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Matrix metalloproteinase (MMP)-9 genotypes and haplotypes in preeclampsia and gestational hypertension

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ABSTRACT

Background: Abnormal production of matrix metalloproteinases (MMPs), especially MMP-9, may play a role in hypertensive disorders of pregnancy. These alterations may result from functional genetic polymorphisms in the promoter region of MMP-9 gene, which are known to change MMP-9 expression. We examined whether 2 MMP-9 polymorphisms ($C^{-1562}T$ and (CA)*n*) and haplotypes are associated with preeclampsia and/or gestational hypertension.
Methods: We studied 476 pregnant women: 176 healthy pregnant (HP), 146 pregnant with gestational hypertension (GH), and 154 pregnant with preeclampsia (PE). Genomic DNA was extracted from whole blood and genotypes for $C^{-1562}T$ and (CA)*n* polymorphisms were determined by PCR-RFLP. Haplotype frequencies were inferred using the PHASE ver. 2.1 program.
Results: For the g.−90(CA)13–25 polymorphism, no significant differences were found in genotype and allele distributions when PE or GH groups were compared with HP group. However, the CT genotype and T allele for g.−1562C>T polymorphism were more commonly found in GH subjects compared with the HP group (both $P<0.05$). Conversely, we found no differences in genotypes or allele distributions for the g.−1562C>T polymorphism when the PE and the HP groups were compared. No significant differences were found in overall distributions of haplotype frequencies when the GH or the PE group was compared with the HP group.
Conclusions: The $C^{-1562}T$ polymorphism in MMP-9 gene is associated with gestational hypertension, but not with preeclampsia. These findings may help to explain the higher plasma MMP-9 levels previously reported in GH compared with HP.

1. Introduction

Preeclampsia is an important hypertensive disorder of pregnancy and its pathophysiology remains unclear. Its origin probably lies in the placenta, since preeclampsia occurs only in the presence of placenta and delivery of the placenta remains the only definitive treatment [1]. Placentation is essential for a successful pregnancy [2]. Early in normal pregnancy, the cytotrophoblastic cells of the developing placenta invade the uterine tissue and disrupt the spiral arteries of the decidua and myometrium. However, the cytotrophoblastic invasion in preeclamptic women is impaired and the spiral arteries remain narrow [3]. This leads to hypoperfusion of the placenta and induces the release of vasopressors and other factors into the maternal circulation, thus involving multiple organs and becoming a systemic condition [4].

Matrix metalloproteinases (MMPs) are a family of structurally related, zinc-dependent enzymes that break down several extracellular matrix components [5]. Imbalanced MMP activity has been reported in clinical conditions affecting the cardiovascular system [6,7] including hypertensive disorders of pregnancy [8–11]. Indeed, altered MMP levels in hypertensive disorders of pregnancy may reflect abnormal invasive ability of trophoblastic cells [12,13], and upregulated MMPs may interact with increased oxidative stress and inflammatory mediators to produce endothelial dysfunction seen in preeclampsia [14].

Genetic polymorphisms in the MMP-9 gene affect MMP-9 transcription, and 2 of them are functional: the g.−1562C>T substitution (rs3918242) and the microsatellite g.−90(CA)13–25 (rs3222264) [15,16]. These functional MMP-9 polymorphisms have been associated with disease conditions, including cardiovascular diseases [15,17].

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However, there are 2 inconclusive studies examining whether the g.−1562C>T polymorphism is associated with hypertensive disorders of pregnancy. One of them shows that the T allele is less frequent in preeclampsia compared with healthy pregnancy [18], whereas another study suggests lack of association between this polymorphism and preeclampsia [19]. In the present study, we aimed at expanding these preliminary findings. We studied whether 2 functional polymorphisms (g.−90(CA)13–25 and g.−1562C>T) in the MMP-9 gene, either alone or combined within haplotypes, are associated with preeclampsia or with gestational hypertension.

2. Materials and methods

2.1. Subjects

Approval for use of human subjects was obtained from the Institutional Review Board at the Faculty of Medicine of Ribeirao Preto. All volunteers were consecutively enrolled in the Department of Obstetrics and Gynecology, University Hospital of the Faculty of Medicine of Ribeirao Preto. We studied 476 pregnant women (176 healthy women with uncomplicated pregnancies, 146 women with gestational hypertension, and 154 women with preeclampsia). Hypertensive disorders were defined in accordance with the guidelines of the NHBPEP (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy) [20]. Gestational hypertension was defined as pregnancy-induced hypertension (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥2 measurements at least 6 h apart) in a woman after 20 weeks of gestation, and returning to normal by 12 weeks post-partum. Preeclampsia was defined as increased blood pressure plus significant proteinuria (≥0.5 g/24 h) in a woman after 20 weeks of gestation. No women with pre-existing hypertension, with or without superimposed preeclampsia, were included in the present study.

At the time of clinic attendance, written informed consent was provided and maternal venous blood samples were collected. Genomic DNA was extracted from the cellular component of 1 ml of whole blood by a salting-out method and stored at −20 °C until analyzed.

2.2. Genotyping

Genotypes for the g.−1562C>T polymorphism (rs3918242) were determined by polymerase chain reaction (PCR) amplification using the primers 5′-GCC TGG CAC ATA GTA GGC CC-3′ (sense) and 5′-CTT CCT AGC CAG CCG GCA TC-3′ (antisense) and the conditions as previously described [21]. The amplified products were digested with Sph I restriction enzyme (New England Biolabs, Ipswich, MA) overnight at 37 °C, producing fragments of 247 bp and 188 bp in the case of a polymorphic variant (allele T), or an undigested 435 bp band in the case of a wild type allele (allele C). Fragments were separated by electrophoresis in 12% polyacrylamide gels and visualized by silver staining.

To determine the genotypes for the g.−90(CA)13–25 polymorphism (rs3222264), a PCR was carried out using the primers 5′-GAC TTG GCA GTG GAG ACT GCG GGC A-3′ (sense) and 5′-GAC CCC ACC CCT CCT TGA CAG GCA A-3′ (antisense) and the conditions as previously described. The amplified products were separated in 7% polyacrylamide–8 M urea gel and visualized by silver staining. Differences in number of bases, from 144 bp (CA 13 repeats) to 168 bp (CA 25 repeats) were determined by comparison with migration of a 10 bp DNA ladder (Invitrogen, Carlsbad, CA) and with some samples from homozygotes that were sequenced. The alleles for the microsatellite g.−90(CA)13–25 polymophism were classified as “low” (L) count when the number of CA repeats was less than 21, and as “high” (H) when the number of CA repeats was ≥21 [22].

2.3. Statistical analysis

Statistical analysis was done using the SPSS 15.0 software (Chicago, IL). The clinical characteristics of women with gestational hypertension or preeclampsia were compared with those of healthy pregnant women by Mann–Whitney *U*-test, chi-square or Fisher exact, as appropriate. The distribution of genotypes for each polymorphism was assessed for deviation from the Hardy–Weinberg equilibrium, and differences in genotype and allele frequencies among groups were assessed using χ^2-tests or Fisher exact tests. A value of $P<0.05$ was considered statistically significant.

The Bayesian statistical based program PHASE ver. 2.1 was used to estimate the haplotypes frequencies in each group [23,24]. The possible haplotypes including genetic variants for 2 MMP-9 polymorphisms studied (C or T variants for the g.−1562C>T and H or L variants for g.−90(CA)13–25) were: H1 (CH), H2 (CL), H3 (TH) and H4 (TL). Differences in haplotype frequency were further tested using a contingency table. The minimum level of statistical significance was corrected for the number of comparisons made. Therefore, we considered significant a probability value of $P<0.05$/number of haplotypes ($P<0.05/4=0.0125$).

3. Results

Table 1 summarizes the characteristics of the 478 pregnant women enrolled in the present study. Healthy pregnant (HP), gestational hypertensive (GH) and preeclamptic (PE) women were matched by age, ethnicity, smoking, % primigravida, heart rate, fasting glucose, hemoglobin, and hematocrit (Table 1; all $P=$NS). As expected, PE and GH presented higher systolic and diastolic blood pressure compared with HP group (both $P=$NS). It should be noted, however, that most patients were receiving pharmacological therapy (methyldopa in most cases). Higher body mass index (BMI) was found in GH group compared with the other study groups ($P<0.05$). Lower gestational ages at delivery were found in GH and PE groups, and lower newborn weights were found only in PE compared with HP group (all $P<0.05$). Significant proteinuria was found in PE women.

Table 2 shows the results of the MMP-9 single-locus analysis. The frequencies of the MMP-9 genotypes in the control subjects were similar to those reported previously in healthy Brazilians [25]. The distribution of genotypes for the two polymorphisms studied here showed no deviation from Hardy–Weinberg equilibrium (all $P=$NS). For the g.−90(CA)13–25 polymorphism, no significant differences were found in genotype and allele distributions when PE or GH groups were compared with HP group (Table 2; all $P=$NS). However, the genotype and allele frequencies for the g.−1562C>T polymorphism were different in GH subjects as compared with HP subjects. The CT genotype and T allele were more commonly found in GH subjects compared with the HP group (Table 2; both $P<0.05$). Conversely, we found no differences in genotype or allele distributions for the g.−1562C>T polymorphism when the PE and the HP groups were compared (Table 2; both $P=$NS).

We estimated MMP-9 haplotype frequencies including the two polymorphisms for the three study groups (Table 3). No significant differences were found in overall distributions of haplotype frequencies when the GH or the PE group was compared with the HP group (Table 3; all $P=$NS).

4. Discussion

While maternal mortality has decreased around the world, the number of delivery hospitalizations with hypertensive disorders in pregnancy has increased [26]. Although preeclampsia is a transient condition, women who have had preeclampsia or gestational hypertension are at increased risk of hypertension, stroke, and coronary artery disease in their later lives [27,28]. In addition, recent studies have shown