Contribution of *TIMP3* polymorphisms to the development of preeclampsia in Han Chinese women

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Abstract

Purpose The aim of this study was to investigate whether polymorphisms in the tissue inhibitor of metalloproteinase 3 gene (*TIMP3*) are associated with the risk of preeclampsia (PE) in Han Chinese women.

Methods Nine single *TIMP3* tag-single nucleotide polymorphisms were selected by Haploview and genotyped using the Sequenom method in 181 preeclamptic and 203 healthy pregnant women from eastern China.

Results The allele frequencies of the tag-single nucleotide polymorphisms were not significantly different between groups ($P>0.05$). However, the genotype distribution of rs135025 was shown to differ between the multigravidity PE subgroup (>3) and controls under additive ($P=0.018$) and recessive models

($P=0.008$), while the genotype distribution of rs80272 differed significantly between the severe PE subgroup and controls under additive ($P=0.014$) and dominant models ($P=0.041$). Moreover, the H2 haplotype (A-C-G-T-A-A-G-C-G) was found to be associated with the risk of PE ($P=0.035$).

Conclusions Genotypes of rs135025 and rs80272 in *TIMP3* may therefore influence susceptibility to PE, and pregnant women carrying the H2 haplotype might be more prone to developing PE.

Keywords Tissue inhibitors of matrix metalloproteinases 3 · Preeclampsia · Genetic polymorphism · Haplotype analysis

Capsule *TIMP3* rs135025 and rs80272 polymorphisms may influence susceptibility to PE in Han Chinese women.

Changlong Guo and Xiaofang Cao These two authors equally contributed to this work.

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Introduction

Preeclampsia (PE), characterized by de novo hypertension and proteinuria in the mother and frequently, growth deficiency in the fetus, is one of the most common adverse pregnancy outcomes [1]. It occurs in approximately 3–8 % of all pregnancies and has been estimated to account for 63,000 deaths worldwide each year [2–4]. Although the precise etiopathogenesis remains elusive, genetic risk factors are believed to be involved [5]. To date, a variety of candidate genes tested in different ethnic groups have been identified associated with PE. Taking studies in Han Chinese population for example, the immune tolerance gene *Foxp3*, vascular remodeling and angiogenesis related gene *CDH13*, and endothelial dysfunction associated gene *IL1* are all found correlated with the occurrence of PE [6–8]. In general, these susceptibility genes have different potential roles in multiple pathophysiological processes, and alterations of their functions are speculated to contribute to the development of PE.

Tissue inhibitor of metalloproteinase 3 (*TIMP3*), a member of the TIMP family, functions as the antagonist of matrix

metalloproteinases (MMPs) to guard homeostasis and affect physiological tissue remodeling and developmental processes by regulating cell growth, invasion, migration, apoptosis, and angiogenesis [9–11]. It has also been shown to have inhibitory effects on angiogenesis and tumor growth [12, 13]. Moreover, elevated expression of *TIMP3* has been observed in the placenta, making it an appealing PE candidate gene [14]. Additionally, the *TIMP3* promoter was found to be hypomethylated in the PE placenta, suggesting that epigenetic alterations may be associated with reduced trophoblastic invasion [15, 16].

The impact of *TIMP3* polymorphisms on multiple complex diseases such as cancer, spontaneous abortion, diabetic nephropathy, hypertension, and macular degeneration has previously been investigated [12, 17–20]. Although methylation differences in *TIMP3* have been observed between preeclamptic and normal placentas in different ethnic groups as well as within a single ethnic population [15, 16], few studies have focused on the association between *TIMP3* polymorphisms and PE in the Han Chinese population. Therefore, in the present study, we used Haploview software to screen nine tag-single nucleotide polymorphisms (tag-SNPs) of *TIMP3* (rs135025, rs135029, rs137487, rs241890, rs242078, rs5754312, rs715572, rs80272, and rs9609643) and explored their relationship with PE risk in Han Chinese women.

Materials and methods

Study participants

A total of 384 subjects, including 181 preeclamptic and 203 healthy pregnant women, were recruited from the Department of Obstetrics and Gynecology at Shengjing Hospital of China Medical University (Shenyang, Liaoning, China) between October 2012 and June 2013. Ethical approval for the study was granted by the Ethics Committee of the National Research Institute for Family Planning. Written informed consent was obtained from each participant. All women enrolled in the study were from eastern China (Liaoning, Heilongjiang, and Jilin provinces), and there were no variations in the genetic background.

Subjects in the control group were normotensive pregnant women who delivered a healthy neonate at term (37 weeks of gestation) without proteinuria, or antenatal medical or obstetric complications; subjects in the case group were pregnant women (previously normotensive and non-proteinuric) with hypertension and proteinuria (blood pressure values $>140/90$ mmHg on two measurements at least 6 h apart; 24 h urinary protein >0.3 g) after the 20th week of pregnancy [21]. Cases and controls were matched for gestational age. Exclusion criteria were major birth defects, alcoholism, smoking, drug use, and preexisting medical conditions such as chronic hypertension, renal insufficiency, and diabetes. To evaluate the

seriousness of illness, PE patients were subsequently divided into two subgroups: mild PE ($n=97$, two separate blood pressure measurements $\geq 140/90$ mmHg and proteinuria ≥ 0.3 g protein/24 h) and severe PE ($n=84$, at least three separate blood pressure measurements $\geq 160/100$ mmHg combined with proteinuria ≥ 2.0 g protein/24 h). Additionally, based on the onset of disease (before or after 34 gestational weeks), the case group was classified as early-onset or late-onset PE.

SNP selection

Tag-SNPs representing genetic variation in *TIMP3* were selected using Haploview version 4.2 based on HapMap Data (Rel 27 Phase II+III) [22, 23]. The full sequence of human *TIMP3* observed in our study contained 5 kb upstream and 5 kb downstream of the gene, all exons and introns, which were pinpointed to chromosome 22, position 31521802–31594027. SNPs with a minimum allele frequency >0.1 and an r^2 threshold of 0.8 were chosen as candidate tag-SNPs. In the following primer design, three tag-SNPs were excluded for failure to obtain suitable primers. Finally, we included nine tag-SNPs (rs135025, rs135029, rs137487, rs241890, rs242078, rs5754312, rs715572, rs80272, and rs9609643) in our study. Among them, only rs137487 is located near the 3' end of the gene. The other eight SNPs are all located in introns.

DNA extraction and genotyping

Peripheral venous blood samples were collected from subjects and stored at -20°C . Genomic DNA was extracted according to the conventional proteinase K digestion and phenol/chloroform extraction method [24]. Primers used in the study were designed at <https://www.mysequenom.com/Tools> and the genotypes of nine tested SNPs were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [25].

Statistical analysis

General characteristics of the cases and controls were expressed as means $\pm$ SD. Statistical evaluation of the clinical data was performed using Student's *t*-test and the chi-square test. The allele frequency and genotype distribution of the nine SNPs were analyzed for deviation from the Hardy–Weinberg equilibrium using the chi-square test. Differences in the distribution of alleles and genotypes between case-control groups were assessed by the Pearson chi-square test with the Statistical Package for the Social Sciences version 13.0 (SPSS Inc., Chicago, IL). Associations between *TIMP3* polymorphisms and PE risk were estimated by odds ratios (OR) and 95 % confidence intervals (95 % CI). Three genetic models: additive (+/+ vs +/- vs -/-), dominant (+/+ plus +/- vs -/-), and recessive (+/+ vs +/- plus -/-) were applied for further

Table 1 Clinical characteristics [mean (S.D.)] of the study population

Characteristics	Control (N=203)	PE (N=181)	PE	
			MPE (N=97)	SPE (N=84)
Maternal age	29.4±4.3	31±6*	29.42±5.2	31.85±6.1* ^{**}
Primiparas	124(61.1 %)	130(71.8 %) ^{a*}	67(69.1 %) ^a	63(75 %) ^{a*}
SBP (mmHg)	117.8±10.9	164.5±23*	149.5±12.4*	181.5±18.9* ^{**}
DBP (mmHg)	77.5±6.9	104.7±16.2	94.9±8.5*	115.8±14.5* ^{**}
Delivery weeks	39.1±1.0	34.3±3.4*	35.4±2.6*	33.1±3.9* ^{**}
Number of pregnancies	1.5±0.6	1.9±1.2*	1.8±1.2*	2.1±1.2* ^{**}
BMI	23.71±0.06	26.48±0.12*	25.76±0.13*	27.39±0.17* ^{**}
Newborn weight (g)	3359±28.81	2201±64.93*	1974±83.00*	2493±121.3*

SBP systolic blood pressure, DBP diastolic blood pressure, PE preeclampsia, MPE mild preeclampsia, SPE severe preeclampsia, BMI body mass index

^a Differences between discrete variables evaluated with Chi square test

**P* value <0.05 vs control

***P* value <0.05 vs MPE

analysis. Finally, SHEsis software was used to analyze haplotypes [26, 27]. All statistical tests were two sided and *P* values less than 0.05 were interpreted as statistically significant.

Results

Clinical characteristics in the PE and control groups

Table 1 presents the clinical characteristics of cases and controls. Compared with healthy pregnant women, PE patients had a significantly higher maternal age, blood pressure, number of pregnancies, and body mass index (BMI) (*P*<0.05), and a significantly lower gestational age at delivery and fetal weight (*P*<0.05). Except for the difference in fetal weight, a similar phenomenon was also observed between mild and severe PE subgroups (*P*<0.05). Moreover, women who were pregnant with their first child seemed to be at a significantly higher risk of developing PE (*P*<0.05).

Allele and genotype frequencies in the PE and control groups

All case and control allele frequency distributions were in accordance with the Hardy–Weinberg equilibrium (*P*>0.05). Moreover, no significant difference was detected between PE patients and controls in the allele frequency distributions of any selected SNPs (Table S1). In the following genotype distribution analysis, we found that the rs135025 and rs80272 differed significantly between the multigravidity (>3) and severe PE subgroup, respectively, compared with controls. No significant differences were observed in the genotype distributions of the

remaining seven SNPs (Table S2). The genotype frequencies of rs135025 and rs80272 were further analyzed under additive, recessive, and dominant models (Tables 2 and 3). Significant differences were identified between the multigravidity PE subgroup (>3) and controls under both the additive (CC vs CT vs TT: *P*=0.018) and recessive models (CC vs CT+TT: *P*=0.008) for rs135025 (Table 2), while a significant difference was observed between the severe PE subgroup and controls under additive (CC vs CT vs TT: *P*=0.014) and dominant models (CC+ CT vs TT: *P*=0.041) for rs80272 (Table 3).

Table 2 Genotype frequencies of rs135025 in women with and without PE

Group	Number	Frequency (CC/CT/TT)	<i>P</i> _{add}	<i>P</i> _{dom}	<i>P</i> _{rec}
Control	186	41/95/50			
PE	172	36/89/47	0.968	0.925	0.798
PE MPE	92	22/46/24	0.940	0.888	0.726
PE SPE	80	14/43/23	0.702	0.754	0.401
Primigravidity	73	14/34/25	0.497	0.240	0.612
Multigravidity (>1)	99	22/55/22	0.669	0.389	0.972
Multigravidity (>3)	18	9/4/5	0.018*	0.935	0.008*

The C allele is the risk allele; *P*_{add}: *P* value of additive model (three genotypes); *P*_{dom}: *P* value of dominant model [(homozygotes of risk allele+heterozygotes) vs homozygotes of non-risk allele]; *P*_{rec}: *P* value of recessive model [homozygotes of risk allele vs (heterozygotes+homozygotes of non-risk allele)]

PE preeclampsia, MPE mild preeclampsia, SPE severe preeclampsia

*Significant difference vs controls

Table 3 Genotype frequencies of rs80272 in women with and without PE

Group	Number	Frequency (CC/CT/TT)	P_{add}	P_{dom}	P_{rec}
Control	185	5/29/151			
PE	173	3/43/127	0.087	0.062	0.536
PE MPE	92	3/19/70	0.554	0.280	0.794
PE SPE	81	0/24/57	0.0134*	0.041*	0.135
Primigravidity	74	2/17/55	0.379	0.188	1.000
Multigravidity (>1)	99	1/26/72	0.072	0.082	0.345
Multigravidity (>3)	19	0/6/13	0.180	0.168	0.468

The C allele is the risk allele; P_{add} : P value of additive model (three genotypes); P_{dom} : P value of dominant model [(homozygotes of risk allele+heterozygotes) vs homozygotes of non-risk allele]; P_{rec} : P value of recessive model [homozygotes of risk allele vs (heterozygotes+homozygotes of non-risk allele)]

PE preeclampsia, MPE mild preeclampsia, SPE severe preeclampsia

*Significant difference vs controls

Haplotypes and PE risk

SHEsis software was used to analyze haplotypes based on the observed genotypes (Table 4), resulting in the following SNP order: rs135025, rs135029, rs137487, rs241890, rs242078, rs5754312, rs715572, rs80272, and rs9609643. Both in controls and cases, haplotypes with frequencies less than 0.03 were automatically excluded to minimize potential false positive associations. Among these eight constructed haplotypes, the frequency of haplotype H2 (A-C-G-T-A-A-G-C-G) was significantly higher in PE patients (OR=3.669; 95 % CI: 1.014~13.271; $P=0.035$), Table 4, especially in multigravidity (OR=4.642; 95 % CI: 1.215~17.731; $P=0.014$), multiparas (OR=5.961; 95 % CI: 1.382~25.707; $P=0.007$), and severe PE (OR=4.918; 95 % CI: 1.254~19.287; $P=0.012$) subgroups,

compared with controls, suggesting that this haplotype might be associated with the risk of PE.

Discussion

Preeclampsia is currently considered to be a pregnancy-specific syndrome instead of a definite disease, and epidemiological research has indicated that inheritance might play a role in its development [28]. To date, a variety of PE susceptible genes with polymorphisms, have been identified. Sowmya et al. revealed that *IL10* T-819 C gene promoter polymorphism could be a potential genetic regulator in the etiology of PE [29]. The renin–angiotensin system (RAS) variants and gene–gene interactions might affect the risk of PE [30]. And the C allele of -786T/C polymorphism in *eNOS* gene might influence the higher susceptibility to severe PE [31].

TIMP3 is an endogenous regulator that stops the proteolytic activities of MMPs against the extracellular matrix (ECM), affects the ECM physiological turnover, and mediates vascular remodeling [32, 33]. TIMP3 is the only TIMP that binds ECM and exerts tissue-specific effects [34, 35]. Increasing evidence suggests that the balance between TIMPs and MMPs has biological importance, so changes in the relative concentration of these molecules may affect a wide range of pathological situations such as reproduction [36–38]. Furthermore, genetic variation in *TIMP3* has been linked with susceptibility to cardiovascular disorders and hypertension [39, 40], both of which share many risk factors with PE. Therefore, it is rational to speculate that TIMP3 may be involved in the development of PE.

TIMP3 inhibits angiogenesis by blocking the binding of the vascular endothelial growth factor to its receptor.

Table 4 Haplotype frequency estimates and their association with PE

Haplotype	SNPs									Freq ^a	Odds ratio [95 % CI]	P value
	1	2	3	4	5	6	7	8	9			
H1	A	C	G	T	A	A	A	T	G	0.03	0.783 [0.288~2.130]	0.631
H2	A	C	G	T	A	A	G	C	G	0.046	3.669 [1.014~13.271]	0.035*
H3	A	C	G	T	G	A	A	T	G	0.187	0.762 [0.482~1.206]	0.246
H4	A	T	G	C	G	A	G	T	G	0.014	0.422 [0.114~1.558]	0.183
H5	G	C	G	T	G	T	A	T	G	0.038	1.191 [0.444~3.197]	0.728
H6	G	C	G	T	G	T	G	C	G	0.064	0.855 [0.416~1.759]	0.671
H7	G	T	T	C	G	A	G	T	A	0.055	0.943 [0.429~2.071]	0.884
H8	G	T	T	C	G	T	G	T	G	0.385	1.231 [0.824~1.840]	0.309

The SNP order was 1=rs135025, 2=rs135029, 3=rs137487, 4=rs241890, 5=rs242078, 6=rs5754312, 7=rs715572, 8=rs80272, and 9=rs9609643

SNP single nucleotide polymorphism

^a Frequency <0.03 in both control and PE has been ignored in analysis

*Significant difference vs controls

Placentation has been considered a model of angiogenesis [10, 36]; therefore, this interaction could represent a placental angiogenesis defect in PE that contributes to PE complications. Moreover, TIMP3 contains an amino acid sequence (PFG) required to inhibit tumor necrosis factor (TNF)- α converting enzyme, and participates in regulating TNF-dependent systemic inflammation [41, 42]. In the inflammatory response that occurs in PE patients, numerous proinflammatory cytokines, such as TNF- α , are highly expressed as part of an excessive maternal inflammatory response [43, 44]. Therefore, it is possible that TIMP3 is involved in the development of PE through its important role in TNF-dependent inflammation.

In the present study, nine tag-SNPs were selected by Haploview and were tested in a cohort of Han Chinese women. Allele frequency analysis suggested that none of the nine individual SNPs were associated with PE. However, the SNP genotyping study indicated that an increased risk of PE was associated with the C allele of rs135025 in the multigravida PE subgroup under a recessive model of inheritance, and with the C allele of rs80272 in the severe PE subgroup under a dominant model of inheritance. Furthermore, the H2 haplotype (A-C-G-T-A-A-G-C-G), with a frequency of 4.6 % in cases and 1.3 % in controls, was shown to be a risk factor in PE patients.

SNPs in *TIMP3* introns were previously speculated to have effects on mRNA stability and transcription and/or translation efficiency, thus possibly influencing *TIMP3* expression or interfering with its biological properties [45]. Both rs135025 and rs80272 are intronic *TIMP3* polymorphisms, so they may disturb the balance between matrix ECM destruction and formation regulated by the relative concentration of TIMPs/MMPs. This could lead to alterations of homeostatic mechanisms that either control blood pressure or affect immune regulation in the mother, promoting the onset of severe pregnancy-associated disorders such as PE [46–49]. Moreover, severe PE patients suffer from higher blood pressure and higher concentration of proteinuria, in addition to other related complications, all of which might be caused by incomplete trophoblast invasion. Such severe defect might make TIMP3 role in severe PE subgroup so prominent that alterations of TIMP3 caused by its certain polymorphisms could be detected associated with PE risk.

Several limitations of the present study should be noted for future work. First, this study was based on a relatively small sample size, especially for multigravida PE and severe PE subgroups. The risk haplotype is also relatively rare compared with other tested haplotypes. Future studies with larger sample sizes and a higher frequency of risk haplotype are therefore required to confirm our association. Second, genetic factors involved in the development of PE differ by ethnic group; therefore, replication studies of different racial groups would be beneficial. Third, the functional relevance of *TIMP3*

genetic variants is unknown, thus underlying molecular mechanisms need to be explored and elucidated. Finally, PE is a complex trait, so genetic variants may not only exert primary effects but also interact with other genes or environmental factors to contribute to its development.

In conclusion, *TIMP3* SNPs rs135025 and rs80272 appear to be associated with the susceptibility to PE in Han Chinese women. Moreover, pregnant women bearing the H2 haplotype (A-C-G-T-A-A-G-C-G) might be more prone to developing PE, although additional studies are required to confirm and extend our findings.

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Conflict of interest The authors declare that they have no conflict of interest.

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