Original Article

Methylenetetrahydrofolate Reductase C677T and *Glutathione S–Transferase P1* A313G Are Associated with a Reduced Risk of Preeclampsia in Maya-Mestizo Women

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Preeclampsia, a common complication of pregnancy, is characterized by elevated blood pressure and proteinuria developing after 20 weeks' gestational age. Susceptibility to this syndrome is believed to have a genetic component. The aim of this study was to investigate whether or not the *5,10-methylenetetrahydrofolate reductase* (*MTHFR*) C677T and *glutathione S–transferase P1* (*GSTP1*) A313G polymorphisms are associated with preeclampsia in Maya-Mestizo women. A case-control study was performed, in which 125 preeclamptic patients and 274 healthy controls were genotyped for the *MTHFR* C677T and *GSTP1* A313G polymorphisms by real-time PCR allelic discrimination. Allele and genotype frequencies were compared using the χ^2 tests. The *MTHFR* 677T allele and the 677TT genotype were significantly more frequent in the controls, suggesting an association with a decreased risk of preeclampsia ($p=0.017$ and $p=0.007$, respectively). Similarly, *GSTP1* 313GG/GC genotypes and the G allele were more frequent in controls, showing a significant association with reduced risk of preeclampsia ($p=0.008$ and $p=0.013$, respectively). Our results suggest, for the first time, that the *MTHFR* 677T and *GSTP1* 313G polymorphisms confer a significantly decreased risk of developing preeclampsia in the Mexican Maya-Mestizo population. (*Hypertens Res* 2008; 31: 1015–1019)

***Key Words*: *glutathione S–transferase P1* (*GSTP1*) A313G, *5,10-methylenetetrahydrofolate reductase* (*MTHFR*) C677T, Maya-Mestizo women, preeclampsia**

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This work was supported by El Fondo del Sistema de Investigación Justo Sierra (FOSISIERRA), Consejo Nacional de Ciencia y Tecnología (CONACYT), México (Grant No. 20020405) and by Coordinación de Investigación en Salud, Instituto Mexicano del Seguro Social, México (Grant No. 2006/1A/I/033).

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Received September 7, 2007; Accepted in revised form December 19, 2007.

Introduction

Preeclampsia (PE) (MIM 189800), a common complication of pregnancy, is characterized by elevated blood pressure and proteinuria developing after 20 weeks' gestational age (*1*). This syndrome occurs in 5–7% of pregnant women, although in some populations various economic, racial, geographic and/or social factors may increase the incidence (*2*). Although preeclampsia is a leading cause of maternal death and a major contributor to maternal and perinatal morbidity, its precise cause has not been completely elucidated (*3*).

Susceptibility to PE is believed to have a genetic component (*4*, *5*). Several studies have reported associations between polymorphisms of oxidative stress, thrombophilia, and hypertension-related genes with PE. Furthermore, an elevated plasma homocysteine level is a known risk factor for endothelial dysfunction and vascular disease, and has been associated with common pregnancy complications (*6*). Hyperhomocysteinemia has also been described as a risk factor for PE and as a promoter of endothelial dysfunction in placental microvascularization disease (*7*, *8*). A common polymorphism in the *5,10-methylenetetrahydrofolate reductase* (*MTHFR*) gene (C677T→A222V) (MIM 607093) has been associated with hyperhomocysteinemia (*9*), where homozygosity for the C677T substitution results in a reduced MTHFR enzyme activity and moderately elevated homocysteine levels (*10*).

On the other hand, oxidative stress is thought to play an important role in the pathophysiology of PE (*11*). The glutathione S–transferases (GSTs) catalyze the nucleophilic addition of glutathione to a large number of electrophilic compounds. In addition, GSTs plays a role in the protection of cells against the cytotoxic effects of these reactive compounds (*12*). An A-to-G transition at nucleotide 313 of the *glutathione S–transferase P1* (*GSTP1*) (MIM 134660) gene isoform results in an isoleucine-to-valine change at residue 105 (I105V), which reduces the catalytic activity of the enzyme (*13*). The lower activity of the *GSTP1* (Val^{105}) allele has been associated with PE, and it has been hypothesized that reduced levels of GSTP1 in PE may be an indicator of decreased capacity of the GST detoxification system (*12*, *13*).

Results of studies seeking associations of the *MTHFR* C677T and *GSTP1* A313G polymorphisms with PE have not always been consistent among different population analyses. Thus in the present study we investigated the possible associations between the latter polymorphisms and preeclampsia in Maya-Mestizo women.

Methods

Patients

The study was approved by the Bioethics Committee of the "Dr. Hideyo Noguchi" Research Center. Informed consent was obtained from all patients and controls before their participation commenced. One-hundred twenty-five preeclamptic women (without a history of PE) and 274 pregnant non-preeclamptic women (controls) were analyzed. All patients were of Maya-Mestizo ethnic origin, resulting from an admixture of Mayan and European (Spanish) populations. Each patient had at least one Maya surname and lived in the State of Yucatan.

Table 1. Clinical Characteristics of Preeclamptic Women and Controls*

	Preeclampsia	Controls	*p*
Patients, *n*	125	274	
Age, years	22.0±5.6	21.9±5.0	0.856
BMI, kg/m^2	33.6±5.4	22.3±3.2	<0.0001
SBP, mmHg	149.2±11.2	113.3±7.9	<0.0001
DBP, mmHg	101.5±8.7	76.8±4.9	<0.0001
Birth weight, g	2,314.4±391.4	3,121.2±373.2	<0.0001

*Data are means±SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

A case-control study was performed to investigate the possible association between the *MTHFR* (C677T→A222V) and *GSTP1* (A313G→I105V) polymorphisms and PE. The study was conducted at the Materno-Infantil Hospital of the Secretaria de Salud from August 2002 to September 2003. That institution is responsible for providing maternity services to low-income women residing in Merida, Yucatan, Mexico. All women admitted with a diagnosis of PE who agreed to participate in the study were recruited and selected consecutively according to their regular visits to this hospital. Upon each visit, blood pressure was measured in the seated position by physicians or obstetrical nurses *via* the auscultatory method using a mercury sphygmomanometer. Korotkoff phase V was generally used for defining diastolic blood pressure.

Preeclampsia was defined as the development of hypertension and proteinuria (>300 mg urinary protein in 24 h) in women with no baseline proteinuria. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg measured on two consecutive occasions at least 24 h apart (*14*). Women with previously diagnosed hypertension were excluded from the study. The control group comprised women with uncomplicated pregnancy admitted for natural childbirth or caesarean section, with normal-length pregnancy, blood pressure ≤120/80 mmHg, and without proteinuria.

Methods: Genotyping

Peripheral blood samples were obtained from all individuals, and genomic DNA was purified as described by Kempter and Grossbadern (*15*). Single-nucleotide polymorphism (SNP) analysis was performed using real-time PCR allelic discrimination TaqMan assays (Applied Biosystems) with minor

Table 2. Genotype and Allele Frequencies of the *MTHFR* C677T and *GSTP1* A313G Polymorphisms in Women with or without Preeclampsia

	Preeclampsia (n=125)	Controls (n=274)	OR (95% CI)	*p-value
MTHFR C677T				
Genotype frequencies, *n* (%)				
CC	36 (0.288)	61 (0.222)	0.261 (0.323–0.705)	0.007
CT	66 (0.528)	131 (0.478)		
TT	23 (0.184)	82 (0.299)		
Allele frequency				
C	138 (0.552)	253 (0.461)	0.696 (0.515–0.940)	0.017
T	112 (0.448)	295 (0.538)		
GSTP1 A313G				
Genotype frequencies, *n* (%)				
AA	44 (0.352)	57 (0.208)	0.316 (0.134–0.744)	0.008
AG	56 (0.448)	150 (0.547)		
GG	25 (0.200)	67 (0.244)		
Allele frequency				
A	144 (0.576)	264 (0.481)	0.684 (0.506–0.925)	0.013
G	106 (0.424)	284 (0.518)		

*p-values and ORs calculated by a logistic regression analysis using a recessive model by *MTHFR* gene (CC+CT *vs*. TT), and a dominant model by *GSTP1* gene (AA *vs*. GG+AG), both adjustment for age and BMI. ORs, odds ratios; CI, confidence interval; BMI, body mass index.

modifications. All PCR reactions contained 20 ng of DNA, 2.5 µL TaqMan Universal Master Mix (Applied Biosystems) (2×), 0.25 µL primers and probes (10×), and water for a final volume of 5 µL, including the appropriate negative controls in all assays. Real-time PCR was performed on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, USA) under the following conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles of amplification (95°C for 15 s and 62°C for 1 min). For each cycle, the software determined the fluorescent signal from the VIC- or FAM-labeled probe (Applied Biosystems). Allelic discrimination was performed using specific primers and probes for each allele. The primer sequences were as follows: forward 5′-GCACTTGAAGGAGAAGGTGTCT-3′ and reverse 5′-CCTCAAAGAAAAGCTGCGTGATG-3′ for C677T *MTHFR*; forward 5′-CCTGGTGGACATGGTGAATGAC-3′ and reverse 5′-CAGATGCTCACATAGTTGGTGTAGA-3′ for A313G *GSTP1*. The probe for each allele is as follows: wild-type (wt) VIC 5′-ATGAAATCGGCTCCCGC-3′ and mutant (mt) FAM 5′-ATGAAATCGACTCCCGC-3′ for the C677T *MTHFR* polymorphism; wt VIC 5′-CTGCAAATA CATCTCC-3′ and mt FAM 5′-CTGCAAATACGTCTCC-3′ for the A313G *GSTP1* polymorphism.

Statistical Analysis

Statistical analyses were performed using SPSS v10 (SPSS, Chicago, USA). Continuous variables were compared by unpaired Student's *t*-test. Deviations from Hardy-Weinberg equilibrium were tested using the χ^2 test. Power calculation of 80%, assuming a 10% difference in genotype percentages, was estimated using the epidemiological data obtained by Duran and Couoh (*16*). Mathematical calculations were performed according to Pértegas Díaz and Pita Fernández (*17*). Allele frequency differences between groups were assessed by χ^2 tests (http://ihg.gsf.de/cgi-bin/hw/hwal.pl). Logistic regression analysis with adjustment for age and body mass index (BMI) was used to test associations between genotype and preeclampsia (SPSS v10.0).

Results

The clinical characteristics of the preeclamptic women and controls are shown in Table 1. Of the 125 patients with PE, 28 presented a severe condition. No cases with HELLP (hemolysis, elevated liver enzymes, and low platelet count) were diagnosed. Preeclamptic women showed significantly higher systolic and diastolic blood pressure as well as BMI ($p<0.0001$), and birth weight was significantly different between the preeclamptic group and the control group ($p<0.0001$).

C677T *MTHFR* and A313G *GSTP1* genotype and allele frequencies are presented in Table 2. Under a recessive model, the *MTHFR* 677TT genotype conferred a significantly decreased risk of PE ([OR]=0.266; 95% confidence interval [CI]: 0.115–0.705; $p=0.007$, adjusted by age and BMI). Moreover, the T allele was significantly less frequent in the PE group (44.8%) than in the controls (53.8%) (OR=0.696, 95% CI: 0.515–0.940; $p=0.017$).

The allele and genotype distributions of the A313G *GSTP1*

polymorphism also differed significantly between groups, as the 313GG and AG genotypes conferred a significantly reduced risk of PE (OR=0.316; 95% CI: 0.134–0.744; $p=0.008$, adjusted by age and BMI). The G allele was also less frequent in the PE group (42.4%) than in controls (51.8%), and thus was associated with a significantly reduced risk of PE (OR=0.684; 95% CI: 0.506–0.925; $p=0.013$).

Both polymorphisms were in Hardy-Weinberg equilibrium, as the observed genotype distribution did not differ from the expected in PE women and controls. The statistical power of the study was 89% at $p<0.05$ to detect previous associations observed in women with PE.

Discussion

Preeclampsia is a complex, multifactorial pregnancy-specific condition involving genetic, environmental, and behavioral factors (*2*, *18*). Hyperhomocysteinemia has been associated with the development of PE (*7*, *8*), and the most common polymorphism associated with this condition is the thermolabile 677T allele of the *MTHFR* gene (*10*). However, several studies have failed to confirm this association (*19*, *20*). In contrast with previous reports associating *MTHFR* 677TT homozygocity with an increased risk of PE (*9*, *21*, *22*), in the present study we found that the 677TT genotype confers a reduced risk of PE in Maya-Mestizo women. The association was observed only under the recessive model, as CC *vs*. CT and TT genotype frequencies (the dominant model) failed to show significant differences ($p=0.157$). The recessive effect of this allele in association with other diseases (leukemia and colorectal cancer) has been previously described (*23–25*). Although there are previous reports of inverse associations of this particular *MTHFR* genotype (677TT) with other diseases, such as colorectal cancer and acute lymphoblastic leukemia (*23–25*), this is the first report to associate the 677TT genotype with a reduced risk of PE. While hyperhomocystinemia is associated with an increased risk of vascular complications during pregnancy, the underlying mechanism has not been fully elucidated and is complex, involving several factors (*7*). The reduced risk of PE in the Maya-Mestizo population could be due to the modifying effect of yet unknown alleles of other genes present in this population or other environmental factors to be determined.

Only two previous studies have been undertaken in Mexican women with PE (*26*, *27*). Pérez-Mutul *et al*. (*26*) investigated the association between the C677T *MTHFR* polymorphism and PE in women from southeastern Mexico. In accordance with our results, the 677TT genotype and allele frequencies were higher in the control group than in women with PE, although the association was not statistically significant. Although the population those authors analyzed was in southeastern Mexico, they did not point out what proportion of these women were Maya-Mestizo. Similarly, in a small study, Dávalos *et al*. (*27*) found no association of 677TT with PE in Mexican-Mestizo women with a different ethnic origin (western Mexico).

Moreover, *GSTP1* A313G (I105V) is a known functional polymorphism (*28*). The amino acid substitution lies within the GSTP1 active site and has been found to alter substrate affinity (*28*, *29*). The Val^{105} allele was associated with an increased risk of developing PE in a Dutch population (*13*) and with severe hypertension in elderly pregnancy in a Japanese population (*30*), although no association was found in a colored population of South Africa (*31*). In contrast, we found an inverse association in which the *GSTP1* 313G allele confers a reduced risk of PE. Furthermore, as opposed to the autosomal recessive model for the *MTHFR* C677T polymorphism described here, the *GSTP1* 313G allele conferred a reduced risk of PE under a dominant model, since the comparison of AG and GG *vs*. AA genotype frequencies was significantly different ($p=0.008$). While decreased protection against oxidative stress due to the presence of the Val^{105} allele would be expected to increase the risk of several diseases (*13*, *30*, *32*), this genotype has been associated with favorable prognosis following chemotherapy with drugs known to be GSTP1-specific substrates in a variety of malignancies, such as pediatric acute lymphoblastic leukemia, myeloma, and Hodgkin's lymphoma (*33–35*). Moreover, these differential effects of *GSTP1* genotypes on the risk of these diseases might result from the enantioselectivity of these genotypes, as previously suggested to explain the inverse association between this variant and endometriosis (*36*).

Our results suggest that both the C677T *MTHFR* and *GSTP1* A313G polymorphisms may play a role in the risk of PE in Maya-Mestizo women. However, additional prospective association studies are required to confirm our findings. On the other hand, these polymorphisms may play a role in the manifestation of PE together with other genetic and environmental factors yet to be determined.

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