Effects of GSTP1 and GPX1 Polymorphisms on the Risk of Preeclampsia in Chinese Han Women

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Key Words
Preeclampsia • GSTP1 • GPX1 • Polymorphism • Chinese

Abstract
Background/Aims: Increasing evidence shows that oxidative stress plays an important part in the pathophysiological mechanisms of preeclampsia (PE). Polymorphic variants of oxidative stress-related candidate genes GST1 and GPX1 can affect the antioxidant activities of their encoded enzymes. Therefore, this study aimed to explore the associational analysis between GSTP1 and GPX1 single nucleotide polymorphisms (SNPs) and susceptibility to PE in Chinese Han women. Methods: DNA from 1130 PE patients and 1226 healthy individuals was genotyped for SNPs rs1695 in GSTP1 and rs1050450 in GPX1 using a predesigned TaqMan SNP genotyping assay. The χ² test compared differences in genetic distributions between the two groups in a case–control study. Results: No significant differences in allelic or genotypic frequencies of GSTP1 rs1695 or GPX1 rs1050450 were detected between cases and controls (GSTP1 rs1695: χ²=1.122, p=0.571 by genotype, χ²=0.138, p=0.710, odds ratio=1.027, 95% confidence interval 0.892–1.183 by allele; GPX1 rs1050450: χ²=0.036, p=0.982 by genotype, χ²=0.002, p=0.960, odds ratio=1.005, 95% confidence interval 0.822–1.229 by allele). Moreover, no significant differences in genetic distribution were found between early/late-onset PE or mild/severe PE and control subgroups. Conclusion: Our results suggest that GSTP1 rs1695 and GPX1 rs1050450 SNPs have no effects on the risk of PE in the Chinese Han population. However, these results should be confirmed by replication in different populations.

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Introduction

Preeclampsia (PE) is a dangerous gestation-specific clinical syndrome characterized by new-onset hypertension and proteinuria that develops after the 20th week of gestation [1-3]. It affects approximately 2%–10% of all pregnant women worldwide, and is one of the leading causes of maternal and perinatal mortality and morbidity, especially in developing countries [4, 5]. Increasing evidence has shown that endothelial cell dysfunction and damage play an important role in the pathophysiological mechanisms of PE [2, 6-8].

During normal gestation, reactive oxygen species (ROS) generation is increased with the increase of oxygen levels resulting from established maternal intraplacental circulation at the end of the first trimester [9, 10]. The placenta must adapt to this increased ROS level to enable normal fetal development [11, 12]. However, increased ROS exposure can also result in lipid peroxidation, protein carboxylation, and DNA oxidation, leading to the intravascular inflammatory responses and endothelial cell dysfunction observed in PE patients [10, 13]. Normally, the effect of ROS can be counterbalanced by that of antioxidants such as glutathione, vitamins C and E, and some enzymes, including glutathione peroxidases (GPxs), glutathione S-transferases (GSTs), and superoxide dismutase [14, 15]. When this balance between the formation of ROS and intrinsic antioxidant defense mechanisms is upset, oxidative stress arises, which is thought to be behind the pathophysiological mechanisms of PE [16, 17].

GSTs can detoxify multiple hazardous substances, such as ROS, by catalyzing the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification [18]. GSTs are encoded by the GST family of genes located on different chromosomes, and can be classified into three groups: cytosolic, mitochondrial, and microsomal [19]. GSTP1 encodes one of the four known main classes of cytosolic GSTs, and is located on human chromosome 11q13. Its single nucleotide polymorphism (SNP) within exon 5, Ile105Val (rs1695), leads to the exchange of valine and isoleucine which results in decreased enzymatic activity of the protein [20, 21].

GPxs can reduce free hydrogen peroxide to water using GSH as an obligate cosubstrate to protect the organism from oxidative damage [22]. GPxs are encoded by the GPx phylogenetically related family of genes located on different chromosomes. Human GPx-1 is located on human chromosome 3p21, and has a common SNP (rs1050450) involving the substitution of leucine for proline at amino acid 198 resulting from a C→T change. Many studies have suggested that this SNP affects GPx-1 activity [23, 24].

Because polymorphic variants of GST and GPX can affect the antioxidant activities of the enzymes they encode, we hypothesized that polymorphic loci might contribute to individual differences in susceptibility to PE. Therefore, we estimated the effects of GPX1 rs1050450 and GSTP1 rs1695 on the risk of PE in a sample of 2356 women living in China with the aim of identifying potential prognostic or predictive tools for women at risk of PE.

Materials and Methods

Subjects

This case-control study enrolled 1130 PE patients and 1226 control subjects recruited from the Affiliated Hospital of Qingdao University, Linyi People's Hospital, LiaoCheng People's Hospital, and Zaozhuang Municipal Hospital between January 2013 and November 2015. All patients provided their informed written consent and the study was approved by the Ethical Committees of the Affiliated Hospital of Qingdao University in accordance with the Code of Ethics of the Declaration of Helsinki.

The following data on each subject were recorded: age, body height, body weight, age of menarche, systolic and diastolic blood pressure, and gestational age (at admission and at delivery). All pregnant women underwent serum and urine specimen analysis before labor.

The diagnostic basis for PE was new-onset hypertension (≥140/90 mmHg) and proteinuria (≥0.3 g/24 h, or ≥1+ by dipstick) that developed after the 20th week of gestation [25]. Some accompanying symptoms were permitted as part of the diagnosis, including upper abdominal discomfort, headache, and blurred
vision. Based on guidelines from the American College of Obstetricians and Gynecologists [26], PE patients were divided into mild and severe groups. Because early-onset PE patients are more severely affected than those with late-onset PE [27, 28], patients in this study were also divided into an early-onset PE subgroup diagnosed before the 34th week of gestation, and a late-onset PE subgroup diagnosed at or after the 34th week of gestation. No individuals with PE had a previous history of PE or systemic diseases such as chronic hypertension, heart disease, diabetes mellitus, thyroid function disorder, kidney disorders, hepatic diseases, blood transfusion, or immunotherapy.

Inclusion criteria for controls were as follows: 1) age ≥26 years; 2) gestational age at admission ≥30 weeks; 3) no previous history of PE or systemic diseases such as chronic hypertension, heart disease, diabetes mellitus, thyroid function disorder, kidney disorders, hepatic diseases, blood transfusion, or immunotherapy; and 4) no obstetric complications. All controls were followed up and none of them developed PE at the end of pregnancy or after birth.

**Genetic studies**

Genomic DNA was isolated from peripheral venous blood using standard methods. Genotyping for SNPs *GSTP1* rs1695 and *GPX1* rs1050450 was conducted with TaqMan assays (Applied Biosystems, Waltham, MA) using the CFX96 real-time PCR detection system. TaqMan probes and primers were synthesized by Applied Biosystems of Life Technologies (New York, NY). *GSTP1* and *GPX1* were amplified using the following primers: 5′-GAT GCT CAC ATA GTT GGT GTA G-3′ and 5′-GGT GGA CAT GGT GAA TGA C-3′ for *GSTP1* and 5′-GCTTCAGACCATTGA CATC-3′ and 5′-CGA GGT GGT ATT TTC TGT AAG ATC-3′ for *GPX1*. PCR was carried out in a final volume of 25 µl containing 1.25 µl 20×SNP genotyping assay, 12.5 µl 2×PCR MasterMix, and 11.25 µl DNA and DNase-free water. Amplifications were carried out in a C1000™ thermal cycler and CFX96™ real-time system (Bio-Rad, Hercules, CA) under the following conditions: initial denaturation at 94°C for 4 min, followed by 45 cycles of 94°C for 15 s and 66°C for 1 min. We detected fluorescent signals from VIC/FAM-labeled probes in each cycle. Genotype analysis was conducted using Bio-Rad CFX manager 3.0 software.

**Statistical analysis**

All data analyses in this study were performed using the Statistical Package for Social Sciences (Version 12.0 for Windows; SPSS Inc., Chicago, IL). The homogeneity χ² test was used to test the Hardy–Weinberg equilibrium (HWE) of the genotype distribution. Differences in genotypic and allelic frequencies of rs1695 and rs1050450 between cases and controls were compared by the χ² test. p<0.05 was considered to denote statistical significance. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to show the degree of relative risk. The analysis of power was performed using the program Power and Sample Size Calculations (PS, Version 3.1.2), considering an alpha of 0.05.

**Results**

**Demographic and clinical characteristics**

Demographic and clinical characteristics of cases and controls are shown in Table 1. We found no significant differences for maternal age, gravidity, number of abortions, or age of menarche between cases and controls (all p > 0.05). However, significant differences in gestational weeks, fetal birth weights, blood pressure, white blood cells, and neutrophils were observed between the two groups (all p < 0.05).

**Genotypic and allelic frequencies**

The allelic and genotypic distribution of *GSTP1* rs1695 and *GPX1* rs1050450 showed that the samples in our study conform to HWE. Table 2 shows that there were no significant differences in allelic or genotypic frequencies of *GSTP1* rs1695 or *GPX1* rs1050450 between PE patients and controls (rs1695: χ²=1.122, p=0.571 by genotype, χ²=0.138, p=0.710, OR=1.027, 95%CI 0.892–1.183 by allele; rs1050450: χ²=0.036, p=0.982 by genotype, χ²=0.002, p=0.960, OR=1.005, 95%CI 0.822–1.229 by allele).
Various models of inheritance were used to ensure sufficient statistical power for the detection of disease susceptibility loci. Therefore, genotype frequencies were further analyzed by additive, dominant, and recessive genetic models, and rs1050450 and rs1695 were found not to be risk factors for PE based on these models (all \( p > 0.05 \)) (Table 3).
Table 4 shows that there were also no significant differences in genotypic or allelic frequencies of rs1695 or rs1050450 between mild/severe PE patients and controls. Similarly, Table 5 lists no significant differences in allelic and genotypic frequencies of rs1695 or rs1050450 between early/late-onset PE and control groups.

Discussion

The etiology and pathogenesis of PE are not completely understood [29-33], and there are currently no prognostic or predictive tools available for women at risk of PE. However, multiple pieces of evidence illustrate that oxidative stress plays an important role in PE pathophysiological mechanisms [16, 17, 34].

Glutathione can neutralize free radicals and ROS in an antioxidation process that is dependent on the activities of antioxidant enzymes such as GPxs and GSTs. When the antioxidant activity of GPxs and GSTs in pregnant women is overwhelmed by the formation of ROS, oxidative stress is more likely to arise.

Cytosolic GSTs can be subdivided into four major classes: alpha, mu, pi, and theta [19]. In recent years, polymorphisms of GSTT1, GSTM1, and GSTP1 have been reported to associate with many pathologies including various cancers and diabetes, as well as PE [35-40]. We were intrigued by the role of GSTP1 in PE. The substitution of G for A at nucleotide 313 of GSTP1 leads to an isoleucine-to-valine change at position 105 (I105V), resulting in decreased enzyme catalytic activity.

In a previous study, Zusterzeel found that 36% of 170 PE white Dutch women with a history of PE carried one rare Val105 allele while 14% carried two Val105 alleles, compared with 41% and 5%, respectively, in controls. This suggested that women with the GSTP1 P1b-1b genotype have a higher susceptibility to PE [41, 42]. Similarly, in a Japanese study, 26% of 97 patients with PE from Hokkaido University Hospital carried one Val105 allele while 74% lacked the Val105 allele, indicating that the Val105 allele is associated with PE risk [43]. However, this is not supported by other studies [44-46]. For example, Maya-Mestizo women with PE from Mexico had an AG genotype frequency of 44.8% and a GG genotype frequency of 20% for the GSTP1 313G allele compared with 54.7% and 24.4%, respectively, in controls, suggesting that both genotypes conferred a reduced risk of PE [46]. Another study by these authors of women from the same ethnicity investigated the GSTP1 polymorphism rs1695. This reported an AG genotype frequency of 47.39% and a GG genotype frequency of 35.66% for women with PE, compared with 47.16% and 32.95%, respectively, in controls, indicating that there is no association between this GSTP1 polymorphism and PE in this population [44].

Table 5. The comparison of genetic distributions between early/late-onset PE and control groups

<table>
<thead>
<tr>
<th>SNP</th>
<th>Allele</th>
<th>PE/control</th>
<th>p</th>
<th>χ²</th>
<th>OR (95% CI)</th>
<th>Genotype</th>
<th>Genotypic test</th>
<th>p</th>
<th>χ²</th>
</tr>
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<tbody>
<tr>
<td>rs1695</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Early-onset</td>
<td>A</td>
<td>517/1940</td>
<td>0.611</td>
<td>0.259</td>
<td>1.058</td>
<td>AA</td>
<td>207/764</td>
<td>0.837</td>
<td>0.356</td>
</tr>
<tr>
<td>PE/control</td>
<td>G</td>
<td>129/512</td>
<td></td>
<td></td>
<td>(0.852-1.313)</td>
<td>AG</td>
<td>103/412</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
<td>13/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late-onset</td>
<td>A</td>
<td>1217/1940</td>
<td>0.994</td>
<td>0.001</td>
<td>1.001</td>
<td>AA</td>
<td>472/764</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE/control</td>
<td>G</td>
<td>321/512</td>
<td></td>
<td></td>
<td>(0.855-1.711)</td>
<td>AG</td>
<td>273/412</td>
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<td></td>
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<td></td>
<td></td>
<td>GG</td>
<td>24/50</td>
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<tr>
<td>rs1050450</td>
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<tr>
<td>Early-onset</td>
<td>C</td>
<td>589/2234</td>
<td>0.957</td>
<td>0.003</td>
<td>1.008</td>
<td>CC</td>
<td>271/1022</td>
<td>0.778</td>
<td>0.502</td>
</tr>
<tr>
<td>PE/control</td>
<td>T</td>
<td>57/218</td>
<td></td>
<td></td>
<td>(0.743-1.368)</td>
<td>CT</td>
<td>47/190</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>5/14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late-onset</td>
<td>C</td>
<td>1402/2234</td>
<td>0.959</td>
<td>0.003</td>
<td>1.006</td>
<td>CC</td>
<td>639/1022</td>
<td>0.691</td>
<td>0.739</td>
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<tr>
<td>PE/control</td>
<td>T</td>
<td>136/218</td>
<td></td>
<td></td>
<td>(0.804-1.259)</td>
<td>CT</td>
<td>124/190</td>
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<td></td>
<td></td>
<td>TT</td>
<td>6/14</td>
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Because of the different viewpoints, it is necessary to test several factors, such as sample size and population, in the analysis of a GSTP1 association with PE. For this reason, we analyzed more than 2000 Chinese Han women in the present study. We observed AA, AG, and GG genotype frequencies in women with PE of 62.39%, 34.34%, and 3.27%, respectively. Although AG and GG genotype frequencies in the East Asian population (Chinese and Japanese individuals) are lower than those of other populations, we found no significant differences in allelic or genotypic frequencies of GSTP1 rs1695 between PE patients and controls in our study. To further understand the relationship between GSTP1 rs1695 and PE, we compared allelic and genotypic frequencies between early/late-onset PE or mild/severe PE and control groups, but again observed no significant differences. Consequently, the GSTP1 rs1695 SNP does not appear to be associated with susceptibility to PE in the Chinese Han population, so is not a suitable predictive tool for Chinese women at risk of PE.

The GPx family of antioxidant enzymes consists of eight groups, GPx1–8 [47, 48]. GPx-1 is present in all cells and is a crucial antioxidant enzyme that is more effective than catalase at removing intracellular peroxides under many physiological conditions [22]. Multiple GPx-1 SNPs have been reported, with rs1050450 being one of the most common, and several studies have associated them with different pathologies. For instance, Hu et al. demonstrated that breast cancer risk was associated with GPx-1 rs1050450, and reported a higher frequency of the Leu/Leu genotype in breast cancer tissues [49]. Tang et al. also observed a significant association between the T allele of GPx-1 rs1050450 and peripheral neuropathy in diabetics [50]. In this study, we compared allelic and genotypic frequencies of rs1050450 between 1130 PE patients and 1226 controls, as well as between early/late-onset PE or mild/severe PE and control groups, but found no significant differences.

In conclusion, this is the first study to investigate the association between GSTP1 and GPx1 SNPs with PE in a Chinese population. Our findings suggest that SNPs rs1695 and rs1050450 are not associated with susceptibility to PE in the Chinese Han population. Moreover, because our post-hoc power calculations for rs1695 and rs1050450 are 6.6% and 5.0% respectively, our results are likely to be credible given the sufficient sample size of our study. However, regional and racial differences are likely to affect the results, and because most subjects in our study were Chinese Han from Shandong Province, larger-scale studies from different regions are necessary to validate our findings.

Acknowledgements

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Disclosure Statement

The authors declare that no competing interests exist. The manuscript has been read and approved by all named authors.

References

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