Polymorphisms of the Endothelial Nitric Oxide Synthase Gene in Preeclampsia in a Han Chinese Population

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Key Words

Endothelial nitric oxide synthase · Polymorphism ·
Preeclampsia · Nitric oxide · Han Chinese

Abstract

Background/Aims: The endothelial nitric oxide synthase (eNOS) gene has been enlisted by previous research as a candidate gene of preeclampsia predisposition. This study investigates the specific roles of 3 polymorphisms of the eNOS gene in a population of Chinese origin from mainland China. **Methods:** We studied the association of 3 commonly studied polymorphisms of the eNOS gene, namely 4b/a, T-786C and Glu298Asp, in a case-controlled sample of 220 patients diagnosed with preeclampsia and 200 healthy controls. The association between eNOS polymorphisms and preeclampsia was evaluated by performing genotyping for the eNOS variants and calculating odds ratios (OR) and 95% confidence intervals. The plasma nitrite concentration in participants was determined to examine how 3 eNOS polymorphisms affect plasma nitric oxide (NO) concentrations in pregnant women. **Results:** The frequencies of both the variant 298Asp allele and eNOS 4a allele were significantly lower in preeclamptic women than in the control group and

had a significantly lower OR. The variant 298Asp allele and eNOS 4a are strongly associated with higher plasma NO concentrations in pregnant women. **Conclusions:** Polymorphisms in the eNOS gene may be protective against preeclampsia in a Chinese population, and this protective effect may be associated with NO formation in plasma in pregnant women.

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Introduction

Preeclampsia is a common complication of pregnancy that has affected up to 10% of all pregnancies in the developing countries and becomes a severe risk factor for both maternal and neonatal morbidity and mortality [1, 2]. Although the exact etiology of preeclampsia has not been thoroughly understood, a variety of genetic and environmental factors have been proposed to play a role in the pathogenesis and development of preeclampsia [3, 4]. The incident risk of preeclampsia for daughters of preeclamptic mothers is reported to be 20–40%, and 11–37% for sisters of preeclamptic women, which is even higher for twin sisters (22–47%) [5].

Mounting evidence suggests that reduced nitric oxide (NO) formation is often linked with the hypertensive disorders of pregnancy, particularly preeclampsia [6–10]. Some previous study has investigated whether genetic mutations in the endothelial NO synthase (eNOS) gene could affect the impaired NO formation which further connects with preeclampsia, and has attempted to establish or dismiss the role of eNOS as a candidate gene for the pathogenesis and development of preeclampsia. Impairment of NO-mediated vasodilatation has been suggested to play important roles in the development of such a condition [11, 12]. In a linkage study, Arngimsson et al. [13] identified the region of chromosome 7q36 which encodes the eNOS gene and could serve as a possible candidate region responsible for pregnancy-induced hypertension.

The eNOS gene consists of 26 exons on chromosome 7. There are several eNOS gene polymorphisms, and at least 3 of these have been proposed to link with distinct NO levels in the blood. A missense mutation in exon 7 of the eNOS gene leads to the replacement of guanine by thymine (G894T), resulting in the single-nucleotide polymorphism in exon 7 (Glu298Asp, rs1799983) [14]. Molecular studies have suggested that intact eNOS Asp298 has an equivalent enzymatic effect with eNOS Glu298, but when it is under selective proteolysis in native cells and tissues the steady activity level of eNOS may be diminished in carriers of this allele [15]. The other two most common variants include: an insertion-deletion polymorphism within intron 4 (4a/b) comprised of 2 alleles (the a*-deletion allele with 4 tandem 27-bp repeats and the b*-insertion allele with 5 repeats) and a T-786C substitute in the promoter region (rs2070744) [16].

The association studies between eNOS variants and preeclampsia have until now produced interesting yet controversial results, and the replication study in this area is far from being sufficient [17, 18]. Therefore, the status of association for the eNOS variants remains less understood. And what is more important, there is regrettably a lack of replication from the Chinese population, except that one study from Taiwan reported that polymorphisms in the eNOS Glu298Asp and intron 4 variant may be protective against preeclampsia in the Chinese population, but the T-786C association study was missing in the same population; in addition, participants in that study are not all from the Han Chinese population, but also from other Chinese ethnic groups. Thus, in our current study, we aimed to replicate similar research on the association between preeclampsia and the three most commonly investigated eNOS polymorphisms (4b/a, T-786C and Glu-298Asp) in a homogeneous population of Han Chinese

origin (mainland China). These variants were selected because of their potential functional roles and their high minor allele frequencies [19–21].

In this study, we also tested the NO concentration in the plasma in all the participants, and aimed to examine how the three eNOS polymorphisms described above affect plasma nitrite concentrations in healthy pregnant women and in preeclamptic women.

Materials and Methods

Participants

A total of 220 cases of preeclampsia patients and 200 healthy female controls were recruited from the outpatient and inpatient sections of the Department of Obstetrics and Gynecology of the First Affiliated Hospital of Wenzhou Medical University. Preeclampsia was defined as significant hypertension level, with systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg and 24-hour proteinuria >300 mg at or after 20 weeks' gestation [22, 23]. The study protocol was reviewed and approved by the Ethics Committee of our hospital, and all participants signed a written informed consent. All controls had a normal pregnancy without complications, and there were no conditions of preeclampsia shown in their medical history. The controls had no blood relations with the test group, and all were delivered at term. All participants included in the study were of Han Chinese origin whereas women with previous medical histories of renal disease, metabolic disorders or diabetes were excluded from the patient sample. Blood samples of all the patients were tested by biochemical measurements and their DNA extractions were also taken for testing.

DNA Analysis

Genomic DNA was extracted from the whole blood of the patients' blood samples using the PureLink® Total RNA Blood Kit (Life Technologies, Invitrogen China Ltd.) according to the instructions on the user manual. Genotyping of each variant was performed by amplification from 50 to 500 μ l of genomic DNA. The primer sequences utilized and the laboratory conditions for genotyping including polymerase chain reaction, restriction enzymes and agarose electrophoresis for each eNOS polymorphism have been previously described [19–21]. Genotyping was performed by laboratory staff blinded to the patients' clinical status.

Measurement of Plasma Nitrite Concentrations

Plasma aliquots were analyzed in triplicate for their nitrite content using an ozone-based chemiluminescence assay as previously described [24–26]. Briefly, 200 μ l of plasma sample was injected into a solution of acidified tri-iodide, purged with nitrogen in line with a gas phase chemiluminescence NO analyzer (Arrow-STRAIGHT™, Shelfscientific, Division of Lazar Research Laboratories, USA). Approximately 8 ml of tri-iodide solution, comprised of 2 g of potassium iodide and 1.3 g of iodine dissolved in 40 ml of water with 140 ml of acetic acid, were put into the purge vessel where the plasma samples were injected. The tri-iodide solution could reduce nitrites to NO gas, which is detectable in the NO analyzer.

Statistical Analyses

Statistical analyses were performed using the statistical package SPSS 19.0. The χ^2 test or an independent Student *t* test was employed to examine the differences between groups, whenever appropriate. The gene-counting method was used in the estimation of the allele frequencies. The frequencies of the genotypes and the alleles were compared between the test group and the control group by the χ^2 test, where appropriate. The 95% confidence intervals (CI) and odds ratio (OR) were also estimated. The χ^2 test was performed for the deviation of genotype distribution from the Hardy-Weinberg equilibrium. Risk factors were adjusted by multivariate logistic regression analysis, in which preeclampsia was a dependent variable and independent variables were history of preeclampsia, age and eNOS polymorphisms.

Results

Characteristics of the Study Patients

The clinical characteristics of the participants are shown in table 1. There were no differences in maternal age between preeclamptic patients and controls. As expected, gestational age was significantly lower in preeclamptic women, and the systolic and diastolic blood pressures and primiparity were significantly higher in the test group than in the control group. Birth weight was slightly lower in preeclamptic women, but the difference was not significant.

eNOS Variant Allele Frequency and Genotype Distribution

The distributions of the eNOS 4b/a, T-786C and Glu-298Asp genotypes were compared between preeclamptic patients and controls and are presented in table 2. Allele frequencies of all three polymorphisms were in Hardy-Weinberg equilibrium ($p > 0.05$).

Frequencies of the bb, ba and aa genotypes were 80.5, 18.5 and 1.0% in preeclamptic patients and 68.9, 28.3 and 2.78% in healthy controls, respectively. The a allele frequencies were 11.2 and 16.9% in preeclamptic patients and in healthy controls, respectively. Significant differences in the genotype distribution or allele frequencies between patients and controls were found ($p < 0.05$). The b allele might be a risk factor for preeclampsia (OR = 1.768; 95% CI = 0.168–2.731; $p < 0.01$).

Genotype frequencies of the Glu298Asp polymorphism in healthy controls were 61.1% for GluGlu, 27.8% for GluAsp and 11.1% for AspAsp. On the other hand, in preeclamptic patients, genotype frequencies were 71% for GluGlu, 24.5% for GluAsp and 4.5% for AspAsp; statistical analysis showed significant differences ($p < 0.05$). The Asp allele frequencies were 25.0 and 16.7% in healthy

Table 1. Clinical characteristics of preeclamptic patients and controls

	Preeclampsia (n = 200)	Controls (n = 180)	p value
Age, years	29.1±6.5	27.2±6.1	>0.05
Gestational age, weeks	36.4±3.4	38.1±3.7	<0.01
Birth weight, g	2,769±832	3,011±756	>0.05
Diastolic blood pressure, mm Hg	96.3±8.2	70.2±8.3	<0.01
Systolic blood pressure, mm Hg	151.3±13.6	112.6±10.7	<0.01
Primiparity, %	90	87	>0.05
Family history of preeclampsia, %	0.42	0.37	>0.05

controls and preeclampsia patients, and the Glu allele conferred a significantly higher risk compared to the Asp allele (OR = 1.657; 95% CI = 1.162–2.362; $p < 0.05$).

As for the T-786C variant, no significant differences in the genotype distribution or allele frequencies between patients and controls were found ($p > 0.05$), so we did not calculate the OR (table 2).

Relationship between Variants and Nitrite Concentration in Plasma

Plasma NO concentration was examined to further understand the underlying mechanism about how the variants of eNOS affected the NO formation in preeclampsia patients and healthy women. Consistent with OR analysis, Glu298Asp and intron 4 polymorphisms had significant effects on the plasma nitrite concentrations; higher nitrite levels were found in both healthy and preeclamptic women with the AspAsp genotype compared with those with the GluGlu genotype and with the 4a4a genotype compared with those with the 4b4b genotype ($p < 0.05$; fig. 1). No effect of T-786C on plasma nitrite concentrations was found in preeclampsia and healthy women in the Chinese population in the current study (fig. 1; all $p > 0.05$).

Discussion

This study has evaluated the relations between preeclampsia and common genetic variants in the eNOS gene in a Han Chinese population from mainland China. The population of mainland China accounts for more than 20% of the population in the world, so it is very im-

Fig. 1. a–c Plasma nitrite concentrations in control (white bars) and in preeclamptic (grey bars) women grouped by genotype for each polymorphism. * $p < 0.05$.

Table 2. Genotype and allele frequencies of the three polymorphisms analyzed in preeclamptic patients and controls

	Preeclampsia (n = 200)	Controls (n = 180)	OR (odds ratio)	p value
4b/a				
bb	161 (80.5)	124 (68.9)	1.864 [1.164–2.986] ¹	0.012
ba	37 (18.5)	51 (28.3)	1 (reference) ¹	
aa	2 (1.0)	5 (2.78)		
b allele	359 (89.8)	299 (83.1)	1.768 [0.168–2.731]	0.008
a allele	41 (11.2)	61 (16.9)	1 (reference)	
T-786C				
TT	81 (40.5)	74 (41.1)	1.095 [0.599–2.002]	0.871
TC	90 (45.0)	77 (42.8)	1.169 [0.643–2.125]	0.649
CC	29 (14.5)	29 (16.1)	1 (reference)	
T allele	252 (63.0)	225 (62.5)	1.022 [0.761–1.371]	0.94
C allele	148 (37.0)	135 (37.5)	1 (reference)	
Glu298Asp				
GluGlu	142 (71.0)	110 (61.1)	2.869 [1.257–6.547]	0.011
GluAsp	49 (24.5)	50 (27.8)	2.178 [0.903–5.250]	0.092
AspAsp	9 (4.5)	20 (11.1)	1 (reference)	
Glu allele	333 (83.3)	270 (75.0)	1.657 [1.162–2.362]	0.005
Asp allele	67 (16.7)	90 (25.0)	1 (reference)	

Results are expressed as numbers with percentages in parentheses; figures in square brackets are 95% CI. The p value was calculated for difference among groups by the χ^2 test.

¹ The OR was referred to 'bb' to 'ba + aa'.

portant to understand the role of eNOS polymorphism in Chinese preeclamptic women. However, so far, only one study from Taiwan reported the relationship between eNOS polymorphisms with preeclampsia in a Chinese population. To further confirm and elaborate this related study, we chose only Han Chinese from mainland China

to repeat part of this similar study; in addition, we added one more single-nucleotide polymorphism (T-786C) and examined the plasma NO concentration in each participant as well. The single locus analysis among the three common variants of the eNOS gene (4b/a, T-786C and Glu894Asp) has revealed that, in a Han Chinese popula-

tion from mainland China, 4b/a and Glu894Asp have a possible association with preeclampsia, whereas T-786C is not associated with this complication, which is consistent with the previous study from Taiwan.

Confusingly, some studies [10, 27–32] have reported a positive association between preeclampsia and the variants of the eNOS gene, whereas the two already published meta-analyses and some other individual studies did not report conformity in the replication of the findings [14, 17, 33–35]. Thus, the current evidence available from the candidate gene approach cannot convince us to accept the view of a major contributory role of eNOS variants in the pathogenesis of preeclampsia.

Preeclampsia is a multifactorial and polygenic disorder. The precise etiology of preeclampsia has yet to be determined, but numerous and extensive analyses in transgenic animal models and in genome-wide human studies have been performed to investigate the relation between environmental and genetic factors of this disorder. Some of these studies focusing on the important role of NO in vascular function have emphasized the relation between eNOS gene polymorphisms and preeclampsia as a vascular disorder. Much attention has been focused on the three of them, namely the T-786C polymorphism in promoter, 4a/b polymorphism in intron 4 and the Glu298Asp polymorphism in exon 7 that may reduce NO bioactivity [24, 36]. In the current study, we examined the plasma NO level in each participant and classified NO levels based on genotypes, by which some novel findings are reported: 2 eNOS polymorphisms (intron 4b/a and Glu894Asp) affect NO formation in both preeclamptic women and healthy women, in whom the 894Asp allele and intron 4a allele are associated with

higher nitrite concentrations, and this is consistent with our genotype analysis that the 894Asp allele and intron 4a are associated with a lower risk of preeclampsia. Our result is partially different from the study of Sandrim et al. [24], which may be due to the different ethnic populations examined.

In conclusion, the present genetic association study replicates a significant association of the eNOS variants with preeclampsia in a Han Chinese population, and these genetic studies confirm that a decreased eNOS activity may be involved in the pathogenesis and development of preeclampsia via its impact on NO production. The results of the present individual gene association study should be interpreted with caution since the participant sample was not large enough. However, preeclampsia is a complex complication with multifaceted etiology and therefore, a contributory pathogenetic role of eNOS variants in the synergistic effect with other genetic or environmental factors seems very impossible [37]. Further collaborative research in preeclampsia may help in elucidating the contributory role of eNOS variants by performing genetic association studies and genome-wide association studies with adequate credibility.

Acknowledgment

We appreciate the funding from Wenzhou Medical University affiliated No. 1 Hospital to support this project.

Disclosure Statement

We declare that all the authors have no conflict of interest.

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