Investigation of the association between matrix metalloproteinase-9 genetic polymorphisms and development of pre-eclampsia in Chinese pregnant women

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ABSTRACT. We carried out a case-control study to evaluate the role of *MMP-9* -1562 C/T (rs3918242) genetic polymorphism in the risk of pre-eclampsia in Chinese pregnant women. Between March 2013 and January 2015, 107 pregnant women with pre-eclampsia were recruited from the Second Affiliated Hospital of Zhengzhou University. Genotyping of *MMP-9* was carried out using polymerase chain reaction-restriction fragment length polymorphism assay. Unconditional logistic regression analyses revealed that women with the CT genotype (OR = 1.81, 95%CI = 1.04-3.11) exhibited significantly higher risk of pre-eclampsia than those with the wild-type CC genotype. Moreover, individuals carrying the T allele were at higher risk of pre-eclampsia than those carrying the C allele; the adjusted OR (95%CI) was 1.62

(1.06-2.47). In conclusion, our study demonstrated that the *MMP-9* -1562 C/T polymorphism plays an important role in the development of pre-eclampsia in Chinese women. Further studies with large sample sizes are greatly needed to confirm our findings.

Key words: MMP-9; Polymorphism; Pre-eclampsia

INTRODUCTION

Pre-eclampsia is a serious disease in pregnant women and is associated with high maternal morbidity and mortality in developing countries (Liang et al., 2010). Although the mechanism of pre-eclampsia remains unclear, the development of coronary artery disease results from multiple complex factors, including various environmental factors and their interactions. A previous study reported familial aggregation of pre-eclampsia, which suggests that genetic factors contribute to the pathogenesis of this disease (Morgan and Ward, 1999). Genetic factors account for 25 to 55% of the risk of developing pre-eclampsia (Thornton and Macdonald, 1999; Salonen Ros et al., 2000).

Matrix metalloproteinases (MMPs) belong to a family of structurally related zinc-dependent enzymes, which have important roles in restructuring of the extracellular matrix by promoting the secretion of collagenases and gelatinases as well as proteolytic enzymes (Lim et al., 1997; Coolman et al., 2007; Palei et al., 2008). MMP-9 is an important member of the MMPs; it is involved in blastocyst implantation and is an important biochemical mediator of Sertoli cell degeneration. Genetic variations of *MMP-9* could alter the expression level and structure of the protein (Aas et al., 2003; Bartek and Lukas, 2003). Here, we report a case-control study to evaluate the role of the *MMP-9* -1562 C/T (rs3918242) polymorphism in the risk of pre-eclampsia in Chinese pregnant women.

MATERIAL AND METHODS

Study subjects

Between March 2013 and January 2015, 107 pregnant women with pre-eclampsia were recruited from the Second Affiliated Hospital of Zhengzhou University. The diagnostic criteria for pre-eclampsia were as follows: systolic pressure □140 mmHg, diastolic pressure □90 mmHg, and proteinuria (urinary protein □0.3 g or urine dipstick protein □ ++ over a 24-h period) after 20 weeks of gestation. Patients who had a history of intrauterine fetal deaths were excluded from this study.

During the same period, 242 pregnant women with gestational age □20 weeks were randomly selected from pregnant women visiting the Second Affiliated Hospital of Zhengzhou University for prenatal examination. Control subjects were free of pre-eclampsia, high blood pressure, and abnormal fetal growth.

Demographic data of the pre-eclampsia patients and control subjects were collected from medical records. Written informed consent forms were signed by both patients and control subjects. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University.

DNA extraction and genotyping

Five fasting venous blood samples were drawn from each study subject, and kept in EDTA-anticoagulant tubes and stored in a freezer at -20°C. Genomic DNA was extracted from the peripheral blood using the TIANamp blood DNA kit (Tiangen, Beijing, China). Genotyping of the *MMP-9* gene was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Forward and reverse primer sequences for *MMP-9* -1562 C/T were 5'-GCCTGGCACATAGTAGGCCC-3' and 5'-CTTCCTAGCCAGCCGGCATC-3', respectively. PCR products were digested at 37°C overnight (~16 h) with 4 U *Sph*I. Amplification was checked using 1.5% agarose gel electrophoresis. In total, 10% random samples were used to confirm the reproducibility of the genotyping results, and the results showed 100% consistency.

Statistical analysis

Statistical differences in the demographic variables and genotype frequencies between the patients and control subjects were determined using the chi-square test for categorical data and the Student *t*-test for continuous variables. Concordance with Hardy-Weinberg equilibrium (HWE) was estimated using the Fisher exact-test. To evaluate the association of pre-eclampsia susceptibility with *MMP-9* -1562 C/T polymorphism, logistic regression analysis was used to calculate adjusted odds ratios (ORs) along with 95% confidence intervals (CIs) with adjustment for possible confounders. SPSS 17.0 package (SPSS Inc., Chicago, IL, USA) was used to carry out all the statistical analyses, and $P < 0.05$ was considered significant.

RESULTS

According to chi-square or *t*-test, patients were comparable with control subjects in terms of gestational age ($\chi^2 = 1.59$, $P = 0.06$), tobacco smoking ($\chi^2 = 0.87$, $P = 0.35$), and alcohol use ($\chi^2 = 1.47$, $P = 0.23$) (Table 1). However, we observed significant differences between patients and controls with respect to age ($t = 3.18$, $P < 0.001$), BMI ($t = 4.70$, $P < 0.001$), systolic blood pressure ($t = 17.78$, $P < 0.001$), diastolic blood pressure ($t = 20.16$, $P < 0.001$), infant birth weight ($t = 9.12$, $P < 0.001$), and model of delivery ($\chi^2 = 18.42$, $P < 0.001$).

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

Variables	Patients	%	Controls	%	*t* or χ^2 test	P value
Age, years	28.85 ± 5.21		26.85 ± 5.50		3.18	<0.001
Gestational age, weeks	27.75 ± 4.50		26.82 ± 5.25		1.59	0.06
BMI, kg/m^2	30.82 ± 4.21		28.40 ± 4.53		4.70	<0.001
Systolic blood pressure, mmHg	154.60 ± 20.80		114.55 ± 18.75		17.78	<0.001
Diastolic blood pressure, mmHg	104.64 ± 18.65		72.57 ± 10.83		20.16	<0.001
Tobacco smoking						
No	99	92.52	230	95.04		
Ever	8	7.48	12	4.96	0.87	0.35
Alcohol consumption						
No	97	90.65	228	94.21		
Ever	10	9.35	14	5.79	1.47	0.23
Infant birth weight, g	2759.53 ± 744.56		3358.52 ± 465.61		9.12	<0.001
Model of delivery						
Normal	44	41.12	159	65.70		
Caesarean	63	58.88	83	34.30	18.42	<0.001

BMI = body mass index.

Of the patients, 67 (62.62%), 33 (30.84%), and 7 (6.54%) had the CC, CT, and TT genotype, respectively. Of the control subjects, 178 (73.55%), 53 (21.90%), and 11 (4.55%) displayed the CC, CT, and TT genotype, respectively (Table 2). The genotype distributions of *MMP-9* -1562 C/T are shown in Table 2. The chi-square test indicted no statistical difference in the genotype frequencies of *MMP-9* -1562 C/T between the two study groups ($\chi^2 = 4.24$, P = 0.12). The genotype distributions of *MMP-9* -1562 C/T in patients were in concordance with the HWE (P = 0.30), whereas those in controls were not (P = 0.01).

Table 2. Genotype distribution of *MMP-9* -1562 C/T in patients with pre-eclampsia and control subjects.

MMP-9 -1562 C/T	Patients	%	Controls	%	χ^2 test	P value	P for HWE	
							In patients	In controls
CC	67	62.62	178	73.55				
CT	33	30.84	53	21.90				
TT	7	6.54	11	4.55	4.24	0.12	0.30	0.01

HWE = Hardy-Weinberg equilibrium.

Unconditional logistic regression analyses revealed that individuals with the CT genotype had significantly higher risk of pre-eclampsia than those with the CC genotype (OR = 1.81, 95%CI = 1.04-3.11) (Table 3). Moreover, individuals carrying the T allele were at increased risk of pre-eclampsia as compared to those having the C allele; the adjusted OR (95%CI) was 1.62 (1.06-2.47).

Table 3. Association between *MMP-9* -1562 C/T genetic polymorphism and development of pre-eclampsia.

MMP-9 -1562 C/T	Patients	%	Controls	%	OR (95%CI)	P value
CC	65	60.75	178	73.55	1.0 (Ref.)	-
CT	35	32.71	53	21.9	1.81(1.04-3.11)	0.02
TT	7	6.54	11	4.55	1.74 (0.55-5.16)	0.27
Allele						
C	165	154.21	409	169	1.0 (Ref.)	-
T	49	45.79	75	31	1.62 (1.06-2.47)	0.02

Ref. = Reference.

DISCUSSION

Previous epidemiological studies have indicated that various polymorphisms in genes such as interleukin-27, transforming growth factor beta-1, estrogen receptor alpha, cystathionine gamma-lyase, and cyclooxygenase 2 might play a critical role in the development of pre-eclampsia (Deepthi et al., 2015; El-Beshbishy et al., 2015; Khani et al., 2015; Mrozikiewicz et al., 2015; Ren et al., 2015; Chen et al., 2016). Here, we carried out a case-control study to estimate the role of the *MMP-9* -1562 C/T polymorphism in the risk of pre-eclampsia, and we observed that the CT genotype and T allele of *MMP-9* -1562 C/T were associated with elevated risk of pre-eclampsia in Chinese pregnant women.

Multiple experimental studies have investigated the association between MMP-9 expression and pre-eclampsia development; however, the results are inconsistent (Mckirdy and Marks, 2012; Wang et al., 2013; Yang et al., 2013; Lockwood et al., 2014; Rasstrigina et

al., 2014). Wang et al. (2013) reported that decreased expression levels of MMP-9 regulate trophoblast invasion and migration, and thus influence the pathogenesis of pre-eclampsia. Yang et al. (2013) indicated that MMP-9 expression might have an important role in the pathogenesis of pre-eclampsia through regulation of trophoblast invasion. Lockwood et al. (2014) suggested that augmented expression of MMP-9 in decidual cells could influence the development of pre-eclampsia. However, Mckirdy and Marks (2012) indicated that high MMP-9 expression was observed in early gestation, but no difference was found in protein levels of MMP-9 between pre-eclampsia and normal pregnant women.

Additionally, several studies have reported an association between *MMP-9* genetic polymorphism and the development of pre-eclampsia; however, the results of these studies are inconclusive (Coolman et al., 2007; Fraser et al., 2008; Palei et al., 2010, 2012; Luizon et al., 2012; Rahimi et al., 2013, 2014, 2015). Some studies suggested that the CC genotype or C allele of *MMP-9* -1562 C/T is associated with increased risk of developing pre-eclampsia in Caucasian pregnant women (Coolman et al., 2007; Luizon et al., 2012; Rahimi et al., 2013, 2014, 2015). However, Fraser et al. (2008) suggested that *MMP-9* -1562 C/T genetic polymorphism failed to present any significant association with pre-eclampsia. Two studies in Brazil reported that *MMP-9* genetic variation is correlated with gestational hypertension, but not with pre-eclampsia (Palei et al., 2010, 2012). In our study, we found that *MMP-9* -1562 C/T genetic polymorphism contributes to the development of pre-eclampsia.

In conclusion, our study demonstrated that the *MMP-9* genetic polymorphism may play an important role in the development of pre-eclampsia in Chinese pregnant women. Further studies with large sample sizes are greatly needed to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

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