokine-mediated endothelia damage may play a role in the pathogenesis of preeclampsia.⁴ Cytokine signals play an important role in the different stages of health and diseases and especially in the genesis of preeclampsia. Potential hypoxia and ischemia of the placenta lead to an increased release of inflammatory cytokines such as interleukin (IL)-1 α , IL-1 β , or tumor necrosis factor (TNF)- α which might trigger endothelial dysfunction and result in preeclampsia.^{5,6} Besides type 1 T helper cell (Th1) cytokines, a number

of inflammatory mediators including IL-5 and IL-6 are also associated with the regulation of inflammatory response involved in the pathogenesis of preeclampsia.⁷ Several studies

¹ Department of Epidemiology, Shanxi Medical University School of Public Health, Taiyuan, China

² Department of Obstetrics, the First Affiliated Hospital, Shanxi Medical University, Taiyuan, China

³ Department of Information, the First Affiliated Hospital, Shanxi Medical University, Taiyuan, China

⁴ Center For Disease Control and Prevention, Taiyuan Railway Administration, Taiyuan, China

⁵ Department of Environmental Health Sciences, Yale School of Public Health, New Haven, CT, USA

Corresponding Authors:

Yawei Zhang, Yale School of Public Health, 60 College Street, New Haven, CT 06520, USA.

Email: yawei.zhang@yale.edu

Suping Wang, Shanxi Medical University School of Public Health, 56 Xin-Jian South Road, Taiyuan, Shanxi, China.

Email: spwang88@163.com

Table 1. Definition of Preeclampsia and Its Subtype.

	Preeclampsia	Preeclampsia Subtypes			
		Mild	Severe	Early Onset	Late Onset
Systolic blood pressure	≥140 mm Hg	140 to <160 mm Hg	≥160 mm Hg	≥140 mm Hg	≥140 mm Hg
Diastolic blood pressure	≥90 mm Hg	90 to <110 mm Hg	≥110 mm Hg	≥90 mm Hg	≥90 mm Hg
Proteinuria ^a	≥1+	1+	≥2+	≥1+	≥1+
Symptoms of severity ^b	Yes/no	No	Yes	Yes/no	Yes/no
Gestational age of diagnosis	>20 weeks	>20 weeks	>20 weeks	21-<34 weeks	≥34 weeks

^aDipstick test in 2 urine samples.

^bIncluding headache, blurred vision, epigastric burning pain, decreased urine output, decreased or absent fetal kick, and so on.

investigated single-nucleotide polymorphisms (SNPs) in inflammatory mediator genes and risk of preeclampsia; however, the results have been inconsistent.^{4,8-15} Differences in ethnic groups and heterogeneity of preeclampsia included in different studies might have contributed to the conflicting results.¹⁶ Additionally, early studies only focused on *IL-1R1*, *IL-1A*, *IL-1B*, and *IL-6* genes. In order to further clarify a potential role of SNPs in inflammatory mediator genes in preeclampsia risk, we investigated 31 SNPs in 12 inflammatory mediator genes, including *IL-1A*, *IL-1B*, *IL-1R1*, *IL-2RA*, *IL-5RA*, *IL-6*, *IL-6R*, *TNFSF11*, *TNFRSF11A*, *IL-28RA*, *IRAK4*, and *KIT* and the risk of preeclampsia in a Chinese Han population.

Materials and Methods

A birth cohort was carried out at the first affiliated hospital of Shanxi Medical University in Taiyuan, China, between March 2012 and November 2013. Eligible women included pregnant women who came to the hospital for delivery with gestational age of 20 weeks or more, who had no mental illness, and who were aged 18 years or older. A total of 4208 eligible women were contacted for participation. Among those, 523 refused to participate and 26 did not complete in-person interviews, which yielded 3659 (87%) women who completed in-person interviews and 3166 women who donated blood samples. All study procedures were approved by the Human Investigation Committee at the Shanxi Medical University. Eligible women were informed of study procedure upon their arrival at the hospital for delivery. After obtaining written consent, an in-person interview was conducted at the hospital by trained study interviewers using a standardized and structured questionnaire. The questionnaire collected information regarding demographic factors, reproductive and medical history, smoking, alcohol and tea consumption, occupational and residential histories, physical activity, and diet. Information on birth outcomes and pregnancy complications were abstracted from medical records.

Among those who had maternal blood samples available, who gave singleton live birth without major birth defects, and who had no chronic hypertension and cardiovascular diseases, a total of 203 had preeclampsia. Preeclampsia was defined as having a blood pressure of 140/90 mm Hg or more (measured twice 6 hours apart) and concurrent proteinuria (2 urine

specimens containing at least 1+ protein by dipstick test) after 20 weeks of gestation.¹⁷ Preeclampsia was further classified as early-onset preeclampsia (EOPE), or late-onset preeclampsia (LOPE), mild preeclampsia, or severe preeclampsia (Table 1). We also randomly selected 233 controls from the same population and frequency matched the cases by age (± 2 years), residency, and time of conception (± 3 months).

DNA was extracted, isolated, and purified from whole blood samples according to a standard phenol-chloroform extraction method. Genotyping was conducted using an Illumina Golden Gate Platform. Duplicate samples (5%) were interspersed throughout the plates used for genotype analysis for quality control purposes. A total of 31 SNPs from 12 cytokine-mediated signaling pathway genes (*IL-1A*, *IL-1B*, *IL-1R1*, *IL-2RA*, *IL-5RA*, *IL-6*, *IL-6R*, *TNFSF11*, *TNFRSF11A*, *IL-28RA*, *IRAK4*, and *KIT*) were considered for this study. The completion rates for all SNPs were over 99% and the concordance rates for quality control samples were greater than 98% for all assays. Hardy-Weinberg equilibrium (HWE) was assessed in controls for each SNP using a χ^2 test. Single-nucleotide polymorphisms with a P value $>.05$ from the χ^2 test were considered to be in HWE. Of the 31 SNPs tested, 3 SNPs were not in HWE and were excluded from the final analyses (Supplementary Table S1).

Unconditional logistic regression was used to estimate the risk and calculate the odds ratio (OR) and 95% confidence intervals (CIs) for individual SNPs adjusting for age, education level, and body mass index. Additional adjustment for other variables including family income, history of preeclampsia, smoking, and alcohol consumption did not result in material changes of the results; therefore, they were not included in the final model. The common homozygous genotypes served as references. A linear trend test was conducted by assigning the ordinal values 1, 2, and 3 to the homozygous common, heterozygous, and homozygous rare alleles, respectively. The minimum P (min P) tests were conducted to examine an association at the gene level. The min P test was based on permutation resampling and was conducted to assess the true statistical significance of the smallest P trend within each gene region.¹⁸ Haplotype analyses were conducted for all genes in which more than 1 SNP was genotyped. Haplotype block structures were evaluated with Haploview using the 4-gamete rule.¹⁹ The individual haplotype frequencies were estimated through the

expectation maximization algorithm under the assumption of the HWE. An unconditional logistic regression model was used to estimate the effect of individual haplotypes, with the most common haplotype as the reference. The false discovery rate method with a significant level of .2 was applied for multiple comparisons. All the statistical analyses were carried out using SAS vision 9.3 software (SAS Institute).

Results

Among 203 preeclampsia cases, 161 cases (79.3%) had severe preeclampsia and 93 (45.8%) had EOPE. As shown in Table 2, preeclampsia cases were likely to have lower educational level, to be overweight or obese, and to have had cesarean section compared to controls. No statistically significant differences were observed in parity, smoking during pregnancy, and infant gender between preeclampsia and control groups.

As shown in Table 3, cases with EOPE were likely to be younger, to give birth to low birthweight infants, to have smaller gestational week, and to have had cesarean section compared to LOPE. Cases with severe preeclampsia were more likely to have lower educational level, to give birth to low birthweight infants, and to have smaller gestational week compared to mild preeclampsia.

At the gene level, *IL-5RA* was significantly associated with the risk of preeclampsia ($\text{min}P = .002$), EOPE ($\text{min}P = .003$), and severe preeclampsia ($\text{min}P = .0003$). *TNFSF11* was associated with EOPE ($\text{min}P = .02$) and mild preeclampsia ($\text{min}P = .002$). After adjustment for multiple comparisons, all these associations remained statistically significant (Table 4).

After adjustment for multiple comparisons, statistically significant associations between inflammatory mediator gene SNPs and risk of preeclampsia or its subtypes are presented in Table 5 and nonstatistically significant associations are presented in Supplementary Table 2. A reduced risk of preeclampsia was observed among women who carried *IL-5RA* rs163550 CC genotype compared to GG genotype ($\text{OR}_{\text{CC}} = 0.31$; 95% CI, 0.17-0.57; $P_{\text{trend}} = .0003$), *IL-5RA* rs163551 GG genotype compared to AA genotype ($\text{OR}_{\text{GG}} = 0.39$; 95% CI, 0.22-0.69; $P_{\text{trend}} = .002$), *IL-6R* rs7549250 AG genotype compared to AA genotype ($\text{OR}_{\text{AG}} = 0.58$; 95% CI, 0.37-0.91; $P_{\text{trend}} = .021$), *IL-6R* rs7549250 GG genotype compared to AA genotype ($\text{OR}_{\text{GG}} = 0.55$; 95% CI, 0.32-0.96; $P_{\text{trend}} = .021$), *TNFSF11* rs9525643 AG genotype compared to AA genotype ($\text{OR}_{\text{AG}} = 0.60$; 95% CI, 0.38-0.96; $P_{\text{trend}} = .020$), and *TNFSF11* rs9525643 GG genotype compared to AA genotype ($\text{OR}_{\text{GG}} = 0.52$; 95% CI, 0.30-0.91; $P_{\text{trend}} = .020$). An increased risk of preeclampsia was observed among women who carried *IL-1R1* rs3771202 CG genotype compared to GG genotype ($\text{OR}_{\text{CG}} = 1.85$; 95% CI, 1.24-2.76; $P_{\text{trend}} = .013$), *TNFSF11* rs2200287 AA&AG genotype compared to GG genotype ($\text{OR}_{\text{AA\&AG}} = 1.61$; 95% CI, 1.07-2.41; $P_{\text{trend}} = .022$), and *TNFSF11* rs2148072 TC&TT genotype compared to CC genotype ($\text{OR}_{\text{TC\&TT}} = 1.64$; 95% CI, 1.09-2.47; $P_{\text{trend}} = .013$).

Although significant associations observed for preeclampsia overall were mainly seen for LOPE (Table 4), *IL-5RA* rs163550

Table 2. Distributions of Selected Characteristics of the Study Population.

	Preeclampsia (n = 203)		Controls (n = 233)		P
	Number	%	Number	%	
Age (years)					.252
<25	52	25.62	54	23.18	
25-29	88	43.35	89	38.2	
≥30	63	31.03	90	38.62	
Education (years)					.004
<10	93	45.81	72	30.9	
11-15	105	51.73	150	64.38	
>15	5	2.46	11	4.72	
Body mass index (kg/m ²)					.002
<24.9	153	75.37	204	87.55	
24.9-30	42	20.69	23	9.87	
≥30	8	3.94	4	1.72	
Missing	0	0	2	0.86	
Parity					.531
Nulliparous	91	44.82	98	42.06	
Parous	111	54.68	135	57.94	
Missing	1	0.50	0	0	
Birthweight (g)					<.001
Low (<2500)	114	56.16	31	13.30	
Normal (2500-4000)	79	38.92	181	77.68	
High (≥4000)	10	4.92	18	7.72	
missing	0	0	3	1.30	
Gestational week					<.001
$\bar{x} \pm s$	36.07 ± 3.28		38.18 ± 2.29		
Intrauterine growth					<.001
SGA	94	46.30	29	12.45	
AGA	91	44.83	168	72.10	
LGA	18	8.87	36	15.45	
Smoking (active) during pregnancy					
Yes	0	0	0	0	
No	201	99.01	233	100	
Missing	2	0.99	0	0	
Smoking (passive) during pregnancy					.214
Yes	44	21.67	40	17.17	
No	159	78.33	193	82.83	
Alcohol drinking during pregnancy					
Yes	0	0	0	0	
No	201	99.01	233	100	
Missing	2	0.99	0	0	
Caesarean section					<.001
Yes	147	72.41	110	47.21	
No	56	27.59	119	51.07	
Missing	0	0	4	1.72	
Gender					.923
Boy	100	49.26	115	49.36	
Girl	101	49.75	114	48.93	
Missing	2	0.99	4	1.71	

Abbreviations: AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age.

and rs163551 showed similar association with LOPE and EOPE. A significant reduced risk was also observed for *IL-6R* rs2229238 TC genotypes compared to CC genotypes and *TNFSF11* rs9525643 GG genotype compared to AA genotype for EOPE but not for LOPE.

Table 3. Distributions of Selected Characteristics of the Preeclampsia Subtypes.

	EOPE (n = 93), N %	LOPE (n = 110), N %	P	Mild (n = 42), N %	Severe (n = 161), N %	P
Age (years)			.026			.080
<25	16 (17.2)	36 (32.7)		6 (14.4)	46 (28.6)	
25-29	42 (45.2)	46 (41.8)		18 (42.8)	70 (43.4)	
≥30	35 (37.6)	28 (25.5)		18 (42.8)	45 (28.0)	
Education (years)			.217			.045
<10	39 (41.9)	54 (49.1)		15 (35.7)	78 (48.4)	
11-15	50 (53.7)	55 (50.0)		24 (57.1)	81 (50.3)	
>15	4 (4.4)	1 (0.9)		3 (7.2)	2 (1.3)	
Body mass index (kg/m ²)			.493			.444
<24.9	68 (73.1)	87 (79.1)		30 (71.4)	125 (77.6)	
24.9-30	20 (21.5)	20 (18.2)		11 (26.2)	29 (18.0)	
≥30	5 (5.4)	3 (2.7)		1 (2.4)	7 (4.4)	
Parity			.184			.182
Nulliparous	37 (39.8)	54 (49.1)		15 (35.7)	76 (47.2)	
Parous	56 (60.2)	55 (50.0)		27 (64.3)	84 (52.2)	
Missing	0	1 (0.9)		0	1 (0.6)	
Birthweight (g)			<.001			.003
Low (<2500)	72 (77.4)	42 (38.2)		14 (33.4)	100 (62.1)	
Normal (2500-4000)	19 (20.4)	60 (54.5)		24 (57.1)	55 (34.2)	
High (≥4000)	2 (2.2)	8 (7.3)		4 (9.5)	6 (3.7)	
Gestational week			<.001			<.001
$\bar{x} \pm s$	34.10 ± 3.51	37.75 ± 1.83		37.45 ± 2.07	35.71 ± 3.45	
Intrauterine growth			.287			.441
SGA	48 (51.6)	46 (41.8)		16 (38.1)	78 (48.4)	
AGA	39 (41.9)	52 (47.3)		21 (50.0)	70 (43.5)	
LGA	6 (6.5)	12 (10.9)		5 (11.9)	13 (8.1)	
Smoking (passive) during pregnancy			.280			.643
Yes	17 (18.1)	27 (24.5)		8 (19.0)	36 (22.4)	
No	76 (81.9)	83 (75.5)		34 (81.0)	125 (77.6)	
Caesarean section			.036			.186
Yes	74 (79.5)	73 (66.4)		27 (64.3)	120 (74.5)	
No	19 (20.5)	37 (33.6)		15 (35.7)	41 (25.5)	
Gender			.534			.914
Boy	48 (51.6)	52 (47.3)		21 (50.0)	79 (49.1)	
Girl	44 (47.3)	57 (51.8)		21 (50.0)	80 (49.7)	
Missing	1 (1.1)	1 (0.9)		0	2 (1.2)	

Abbreviations: AGA, appropriate for gestational age; EOPE, early onset of preeclampsia; LGA, large for gestational age; LOPE, late onset of preeclampsia; SGA, small for gestational age.

Table 4. Associations Between Evaluated Gene Regions and Risk of PE and Subtype.

Gene	SNP Number	SNP Database ID	MinP test P				
			PE	EOPE	LOPE	Mild	Severe
<i>IL6R</i>	2	rs7549250 rs2229238	.040	.035	.076	.300	.061
<i>TNFSF11</i>	3	rs2200287 rs2148072 rs9525643	.049	.020	.016	.002	.060
<i>TNFSF11A</i>	2	rs9646629 rs9951012	.792	.695	.930	.232	.614
<i>IL1A</i>	3	rs2071374 rs3783550 rs17561	.807	.293	.737	.206	.946
<i>IL1B</i>	2	rs1143633 rs1143643	.870	.831	.477	.770	.694
<i>IL28RA</i>	2	rs6696471 rs16860738	.863	.443	.493	.491	.927
<i>IRAK4</i>	5	rs4251513 rs1461567 rs4251569 rs6582484 rs17121283	.963	.894	.868	.513	.856
<i>IL5RA</i>	5	rs6794523 rs334809 rs340813 rs163551 rs163550	.002	.003	.058	.176	.0003

Abbreviations: EOPE, early onset of preeclampsia; LOPE, late onset of preeclampsia; minP, minimum P; PE, preeclampsia; SNP, single nucleotide polymorphism.

After being stratified according to the severity of preeclampsia, the majority of significant associations remained for severe preeclampsia but not for mild preeclampsia (Table 5).

Two SNPs of *TNFSF11* (rs2200287 and rs2148072), however, showed significantly increased risk of mild preeclampsia if women carried *TNFSF11* rs2200287 AA genotype compared

Table 5. Associations between Inflammatory Mediator Genes Polymorphisms and Risk of PE Overall and Its Common Subtypes Including EOPE, LOPE, Mild PE, and Severe PE.

SNPs	PE overall					EOPE					LOPE					Mild					Severe							
	Controls	Cases	OR ^a	95% CI	P	Cases	OR ^a	95% CI	P	Cases	OR ^a	95% CI	P	Cases	OR ^a	95% CI	P	Cases	OR ^a	95% CI	P	Cases	OR ^a	95% CI	P			
IL1R1	rs3771202	148	102	1		49	1			53	1			24	1			78	1			78	1					
		GG	77	95	1.85	(1.24-2.76	.003	39	1.58	0.95-2.62	.078	56	2.08	1.28-3.38	.003	17	1.40	0.70-2.77)	.340	78	1.95	1.27-3.00)	.002	78	1.95	1.27-3.00)	.002	
		CC	7	6	1.10	0.35-3.44)	.865	5	1.95	0.59-6.50)	.276	1	0.33	0.04-2.79)	.306	1	0.87	0.10-7.44)	.900	5	1.13	0.34-3.77)	.844	5	1.13	0.34-3.77)	.844	
		Trend				.013			.056					.046				.486				.012				.012		
		CC and CG	84	101	1.78	1.20-2.63	.004	44	1.61	0.99-2.64	.056	57	1.91	1.18-3.08	.008	18	1.35	0.69-2.64	.382	83	1.87	1.23-2.85	.004	83	1.87	1.23-2.85	.004	
IL6R	rs7549250	59	76	1		34	1			42	1			13	1			63	1			63	1					
		AA	118	87	0.58	0.37-0.91	.018	40	0.61	0.35-1.06	.082	47	0.53	0.31-0.91	.022	24	0.93	0.44-1.96	.845	63	0.51	0.31-0.82	.0006	63	0.51	0.31-0.82	.0006	
		AG	53	38	0.55	0.32-0.96	.034	18	0.60	0.30-1.20	.150	20	0.51	0.26-0.99	.046	5	0.43	0.14-1.29	.134	33	0.59	0.33-1.05	.071	33	0.59	0.33-1.05	.071	
		Trend				.021			.115					.028				.162				.039				.039		
		AG and GG	171	125	0.57	0.38-0.87	.009	58	0.61	0.36-1.02	.061	67	0.52	0.32-0.87	.012	29	0.77	0.38-1.59	.488	96	0.53	0.34-0.83	.006	96	0.53	0.34-0.83	.006	
IL6R	rs2229238	191	179	1		86	1			93	1			36	1			143	1			143	1					
		CC	39	21	0.59	0.33-1.04	.068	5	0.28	0.11-0.75	.011	16	0.88	0.46-1.68	.689	6	0.8	0.31-2.03	.640	15	0.55	0.29-1.04	.068	15	0.55	0.29-1.04	.068	
		TC	3	1	0.42	0.04-4.23	.462	1	0.71	0.07-7.05	.771	0			.985	0		.990	1	0.57	0.05-5.86	.634	1	0.57	0.05-5.86	.634		
		TT				.054			.021					.437				.440				.071				.071		
		Trend				.054			.021					.437				.440				.071				.071		
TNFSF11	rs2200287	42	22	0.58	0.33-1.01	.054	6	0.31	0.13-0.77	.011	16	0.82	0.43-1.57	.555	6	0.74	0.29-1.88	.527	16	0.55	0.29-1.03	.061	16	0.55	0.29-1.03	.061		
		GG	163	123	1		61	1			62	1			23	1			100	1			100	1				
		AG	62	68	1.53	1.00-2.35	.048	27	1.23	0.71-2.13	.451	41	1.82	1.09-3.02	.022	12	1.40	0.65-2.99	.389	56	1.59	1.01-2.51	.044	56	1.59	1.01-2.51	.044	
		AA	8	12	2.18	0.85-5.58	.105	5	1.75	0.55-5.60	.347	7	2.70	0.91-8.04	.074	7	6.22	2.06-18.80	.001	5	1.19	0.37-3.87	.768	5	1.19	0.37-3.87	.768	
		Trend				.017			.267					.007				.005				.089				.089		
AA and AG	rs2148072	70	80	1.61	1.07-2.41	.022	32	1.29	0.77-2.17	.331	48	1.91	1.17-3.11	0.009	19	1.96	1.00-3.85	.050	61	1.55	1.00-2.40	.051	61	1.55	1.00-2.40	.051		
		CC	162	118	1		59	1			59	1			21	1			97	1			97	1				
		TC	62	67	1.56	1.02-2.39	.041	26	1.23	0.71-2.14	.467	41	1.87	1.12-3.12	0.016	13	1.67	0.78-3.56	.183	54	1.56	0.99-2.47	.055	54	1.56	0.99-2.47	.055	
		TT	8	12	2.25	0.88-5.77	.091	5	1.81	0.56-5.81	.319	7	2.77	0.93-8.22	0.067	7	6.83	2.24-20.85	.0007	5	1.21	0.37-3.90	.752	5	1.21	0.37-3.90	.752	
		Trend				.013			.260					0.006				.002				.102				.102		
TC and TT	rs9525643	70	79	1.64	1.09-2.47	.018	31	1.30	0.77-2.19	.334	48	1.97	1.21-3.21	0.007	20	2.28	1.15-4.49	.018	59	1.52	0.98-2.37	.061	59	1.52	0.98-2.37	.061		
		TNFSF11																										
		AA	49	61	1		29	1			32	1			15	1			46	1			46	1				
		AG	126	100	0.60	0.38-0.96	.035	50	0.67	0.38-1.19	.177	50	0.54	0.30-0.95	0.034	14	0.37	0.16-0.82	.015	86	0.67	0.41-1.11	.120	86	0.67	0.41-1.11	.120	
		GG	58	40	0.52	0.30-0.91	.022	13	0.38	0.18-0.80	.012	27	0.63	0.32-1.21	0.167	12	0.68	0.29-1.58	.367	28	0.46	0.25-0.86	.015	28	0.46	0.25-0.86	.015	
Trend					.020			.011					0.169				.357				.014				.014			
		AG and GG	184	140	0.58	0.37-0.90	.015	63	0.58	0.33-1.00	.050	77	0.57	0.33-0.97	0.039	26	0.47	0.23-0.95	.036	114	0.60	0.37-0.98	.040	114	0.60	0.37-0.98	.040	
		IL5RA																										
		AA	64	75	1		38	1			37	1			11	1			64	1			64	1				
		AG	108	97	0.75	0.49-1.17	.209	41	0.64	0.37-1.09	.102	56	0.89	0.52-1.52	0.677	22	1.19	0.54-2.62	.666	75	0.68	0.43-1.08	.104	75	0.68	0.43-1.08	.104	
GG		60	28	0.39	0.22-0.69	.001	12	0.33	0.16-0.70	.004	16	0.45	0.23-0.91	0.026	8	0.76	0.28-2.02	.581	20	0.32	0.17-0.60	.0004	20	0.32	0.17-0.60	.0004		
		Trend				.002			.003					0.035			.620				.0005				.0005			
		AG and GG	168	125	0.62	0.41-0.94	.026	53	0.53	0.32-0.88	.014	72	0.73	0.44-1.21	0.226	30	1.03	0.49-2.19	.930	95	0.55	0.35-0.85	.008	95	0.55	0.35-0.85	.008	
		IL5RA																										
		GG	65	77	1		39	1			38	1			10	1			67	1			67	1				
GC	rs163550	109	101	0.77	0.50-1.19	.243	43	0.66	0.39-1.14	.135	58	0.90	0.53-1.52	0.690	25	1.52	0.68-3.37	.305	76	0.66	0.42-1.05	.080	76	0.66	0.42-1.05	.080		
		CC	57	22	0.31	0.17-0.57	.0002	9	0.26	0.11-0.58	.001	13	0.37	0.18-0.78	0.008	6	0.67	0.23-1.96	.462	16	0.26	0.13-0.50	.00007	16	0.26	0.13-0.50	.00007	
		Trend				.0003			.001					0.014			.58				.00008				.00008			
		CC and GC		166	123	0.61	0.41-0.92	.019	52	0.52	0.31-0.87	.012	71	0.71	0.43-1.17	0.182	31	1.22	0.56-2.63	.614	92	0.52	0.34-0.81	.003	92	0.52	0.34-0.81	.003

Abbreviations: CI, confidence interval; EOPE, early onset of preeclampsia; LOPE, late onset of preeclampsia; PE, preeclampsia.

^aAdjusted for maternal age by years, education, and body mass index.

to GG genotype ($OR_{AA} = 6.22$; 95% CI, 2.06-18.80; $P_{trend} = .005$) or *TNFSF11* rs2148072 TT genotype compared to CC genotype ($OR_{TT} = 6.83$; 95% CI, 2.24-20.85; $P_{trend} = .002$).

We further examined the combination of significant SNPs in relation to the risk of preeclampsia and its subtypes (data not shown). We found that combination of *IL5RA* rs163551 GG genotype and *IL5RA* rs163551 CC genotype was associated with a reduced risk of EOPE compared to other genotypes ($OR = 0.32$; 95% CI, 0.15-0.68; $P = .003$; 9 exposed cases and 57 controls). Combination of *LIR1* rs3771201 GG genotype, *IL6R* rs7549250 AG genotype, *TNFSF11* rs2200287 AG genotype, and *TNFSF11* rs2148072 AG genotype was associated with an increased risk of LOPE ($OR = 2.46$; 95% CI, 1.06-5.70; $P = .036$; 14 exposed cases and 12 controls). *IL5RA* rs163551 GG genotype and *IL5RA* rs163551 CC genotype together was associated with a reduced risk of LOPE ($OR = 0.40$; 95% CI, 0.21-0.78; $P = .003$; 13 exposed cases and 57 controls). Additionally, women who carried both *TNFSF11* rs2200287 AA genotype and *TNFSF11* rs2148072 TT genotype experienced an increased risk of mild preeclampsia compared to other genotypes ($OR = 5.61$; 95% CI, 1.91-16.46; $P = .002$; 7 exposed cases and 8 controls). Women who carried both *IL5RA* rs163551 GG genotype and *IL5RA* rs163551 CC genotype had a reduced risk of mild preeclampsia ($OR = 0.33$; 95% CI, 0.18-0.61; $P = .0003$; 16 exposed cases and 57 controls).

We observed strong linkage disequilibrium of 5 blocks in 28 SNPs (data not shown). Haplotype analyses were consistent with the results of the individual SNP analyses and did not provide additional insight into these associations (data not shown).

Discussion

The present study found that genetic polymorphisms in *IL-1R1*, *IL-6R*, *TNFSF11*, and *IL-5RA* were associated with the risk of preeclampsia in a Chinese Han population, and the risk varied by preeclampsia subtypes. Specifically, genetic polymorphisms in *IL-6R* (rs2229238), *TNFSF11* (rs9525643), and *IL-5RA* (rs163551, rs163550) were associated with EOPE risk, *IL-6R* (rs7549250), *TNFSF11* (rs2200287, rs2148072), and *IL-5RA* (rs163551, rs163550) were associated with LOPE risk, *TNFSF11* (rs2200287, rs2148072) were associated with mild preeclampsia, whereas *IL-1R1* (rs3771202), *TNFSF11* (rs9525643), and *IL-5RA* (rs163551, rs163550) were associated with severe preeclampsia risk in this population.

No published studies have investigated genetic polymorphisms in *TNFSF11*, *TNFSF11A*, *IL-2RA*, *IL-5RA*, *IL-28RA*, *IRAK4*, and *KIT* genes in relation to preeclampsia. An early study by Goddard et al from the United States including 394 preeclampsia and 602 normotensive pregnant women examined the association between *IL-1A* (rs3783550, rs2071374, rs17561) and preeclampsia and found no association between them.¹³ Another study from the United States by Haggerty et al involving 150 preeclampsia and 661 normotensive pregnant women reported that genetic polymorphisms in *IL-1A* rs17561 were associated with preeclampsia risk both in black

women and white women.¹⁴ A study conducted by Andraweera et al including 175 preeclampsia and 171 normal pregnant women from Sri Lanka investigated polymorphisms in *IL-1A* (rs1800587, rs17561) and found *IL-1A* rs1800587, but not rs17561, was associated with preeclampsia risk.⁹ Ghasemi et al conducted a study in Iran involving 305 preeclampsia women and 325 controls and reported that carriage of the variant C allele in *IL-1A* rs1800587 was associated with preeclampsia.⁸ The only study conducted in China, which included 402 preeclampsia cases and 554 controls, investigated polymorphism *IL-1A* rs17561 and found that carriage of the variant C allele in *IL-1A* rs17561 was associated with preeclampsia.¹⁰ However, our study did not find that polymorphisms in *IL-1A* (rs3783550, rs2071374, rs17561) were associated with preeclampsia risk.

Five studies also investigated genetic polymorphisms in *IL-1B* genes in relation to preeclampsia risk^{9,11-13,15}; none found any association, which was consistent with our study.

Only one study conducted by Franchim et al, which included 109 preeclampsia cases and 174 controls, investigated polymorphisms in *IL-1R1* rs2234650 and preeclampsia and found no association.⁴ Although our study did not examine the SNP rs2234650, we investigated *IL-1R1* rs3771202 and found it was associated with the risk of preeclampsia, and the association was mainly seen for severe preeclampsia. The *IL-1R1* encodes a receptor located on type 2 T helper cell (Th2) cells and binds with IL-1R AcP and IL-1A or IL-1B to form a trimeric complex. In this complex, the Toll- and IL-1R-like domains get close to each other, which subsequently results in the recruitment of myeloid differentiation primary response gene 88 (MYD88), toll-interacting protein, and IL-1 receptor-associated kinase 4 (IRAK4). This then affects inflammatory response through the protein-related pathways involved in innate immunity.^{20,21} Additionally, rs3771202 was located in the noncoding intron, where it may regulate exon transcription. To confirm our results and identify potential regulatory SNPs, we used ESEfinder²² to look for potential ESEs and modifications of the splicing factors binding site pattern resulting from the mutation. Although rs3771202 was associated with the risk of severe preeclampsia in our population, we did not find rs3771202 could change binding sites on RNA splicing. Its function still needs further study.

Substantial evidence supports a role of cytokines in the etiology of preeclampsia during both the early placental stage and the later systemic stage. The Th1-type cytokines including IL-2, TNF- α , and IFN- γ can induce several cell-mediated inflammatory responses. On the other hand, Th2-type cells secrete the Th2 cytokines IL-4, IL-5, IL-6, and IL-10, which are associated with help for humoral immunity.²³ Th2-type immunity is associated with normal pregnancy, whereas an excessive Th1 reactivity is associated with adverse pregnancy complications including spontaneous miscarriage and preeclampsia.^{24,25} It is biologically plausible that polymorphisms in inflammatory mediator genes involved in cytokine-mediated signaling pathways might influence preeclampsia risk.

An early study conducted in China found that alterations in IL-6 and its corresponding receptors IL-6R play a role in the

etiology of severe preeclampsia.²⁶ There were 6 studies that investigated genetic variation in *IL6* rs1800795 and risk of preeclampsia and none found an association.²⁷⁻³² Although our study did not examine the SNP rs1800795, we investigated *IL-6* rs7805828 and also found no association. Although no existing study has investigated polymorphisms in *IL-6R* and preeclampsia, our study examined *IL-6R* (rs7549250 and rs2229238) and found that *IL-6R* rs7549250 was associated with LOPE and that *IL-6R* rs2229238 was associated with EOPE. Using ESEfinder, we found that rs7549250 could change SF2/ASF (IgM-BRCA1) binding sites, whereas rs2229238 could modify SC35 binding sites on splicing. These altered splicing binding site patterns may induce splicing switching or exons skipping³³ and may subsequently result in affected synthesis of IL-6R.

The ligand-binding component IL-6R together with signal-transducing component gp130 form a cell surface cytokine receptor complex, which plays a key role in the IL-6 signaling.³⁴ The complex can also activate multiple intracellular signaling cascades including janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway and mitogen-activated protein kinase pathway, affecting maternal inflammation that is associated with preeclampsia.

Tumor necrosis factor receptor superfamily, member 11a (*TNFRSF11A*), is also known as a receptor activator of nuclear factor- κ B (RANK). *TNFRSF11A* and its ligand (*TNFSF11* or RANKL) are 2 functionally linked genes and are frequently considered together when considering their role in determination of various traits, such as dendritic cell/T-cell communication, T-cell activation, or early B-lymphocyte development. Their primary function is to activate the transcription factor nuclear factor κ B (NF- κ B),³⁵ which is implicated with dendritic cells, T-cell activation, and subsequently proinflammatory cytokines secretion, such as IL-1, IL-6, and IL-12.³⁶ Furthermore, activation of NF- κ B is also a key process involved in the toll-like receptor associated with innate immunity, including Myd88-dependent or independent pathways. These biological functions are implicated in the pathogenesis of preeclampsia.³⁷ Our study found that *TNFSF11* (rs2200287 and rs2148072) was associated with LOPE and mild preeclampsia, whereas *TNFSF11* rs9525643 was associated with EOPE and severe preeclampsia. Using ESEfinder, we found that rs2200287 could change SF2/ASF (IgM-BRCA1)-binding site, whereas rs9525643 could change SRp40-binding sites on splicing. These altered splicing binding site patterns may induce splicing switching or exon skipping,³³ subsequently resulting in affected synthesis of TNFSF11. Although rs2148072 was associated with the risk of preeclampsia in our population, its function was unclear.

The *IL-5RA* encodes an important receptor of IL-5, which binds to IL-5 and is activated in at least 3 signaling pathways including JAK2/STAT5, Btk, and Ras/ERK pathways.³⁸ Among them, the activation of the Ras/ERK pathway can promote cell survival and proliferation against trophoblastic apoptosis and aponecrosis, which have been implicated as key pathologic events in preeclampsia.³⁹ Our study found that polymorphisms in *IL5-RA* (rs163551, rs163550) were associated with EOPE, LOPE, and severe preeclampsia. We found that

rs163551 could change SF2/ASF and SF2/ASF (IgM-BRCA1) binding sites, whereas rs163551 could change SF2/ASF and SC35 binding sites on splicing, suggesting that these SNPs have the potential to affect the synthesis of IL-5RA.

The present study was a candidate gene study. We recognized the possibility that one or more additional SNPs, in cis, could be causal SNPs. Additional studies are needed to survey the common genetic variation in these 4 genes, explore more functional SNPs associated with the risk of preeclampsia, and determine the pattern of linkage disequilibrium between each other. Relatedly, functional studies should investigate the functional consequences of these SNPs. Future experimental studies are needed to characterize these SNPs biological function.

Strengths and limitations should be considered when interpreting the study results. Diagnosis of preeclampsia in our study was based on medical records not self-reporting, which minimized potential disease misclassification. We have collected detailed information on various potential confounding factors and have controlled them in the model. Our study sample size was moderate and might have limited power for subtype analyses. Future larger studies are warranted to replicate the observed associations. The current study was hospital based, which might have limited generalizability. However, the rate of preeclampsia (6.4%) in our study population was within the range (2%-8%) reported worldwide.¹

In conclusion, our study provided the first evidence that genetic polymorphisms in *IL-1R1*, *IL-6R*, *TNFSF11*, and *IL-5RA* were associated with the pathogenesis of preeclampsia and the association varied by preeclampsia subtypes. These findings warrant further investigation in larger population-based epidemiologic studies among different populations.

Authors' Note

SW and YZ contributed equally to this work.

Declaration of Conflicting Interests

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Supplemental Material

The online supplemental tables are available at <http://journals.sagepub.com/doi/suppl/10.1177/1933719116660844>.

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