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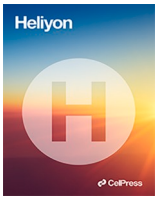

Research article

Polymorphisms in myeloperoxidase and tissue inhibitor of metalloproteinase-1 genes and their association with preeclampsia in the Chinese Han population

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ABSTRACT

Hypertensive disorders of pregnancy (HDP) are multifaceted syndromes unique to pregnancy, characterized by increased blood pressure, edema, and proteinuria. Patients with HDP exhibit signs of endothelial dysfunction, possibly linked to increased myeloperoxidase (MPO) level and aberrant oxidative stress. Additionally, altered level of tissue inhibitor of metalloproteinase-1 (TIMP1) protein is associated with placental ischemia, hypoxia, and maternal vascular endothelial damage. Preeclampsia (PE) represents a critical stage of HDP that poses severe threats to maternal and fetal safety. This study aimed to determine the relationship between MPO and TIMP1 polymorphisms and the risk of PE in the Chinese Han population. Single nucleotide polymorphisms (SNPs), including MPO rs7208693, MPO rs2243828, and TIMP1 rs6609533, were genotyped in 170 patients with PE and 303 control participants. No significant association was observed between MPO polymorphisms (rs7208693 and rs2243828) and the risk of PE, whereas significant association between the TIMP1 rs6609533 A > G SNP and PE susceptibility was found. Specifically, individuals with the GG or AG genotypes had elevated risk of PE compared to those harboring the AA genotype. Furthermore, in the PE group, patients carrying the G allele were more likely to experience fetal growth restriction (FGR). In the non-PE group, the association between the G allele and the risk of FGR was not evident. In conclusion, the TIMP1 rs6609533 G allele in Chinese Han women was identified as a risk factor for PE. Our results indicated that the TIMP1 rs6609533 SNP can serve as a biomarker for the clinical diagnosis and treatment of PE.

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1. Introduction

Hypertensive disorders of pregnancy (HDP) are distinct conditions that can occur during pregnancy. Unlike hypertension associated with obesity, primary hypertension, or diabetes mellitus, HDP present with clinical symptoms such as hypertension, edema, and proteinuria. This condition can progress rapidly during pregnancy, leading to severe complications that threaten maternal and fetal safety [1]. HDP are generally categorized into gestational hypertension, preeclampsia/eclampsia, chronic hypertension complicating pregnancy, and chronic hypertension with superimposed preeclampsia/eclampsia [2]. Of these, preeclampsia (PE) impacts 2 %–8 % of all pregnancies and is one of the leading causes of maternal complications [3]. PE is a leading cause of maternal morbidity and mortality worldwide. It is frequently associated with maternal factors such as age, weight, reproductive history, and ethnicity and exhibits a postpartum self-limiting nature [4]. As a common obstetric complication, PE significantly increases the risk of intrauterine growth retardation, preterm birth, and even intrauterine death [5]. Because PE has emerged as a global public health concern, proactive prevention and treatment are crucial for optimizing maternal and infant outcomes and improving the overall health of patients with PE. Gestational hypertension can be controlled with antihypertensive medication. However, the curative treatment for PE and eclampsia is delivery of the fetus, which may be associated with poorer fetal outcomes [6]. Consequently, it is necessary to elucidate the pathogenesis of PE to improve the diagnosis and treatment of this disease.

Advances in molecular biology have revealed substantial genetic evidence, tentatively establishing a link between gene function disparities and HDP. Single nucleotide polymorphisms (SNPs) represent functional genetic loci prevalent in genes that can induce changes in gene function [7]. Notable variations in the drivers of HDP include mutations in endothelial nitric oxide synthase (eNOS) [8], alpha-ketoglutarate-dependent dioxygenase (FTO)/zinc finger protein 831 (ZNF831) [9], and aberrant DNA methylation [10]. Extensive genetic association analyses have been performed in numerous case–control studies, identifying several susceptibility loci associated with HDP, with PE studies being the most prevalent [11–15]. In clinical practice, SNPs may be used for early detection by combining screening and risk assessment [16,17]. They may also be used in personalized treatment approaches and for predicting treatment outcomes [18,19]. PE is a disease with a strong familial predisposition that also varies according to geographical, socioeconomic, and ethnic factors [20]. A meta-analysis evaluated the overall effect of genetic variants and identified seven variants associated with PE, highlighting the importance of genetic factors in its pathogenesis [21]. However, the identification of mutations and SNPs alone is insufficient to establish their role in the pathogenesis of PE. Therefore, the identification and characterization of additional functional SNPs represent an effective strategy for gaining further insight into the pathological mechanisms underlying PE.

Regarding the pathogenesis of PE-related genes, the roles of myeloperoxidase (MPO) and tissue inhibitors of metalloproteinase (TIMPs) have attracted increased attention. However, related studies are limited. MPO is a heme enzyme primarily produced and released by activated myeloid cells, such as monocytes and neutrophils, and is involved in inflammation and oxidative stress [22]. MPO activity reflects the degree of neutrophil activation, is associated with impaired endothelium-dependent vasodilation, and is becoming a risk factor for cardiovascular diseases [22–24]. A significant increase in circulating and placental level of MPO has been observed in patients with PE [25]. Variations in MPO are strongly correlated with alterations in plasma MPO level and associated with susceptibility to various vascular disorders, including PE [26–28]. Moreover, MPO rs2243828 A > G polymorphism was reported to regulate inflammatory gene expression networks by affecting sex hormone-sensitive elements [29]. TIMPs are a class of small proteins that serve as endogenous inhibitors of matrix metalloproteinases (MMPs) and influence extracellular matrix degradation and remodeling [30]. This important biological function is necessary for placental formation [31]. Genetic variations in TIMP genes alter gene expression and protein production, contributing to the etiology and progression of obstetric and gynecologic diseases [32,33]. Correlation analyses have confirmed a significant association between genetic variations in TIMP genes and an increased risk of cardiovascular diseases [34,35]. The TIMP1 rs6609533 A > G polymorphism has been implicated in multiple diseases associated with extracellular matrix dysregulation, such as conical corneal disease. This indicates that TIMP1 rs6609533 A > G locus variants can be important influencing factors in these diseases by affecting protein expression [36]. Of the three SNPs examined in the present study,

Table 1
Clinical and biochemical characteristics of the study population.

Characteristics	Control(n = 303)	Case(n = 170)	Case/Control	p
BMI(kg/m^2)	20.70(18.83–22.94)	23.03(20.91–26.26)	1.11	**< 0.001**
Maternal age(years)	28.00(26.00–32.00)	33.00(29.00–37.00)	1.18	**< 0.001**
systolic BP(mm Hg)	118.00(112.50–123.00)	141.06(130.89–149.25)	1.20	**< 0.001**
Diastolic BP(mm Hg)	73.00(70.00–77.00)	89.78(82.44–93.92)	1.23	**< 0.001**
Gestational age(weeks)	39.00(38.00–40.00)	35.00(31.00–38.00)	0.90	**< 0.001**
Newborn birth weight(g)	3200.00 (2920.00–3500.00)	2185.00 (1377.50–3098.75)	0.68	**< 0.001**
FGR	5	38	7.60	**< 0.001**
ALB(g/L)	35.70(33.90–37.30)	29.85(26.28–32.63)	0.84	**< 0.001**
AST(U/L)	14.50(12.80–17.30)	18.60(13.90–27.20)	1.28	**< 0.001**
PLT ($\times 10^9$/L)	227.00(191.00–269.00)	207.00(159.50–250.25)	0.91	**< 0.001**
ALT (U/L)	8.40(6.90–11.00)	12.60(7.88–19.95)	1.50	**< 0.001**
Creatinine (mg/dl)	50.00(45.00–57.00)	62.00(53.00–74.00)	1.24	**< 0.001**
serum UA (μmol/L)	323.40(271.00–382.20)	447.50(347.70–534.58)	1.38	**< 0.001**

All parametric data do not meet the parametric test conditions and are expressed as median (interquartile range). BMI, Body mass index; FGR, fetal growth restriction; ALB, albumin; AST, aspartate aminotransferase; PLT, blood platelet; ALT, alanine aminotransferase; UA, uric acid. Case/Control, Ratio of median. Bold, statistically significant difference ($p < 0.05$).

MPO rs7208693 C > A and TIMP1 rs6609533 A > G are polymorphisms causing missense mutations, whereas rs2243828 A > G is a variant in the promoter region that may affect the transcription and stability of mRNAs as well as proteins.

To date, there have been relatively few studies assessing the effect of SNPs in MPO and TIMP1 on PE. In the present study, we enrolled 170 patients diagnosed with PE and 303 healthy pregnant women to determine the correlation between polymorphisms in MPO and TIMP1 and susceptibility to PE. Our findings offer novel insights into the genetic variants associated with PE, which will contribute to its clinical diagnosis and treatment.

2. Results

Clinical and Biochemical Characteristics of the Study Population. A total of 170 patients with PE and 303 women with normal pregnancy were enrolled in the study. The clinical and biochemical characteristics of the study population are summarized in Table 1. Compared with the control group, the PE group exhibited significantly elevated systolic/diastolic blood pressure (SBP/DBP), higher maternal age, and an increased pre-pregnancy body mass index (BMI). Maternal age and BMI were selected as covariates for subsequent risk assessment analysis to estimate the associations between alleles and genotypes of the SNPs and PE. Conversely, gestational age and fetal birth weight were significantly lower in the PE group than in the normal pregnancy group. The prevalence of fetal growth restriction (FGR) was 5 out of 303 (1.7 %) for women without PE, which was significantly lower than 38 out of 170 (22.4 %) for the PE cohort. Level of biochemical markers of PE, including serum creatinine, uric acid indicators, alanine aminotransferase, and aspartate aminotransferase, was higher, whereas blood platelet count and albumin level was lower compared with the normal pregnancy group.

Association between SNPs and PE Risk. The genotyping of MPO and TIMP1 SNPs was conducted in 170 patients with PE and 303 control individuals. The distribution of the three polymorphisms adhered to the Hardy–Weinberg equilibrium (all $p > 0.05$) (Table 2). The occurrence of PE is highly correlated with maternal age and BMI, which was also demonstrated in the present study (33.00 [29.00–37.00] vs. 28.00 [26.00–32.00] years for maternal age [$p < 0.001$] and 23.03 [20.91–26.26] vs. 20.70 [18.83–22.94] for BMI [$p < 0.001$]). The frequencies of MPO rs2243828, MPO rs7208693, and TIMP1 rs6609533 and their associations with PE susceptibility were listed in Table 2. Risk analysis revealed that neither rs7208693 nor rs2243828 showed a significant association with differences in susceptibility to PE in codominant, dominant, or recessive genetic models or in allele frequency distribution (Table 2).

The TIMP1 rs6609533 genotype showed a significant association with PE susceptibility. In the PE group, the number of AA, AG, and GG genotypes for the rs6609533 A > G polymorphism was 7 (4.1 %), 40 (23.5 %), and 123 (72.4 %), respectively, compared to 69

Table 2
Genotype distribution of the MPO and TIMP1 gene polymorphisms in PE patients and healthy controls.

Gene type	Control (n = 303)(%)	Case (n = 170)(%)	Crude OR (95 % CI)	p value	Adjusted OR (95 % CI)[a]	adj.p.val
rs7208693 C > A (HWE = 0.413)						
Codominant						
CC	224(73.9)	119(70.0)	1.00		1.00	
CA	75(24.8)	48(28.2)	1.21(0.79–1.84)	0.390	1.13(0.70–1.84)	0.612
AA	4(1.3)	3(1.8)	1.41(0.31–6.41)	0.655	0.571(0.10–3.14)	0.519
Dominant	79(26.1)	51(30.0)	0.82(0.54–1.25)	0.359	0.92(0.57–1.47)	0.722
Recessive	299(98.7)	167(98.2)	0.75(0.17–3.37)	0.702	1.82(0.33–9.94)	0.492
Allele						
C	523(86.3)	286(84.1)	1.00		1.00	
A	83(13.7)	54(15.9)	1.19(0.82–1.73)		1.03(0.68–1.58)	0.887
rs2243828 A > G (HWE = 0.518)						
Codominant						
AA	233(76.9)	122(71.8)	1.00		1.00	
AG	64(21.1)	44(25.9)	1.31(0.84–2.04)	0.227	1.51(0.91–2.50)	0.112
GG	6(2.0)	4(2.4)	1.27(0.35–4.60)	0.712	1.06(0.23–5.00)	0.938
Dominant	70(23.1)	48(28.2)	1.31(0.85–2.01)	0.216	1.47(0.90–2.39)	0.126
Recessive	297(98.0)	166(97.6)	1.19(0.33–4.49)	0.787	0.96(0.21–4.49)	0.963
Allele						
A	530(87.5)	288(84.7)	1.000		1.00	
G	76(12.5)	52(15.3)	1.26(0.86–1.84)	0.236	1.35(0.88–2.09)	0.174
rs6609533 A > G (HWE = 0.842)						
Codominant						
AA	69(22.8)	7(4.1)	1.00		1.00	
AG	153(50.5)	40(23.5)	2.58(1.10–6.04)	**0.029**	2.84(1.15–7.04)	**0.024**
GG	81(26.7)	123(72.4)	14.97(6.55–34.21)	**< 0.001**	15.42(6.35–37.47)	**< 0.001**
Dominant	234(77.2)	163(95.9)	6.87(3.08–15.32)	**< 0.001**	7.32(3.10–17.29)	**< 0.001**
Recessive	222(73.3)	47(28.8)	7.17(4.71–10.93)	**< 0.001**	6.87(4.28–11.01)	**< 0.001**
Allele						
A	291(48.0)	54(15.9)	1.00		1.00	
G	315(52.0)	286(84.1)	4.89(3.51–6.82)	**< 0.001**	4.83(3.35–6.97)	**< 0.001**

rs7208693 C > A C, wild gene, A, mutant gene; rs2243828 A > G A, wild gene, G, mutant gene; rs6609533 A > G A, wild gene, G, mutant gene; OR, odds ratio; 95 % CI, 95 % confidence interval; HWE, Hardy-Weinberg equilibrium.

[a] Adjusted for age and BMI; adj.p.val, adjusted p value; Bold, statistically significant difference ($p < 0.05$).

Table 3
Effect of TIMP1 rs6609533 A > G gene polymorphisms on PE complicated FGR.

Disease state	Genotype(%)			Genotype(Codominant)				Genotype(Dominant)		Genotype(Recessive)		Allies	
	AA	AG	GG	AG VS. AA		GG VS. AA		AG/GG VS. AA		GG VS. AA/AG		G VS. A	
				Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p	Crude OR (95% CI)	p
All patients (PE and Non-PE)													
Non-FGR	73(96.1)	189(97.9 %)	168(82.4 %)	1.00		1.00		1.00		1.00		1.00	
FGR	3(3.9 %)	4(2.1 %)	36(17.6 %)	0.52 (0.11–2.36)	0.393	5.21 (1.56–17.48)	**0.007**	2.73 (0.82–9.05)	0.101	8.02 (3.49–18.4)	**< 0.001**	4.85 (2.47–9.51)	**< 0.001**
Subgroup 1(Non-PE)													
Non-FGR	67(97.1 %)	153(100.0 %)	78(96.3 %)	1.00		1.00		1.00		1.00		1.00	
FGR	2(2.9 %)	0(0.0 %)	3(3.7 %)	0.00 (0.00–0.00)	0.996	0.00 (0.00–0.00)	0.785	0.44 (0.07–2.66)	0.367	4.23 (0.69–25.7)	0.118	1.39 (0.39–4.99)	0.610
Subgroup 2(PE)													
Non-FGR	6(85.7 %)	36(90.0 %)	90(73.2 %)	1.00		1.00		1.00		1.00		1.00	
FGR	1(14.3 %)	4(10.0 %)	33(26.8 %)	0.67 (0.06–7.03)	0.736	2.20 (0.26–18.97)	0.437	1.76 (0.21–15.10)	0.605	3.08 (1.12–8.45)	**0.029**	2.59 (1.06–6.32)	**0.036**

rs6609533 A > G A, wild gene, G, mutant gene; PE, preeclampsia; Non-PE, No-preeclampsia; FGR, fetal growth restriction; Non-FGR, No fetal growth restriction. Bold, statistically significant difference ($p < 0.05$).

(22.8 %), 153 (50.5 %), and 81 (26.7 %) in the normal pregnancy group. The statistical analysis results showed that there were significant association between rs6609533 GG and AG genotypes and PE risk (GG/AA: adjusted odds ratio [OR] = 15.42, 95 % confidence interval [CI] = 6.35–37.47, adjusted $p < 0.001$; AG/AA: adjusted OR = 2.84, 95 % CI = 1.15–7.04, adjusted $p = 0.024$) (Table 2). However, the association of the GG genotype with a high risk of PE should be interpreted cautiously due to the relatively small sample size, necessitating confirmation with larger studies. Furthermore, in both dominant and recessive genetic models, the TIMP1 rs6609533 A > G polymorphism was significantly associated with PE susceptibility (dominant model: AG/GG vs. AA, adjusted OR = 7.32, 95 % CI = 3.10–17.29, adjusted $p < 0.001$; recessive model: GG vs. AG/AA, adjusted OR = 6.87, 95 % CI = 4.28–11.01, adjusted $p < 0.001$) (Table 2). In addition, allelic frequency analysis revealed that the G allele was associated with increased PE susceptibility compared with the A allele (adjusted OR = 4.83, 95 % CI = 3.35–6.97, adjusted $p < 0.001$) (Table 2). These findings indicated that the G allele at the rs6609533 locus was associated with an increased risk of PE and may be a potentially detrimental factor that contributing to its occurrence.

Promotion of FGR Risk by TIMP1 rs6609533 is D*ependent on PE progression.* The incidence of FGR was significantly higher in the PE group than in the normal pregnancy group (38/170 [22.4 %] vs. 5/303 [1.7 %], $p < 0.001$) (Table 1). Although there is evidence supporting a pathogenic role of TIMP1 in PE, some studies indicate that TIMP1 is essential for normal fetal development and growth in utero and may exacerbate FGR. This suggests that inadequate TIMP1 infiltration and function in the trophoblast invasion process can lead to dysregulation and directly contribute to FGR development [31]. However, one unclear aspect of the current study was whether TIMP1 played an indirect role through PE or a direct role in the development of FGR, in which G of TIMP1 rs6609533 was associated with an increased susceptibility to PE, leading to uteroplacental circulatory dysplasia; this would indirectly affect the main pathophysiological mechanisms of FGR as directly ascribed to rs6609533 SNP. Therefore, it was necessary to perform a stratified analysis of FGR in PE.

The results indicated that, in all participants, those with the GG genotype exhibited a significantly elevated risk of FGR compared with those harboring the AA genotype (OR = 5.21, 95 % CI = 1.56–17.48, $p = 0.007$) (Table 3). In the recessive model, individuals carrying the GG genotype had an even higher risk of FGR compared with carriers of the AA/AG genotype (OR = 8.02, 95 % CI = 3.49–18.4, $p < 0.001$) (Table 3). Moreover, the G allele in the allele model was significantly associated with an increased risk of FGR compared with the A allele (OR = 4.85, 95 % CI = 2.47–9.51, $p < 0.001$) (Table 3). Furthermore, all participants were further categorized into non-PE (subgroup 1) and PE (subgroup 2) groups to determine the association between TIMP1 rs6609533 and the risk of FGR. The results showed no significant association between TIMP1 rs6609533 A > G polymorphism and the risk of FGR in the non-PE group. In contrast, in the PE group (subgroup 2), TIMP1 rs6609533 in the recessive model and allele frequency distribution exhibited significant differences between the FGR and non-FGR groups (recessive model: GG vs. AA/AG, OR = 3.08, 95 % CI = 1.12–8.45, $p = 0.029$; allele frequency distribution: G vs. A, OR = 2.59, 95 % CI = 1.06–6.32, $p = 0.036$) (Table 3).

Overall, these results indicated that the rs6609533 A > G genotype was associated with an increased risk of PE, but did not directly affect the risk of FGR. We hypothesized that the rs6609533 G allele may be associated with an increased risk of FGR by potentially disrupting normal fetal growth and development, thereby promoting PE progression.

Effect of rs6609533 *A > G on Gene Transcription.* To confirm the functional relevance of rs6609533 A > G in the expression of TIMP1, cis-expression quantitative trait loci (eQTL) analysis was performed using GTEx data to estimate its effect on TIMP1 gene transcription. The results indicated that the rs6609533 G allele significantly increased TIMP1 mRNA level in brain tissue ($p = 3.27 \times 10^{-5}$) (Fig. 1). This indicated that the rs6609533 G allele may increase the risk of PE by promoting TIMP1 expression.

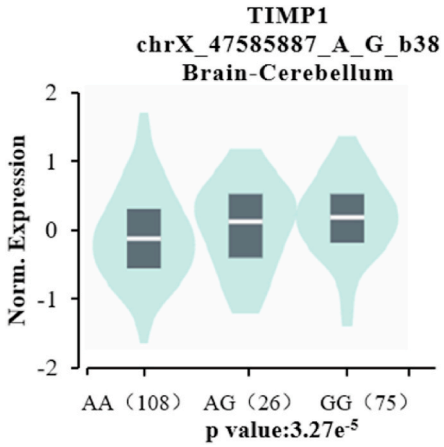

Fig. 1. Genotype-based mRNA expression of TIMP1 gene rs6609533 polymorphisms in brain tissue ($p = 3.27 \times 10^{-5}$) via the GTEx portal database. Y-axis indicates the expression of the TIMP1 gene after normalization. The p-value was corrected with the Bonferroni method to eliminate the effects of multiple tests.

3. Discussion

The diagnostic criterion for blood pressure in patients with PE is SBP/DBP ≥140/90 mmHg; however, in this study, the SBP ranged from 130.89 to 149.25 mmHg and DBP from 82.44 to 93.92 mmHg within the interquartile spacing of the PE group. In the diagnostic criteria, either SBP or DBP higher than the baseline is defined as hypertension. However, in actual clinical diagnosis, there are cases where only high SBP or high DBP occurs, leading to the blood pressure statistics in Table 1. Therefore, the conclusions of this paper did not contradict the diagnostic requirements.

Numerous studies have confirmed the critical of MPO and TIMPs in vascular diseases. A previous study reported a significant increase in serum MPO level among individuals with hypertension compared with normal individuals [37]. Moreover, abnormally elevated level of plasma MPO protein has been observed in patients with PE, where it reduces nitric oxide (NO) bioavailability and contributes to endothelial dysfunction [38]. One study found that PE is associated with an increase in advanced oxidative protein products, which is considered oxidative damage; however, no changes in MPO were observed [39]. This result is similar to another study's findings, where it was speculated that the increase in maternal circulating MPO level may result from increased systemic inflammation due to an identified disease, rather than being a major pathophysiological factor. This observation may clarify why MPO level is not always increased in patients with PE [40]. The results from a cohort study confirmed a substantial increase in serum TIMP1 level in hypertensive patients compared with the control group [41], with a positive correlation observed between TIMP1 and the severity of HDP. This association may be attributed to its impact on the balance of MMPs/TIMPs, suppression of trophoblast invasion capability, and involvement in uterine artery remodeling. Consequently, this interaction may lead to vascular dysfunction in the fetal/placental system, thereby triggering maternal hypertension [42,43]. In a study involving Uyghurs, where the association of MMP9 and TIMP1 with PE was analyzed, no significant differences in placental TIMP1 level were observed between the groups, possibly due to the complex regulation of MMPs/TIMPs [44]. We did not further confirm the differences in the protein expression level of MPO and TIMP1 between the PE and normal populations. Genetic polymorphisms, which are the most common genetic variations, have shown a significant association with the risk of developing PE [28,30]. However, few studies have examined the impact of key SNP sites within MPO and TIMP1 on PE risk. Based on this analysis, we aimed to determine the association between the aforementioned SNP sites and PE susceptibility.

In addition to functional SNP studies, several investigations have examined the correlation between MPO gene polymorphisms and disease susceptibility, such as essential hypertension and coronary artery disease [27,45]. MPO gene polymorphisms also serve as risk factors for pregnancy-related conditions, such as MPO rs2333227 G > A, which is associated with an increased risk of gestational diabetes and PE among Chinese women [28,46]. However, a study that genotyped oxidative stress-related genes in 121 patients with PE and 214 normal pregnant women in the Korean population revealed that the genetic variation of MPO rs2333227 G > A was not associated with individual differences in susceptibility to PE [47]. This indicates that even when studying genetic polymorphisms at the same locus across different populations, different results may be obtained. The effects of genetic polymorphisms on individuals show significant regional and ethnic differences. Furthermore, in a previous study involving patients with acute kidney injury, carriers of minor alleles for rs7208693 and rs2243828 in MPO exhibit higher plasma MPO level, which correlated with subsequent oxidative stress and kidney injury [48]. However, in the present study, we did not observe a significant association between MPO SNPs (rs7208693 and rs2243828) and PE risk (all $p > 0.05$) (Table 2). Our cohort analysis involving 170 patients with PE and 303 control participants confirmed that MPO SNPs (rs7208693 and rs2243828) were not associated with PE susceptibility in Han Chinese pregnant women. These results indicated that different genetic polymorphic sites in MPO exhibit diverse biological functions and that their effects on susceptibility to different diseases vary.

Previous studies have analyzed the potential relationship between key SNP sites in TIMP1 and susceptibility to multiple pathological states. Specifically, the TIMP1 rs2070584 G allele and GG genotype have been found to be associated with PE and the response to antihypertensive therapy [49]. Another important TIMP1 SNP rs4898 exhibits a significant association with the pathological progression of PE. Specifically, the TIMP1 rs4898 C allele is associated with an increased risk of early-onset PE. Carriers of the CC genotype tends to have lower placental weight [50]. In addition, TIMP1 rs6609533, the focus of the present study, has been implicated in the development of specific diseases. The intronic SNP rs6609533 A allele is associated with an increased risk of chronic obstructive pulmonary disease susceptibility [51]. Moreover, the rs6609533 G allele significantly increases the risk of early aseptic loosening and may serve as a genetic marker for its diagnosis [52].

Our findings indicated that the TIMP-1 rs6609533 A > G polymorphism was associated with susceptibility to PE, consistent with the findings of previous studies on essential hypertension or intracerebral hemorrhage [53,54]. This suggested that the rs6609533 A > G was a risk factor for various diseases, including PE. In the present study, both GG and AG genotypes were significantly associated with an increased risk of PE (GG, OR = 15.42, $p < 0.001$; AG, OR = 2.84, $p = 0.024$) (Table 2). In addition, the frequency of the TIMP1 G allele in the PE group was significantly higher than that in the control group ($p < 0.001$) (Table 2). This indicated that the G allele was associated with an increased risk of PE in pregnant women. Further analyses, adjusting for pre-pregnancy BMI and age, revealed a significant correlation between the occurrence of PE and TIMP1(Table 2). Overall, the GG and AG genotypes influenced the risk of PE independently of well-established risk factors such as pre-pregnancy rs6609533 BMI and age. The GG and AG genotypes would have the potential to serve as biomarkers for assessing the risk of PE.

In conclusion, the present study revealed an association between the TIMP1 rs6609533 A > G polymorphism and susceptibility to PE in a Chinese pregnant population. Our data indicated that the TIMP1 rs6609533 G allele and GG and AG genotypes may be linked to an increased risk of PE. Gene expression predictions for rs6609533 A > G revealed that the G allele was associated with increased TIMP1 mRNA level in brain tissue ($p = 3.27 \times 10^{-5}$) (Fig. 1). In addition, abnormally elevated level of TIMP1 protein is closely associated with the development of PE [53–55]. Therefore, there was reason to speculate that the TIMP1 G allele may exert pathogenic

effects in PE by upregulating TIMP1 mRNA and protein.

This study had some limitations, and improvements could be made in three aspects. First, the study included a relatively limited number of pregnant samples, and larger samples are needed to confirm the association between MPO and TIMP1 SNPs and PE susceptibility. Furthermore, protein expression level was not measured; thus, the effect of genotype on expression level was not determined. Second, the factors influencing PE risk extend beyond genetic variation and include environmental factors and various other risk factors. Analyzing a combination of multiple risk factors and genetic variations may provide a more accurate assessment of the impact of TIMP1 rs6609533 on PE risk. Finally, we focused exclusively on the Chinese Han population. Therefore, the conclusion that the rs6609533 G allele was associated with increased PE pathogenicity may not apply to other ethnic groups. Additional risk analyses are needed to determine the relationship between TIMP1 rs6609533 and PE susceptibility in patients from diverse ethnic backgrounds.

4. Materials and methods

Study Cohort. This study was approved by the institutional review board of the Third Affiliated Hospital of Guangzhou Medical University. Written informed consent was obtained from the patients or their guardians. A total of 170 patients with PE and 303 control participants were recruited among pregnant women who visited the Third Affiliated Hospital of Guangzhou Medical University from January 2016 to December 2021. All participants were Han Chinese women. The clinical diagnosis of PE in the case group followed the ACOG criteria (SBP/DBP $\geq$140/90 mmHg after the 20th week of pregnancy, plus proteinuria $\geq$300 mg per 24 h urine collection or $\geq$ 1+ urine dipstick; in the absence of proteinuria, hypertension in pregnancy with any of the following features: pulmonary edema, platelet count $\leq 10 \times 10^{10}$/L, impaired liver function, SBP/DBP $\geq$160/110 mmHg, renal failure and visual disturbances) [3]. Normotensive pregnant women without a history of chronic hypertension and complications during pregnancy were defined as the control population. Women with infectious diseases, severe organic or systemic diseases, essential chronic hypertension, pregnancy complications or complications, and multiple pregnancies, were excluded from both the case and control groups. Clinical characteristics and biochemical indicators of all participants were collected for stratified analysis. Blood and urine samples were analyzed before any medication or other special treatment was administered. The age range of all patients with PE and control group pregnant women was 18–50 years. The sampling of the control and case groups was nonprobabilistic, continuous, and random.

Polymorphism Selection and Genotyping. We used the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), the online tool SNPinfo (http://snpinfo.niehs.nih.gov/), and LDlink (https://ldlink.nci.nih.gov/) to identify putative functional SNPs for MPO and TIMP1 and to establish the wild genotype or allele as a reference index. The 1000 Genomes Project (https://www.ncbi.nlm.nih.gov/bioproject/28889) was used to determine the frequencies of MPO and TIMP1 variants in the Han population of southern China. The SNP selection criteria were as follows: (1) minor allele frequencies (MAFs) $>$ 5 % in Han Chinese participants as reported in HapMap; (2) SNPs predicted to be functional variations by SNPinfo; and(3) SNPs with low-linkage disequilibrium ($R^2 < 0.8$) with each another. Finally, we selected MPO rs7208693 C $>$ A (MAF = 0.1179), MPO rs2243828 A $>$ G (MAF = 0.2319), and TIMP1 rs6609533 A $>$ G (MAF = 0.4764) for further study, in which rs2243828 is a variant in the promoter region and rs7208693 and rs6609533 are missense mutations. According to the 1000 Genomes Project, the allele frequencies in the Han population of southern China were as follows: for MPO rs7208693 C $>$ A, the frequencies were 0.876 for C and 0.124 for A; for MPO rs2243828 A $>$ G, the frequencies were 0.841 for A and 0.159 for G; and for TIMP1 rs6609533 A $>$ G, the frequencies were 0.524 for A and 0.476 for G.

DNA Extraction and Genotype Identification. Maternal peripheral venous blood samples (200 μL) were collected and preserved at −80 °C until analysis. DNA extraction was performed in accordance with the manufacturer's protocol using the TIANamp Blood DNA Kit (TianGen Biotech, Beijing, China).Following the screening of qualified DNA, TaqMan® SNP genotyping assays (Applied Biosystems) and TaqPath ProAm Master Mix were used for the SNP genotyping assay. PCR amplification conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Standard positive and negative samples from the manufacturer were used as references to avoid false-positive and false-negative results. In addition, 5 % of the samples were randomly selected and amplified to ensure reliability, and the retest results were completely consistent with the original experimental data.

Statistical Analysis. The Kolmogorov–Smirnov test was used to evaluate the normality of data for clinical and biochemical characteristics. The characteristics of the case and control groups were determined by applying a *t*-test for quantitative data that met normality and variance assumptions. The nonparametric rank-sum test was used for data that did not meet these assumptions, whereas the chi-square test was used for qualitative data. The Hardy–Weinberg equilibrium test (HWE) was used to calculate allele frequencies in the control group using the chi-square test. ORs and 95 % CIs were calculated via binary logistic regression analysis to evaluate the effect of each polymorphism on PE susceptibility, adjusting for age and BMI. Influence analyses of the FGR and non-FGR groups based on genotype, as well as haplotype analyses, were performed using binary logistic regression analyses. SPSS (Version 27; IBM Corp., Armonk, NY, USA) was used for statistical analysis, with statistical significance set at $p < 0.05$.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Third Affiliated Hospital of Guangzhou Medical University on August 8, 2014 (REC ref. No. 2014 (085)).

Informed consent statement

All participants or their guardians provided written informed consent before the study.

Data availability statement

The datasets analyzed during the current study are available from the corresponding authors upon reasonable request.

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CRediT authorship contribution statement

Liu Li: Writing – original draft, Formal analysis, Data curation. **Dong He:** Writing – review & editing, Validation, Methodology. **Weilin Zhou:** Visualization, Software, Investigation. **Zhiyang Guo:** Visualization, Software, Investigation. **Ma Yue:** Visualization, Software, Investigation. **Lingjie Liu:** Visualization, Software, Investigation. **Hong He:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Shuqi He:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Huang Yi:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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