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Angiotensin-converting enzyme gene polymorphism in preeclampsia and normal pregnancy

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KEY WORDS
Hypertension
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Objective: The purpose of this study was to evaluate the angiotensin-converting enzyme gene polymorphism in pregnant women with and without preeclampsia.
Study design: Preeclampsia was defined as hypertension and pathologic proteinuria in pregnant women after gestational week 20. Genomic DNA was isolated from leukocytes. The insertion-deletion polymorphism in intron 16 of the angiotensin-converting enzyme gene was detected in DNA samples with the use of the polymerase chain reaction. Chi-squared and Student *t* tests were used for statistical analysis.
Results: In preeclampsia (n = 51 women) angiotensin-converting enzyme genotypes were deletion-D (DD) in 16 women (31%), insertion-I (II) in 12 women (24%), and insertion-deletion in 23 women (45%); in the control group (n = 71), the angiotensin-converting enzyme genotypes were DD in 21 women (30%), II in 17 women (24%), and insertion-deletion in 33 women (46%). Angiotensin-converting enzyme genotype distribution and allelic frequencies were not different between groups.
Conclusion: No difference in the angiotensin-converting enzyme genotype distribution was found between preeclampsia and normal pregnancy. The results showed no association between angiotensin-converting enzyme polymorphism and the development of preeclampsia.

Hypertensive disorders of pregnancy complicate approximately 6% to 8% of all pregnancies and constitute 1 of the major causes of maternal and fetal morbidity and death. Preeclampsia is characterized by high blood pressure and pathologic proteinuria that develops after 20 weeks of gestation in otherwise previously normotensive pregnant women.[1] Its cause remains unclear despite intense research. Evidences for an inherited predisposition to preeclampsia are well known, and studies have reported an increased risk in relatives of affected women.[2]

The renin-angiotensin-system has a critical role in controlling blood pressure, and its genes are potential candidates for the genetic susceptibility to preeclampsia.

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The angiotensin-converting enzyme (ACE) is an important component of the renin-angiotensin-system. It is a dipeptidyl carboxypeptidase that is encoded by the ACE gene, located on chromosome 17q23, and comprising 26 exons and 25 introns.[3] This enzyme catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and also inactivates the vasodilator bradykinin.[4] Normal pregnant women experience a refractoriness to the pressure effects of infused angiotensin II,[5] and there is an increased sensitivity to angiotensin II in patients with or destined to experience the preeclampsia syndrome.[6,7]

A polymorphism in intron 16, which involves the presence (insertion-I [II]) or absence (deletion-D [DD]) of a 287 base pair (bp) nucleotide sequence, has been identified on the ACE gene. As a result, 2 alleles are formed: I and D, generating 3 different genotypes, DD, II, and insertion-deletion (ID).[8] Usually, seric ACE levels show marked interindividual variation, which is the DD genotype that is associated with higher ACE concentration.[8]

The role of the ACE gene polymorphism has not been established clearly in preeclampsia; some authors report an association with the disease,[9,10] but not all authors.[11-13] The aim of the present study was to evaluate the ACE gene polymorphism in women with and without preeclampsia.

Material and methods

Subjects

Fifty-one women with preeclampsia and 71 women with normotensive pregnancy under medical assistance at the Department of Obstetrics and Gynecology, Hospital São Lucas of Pontifícia Universidade Católica do Rio Grande do Sul (Porto Alegre, Brazil) were included. According to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy,[1] preeclampsia was defined as systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg that was associated with proteinuria of >300 mg/24 hours. Urinary protein excretion was estimated in a 24-hour urine collection, measured by acid precipitation with turbidimetry, or was estimated as the ratio of protein:creatinine in a urine random sample. Only nulliparous women who had a normal blood pressure and a normal urinary sediment 12 weeks after delivery were included in the preeclamptic group.

The control group consisted of normotensive pregnant women with a negative dipstick test result for urinary protein and no history of hypertension. Patients with a history of essential hypertension, secondary hypertension, diabetes mellitus, renal disease, and infection were excluded, as were women with signs of altered fundi (hypertensive retinopathy).

This study was approved by the Hospital Scientific and Ethics Committees. All patients were clarified of the research aims, risks, and benefits and were included only after informed consent was signed.

ACE genotyping

Peripheral venous blood samples were collected from the antecubital vein in vacutainer tubes that contained EDTA. Genomic DNA was extracted from leucocytes by proteinase K digestion followed by phenol:chloroform extraction. The reaction mixture (25 μL total) contained 17.5 pmol of each primer (aceF 5′- CTG GAG ACC ACT CCC ATC CCT TCT-3′ and aceR 5′- GAT GTG GCC ATC ACA TTC GTC AGA T-3′), 0.5 μL of 10 mmol/L deoxyribonucleoside triphosphate mix, 1.75 U Taq DNA polymerase (Invitrogen, São Paulo, Brazil), 2.5 μL of 10× polymerase chain reaction (PCR) buffer (200 mmol/L Tris-HCl [pH 8.4], 500 mmol/L KCl), 1 μL of 50 mmol/L $MgCl_2$, 2.5 μL of 10% DMSO, and 1.5 μL sample DNA. The amplification program was performed according to Rigat et al,[8] which consisted of an initial denaturation (94°C/3 min), 30 cycles of denaturation (94°C/1 min), annealing (52°C/1 min), and extension (72°C/1 min) and a final extension step (72°C/5 min). PCR products were electrophoresed in 2% agarose gel and analyzed by ethidium bromide stain under ultraviolet light. The I/D polymorphism was identified by the presence of a band of 480 bp (corresponding to the I allele) or 190 bp (D allele).

Specimens that were genotyped as DD (n = 41 patients) by the standard amplification procedure that was described earlier were also assayed by a triple primer method.[14,15] This method consisted on the use of a third primer that annealed at the 5′ end of the insertion sequence (5′-TGG GAT TAC AGG CGT GAT ACA G-3′), along with the standard primers, which resulted in the amplification of an extra 160-bp fragment when the I allele was present. This allowed the correct identification of some ID genotypes that were mistyped initially as DD. The use of this additional amplification procedure seems to be the most reliable PCR strategy to identify DD and ID genotypes, despite the presence of 10% DMSO.[15] Four of 41 individuals were found to be ID (9.8%).

Statistics

Data were analyzed with the EPI-Info 2000 software (Centers for Disease Control, Atlanta, Ga) with the use of descriptive statistics, the Student *t* test, and chi-squared test.

Table I Clinical characteristics of preeclamptic and control groups

Characteristic	Preeclampsia group (n = 51)	Control group (n = 71)	*P* value*
Maternal age (y)	21 ± 4.1	25 ± 6.2	< .001
Gestational age at delivery (wk)	36.2 ± 3	39 ± 1.9	< .001
White race (n)	34 (66.6%)	54 (76.1%)	.256†
Gestation (n)	1.1 ± 0.2	1.6 ± 1.1	< .001
Systolic blood pressure (mm Hg)	157 ± 17.5	111 ± 10.2	< .001
Diastolic blood pressure (mm Hg)	103 ± 11.9	70 ± 7.7	< .001
Proteinuria (g/24 h)	2.35 ± 2.40	—	—
Creatinine (mg/dL)	0.64 ± 0.13	0.58 ± 0.08	.013
Uric acid (mg/dL)	5.7 ± 1.5	4.2 ± 1.1	< .001

Results are shown as mean ± standard deviation.
* Student *t* test was used.
† Chi-squared test was used in this instance.

Results

Clinical characteristics of both normotensive and preeclamptic groups are presented in Table I. The distribution of the ACE genotypes and allelic frequencies is given in Table II; no significant difference was disclosed between the 2 groups ($P = .977$). The allelic frequencies for preeclamptic and control groups were also not different ($P = .968$).

Table II ACE polymorphism genotype distribution and allele frequency in preeclampsia and normal pregnancy

	Preeclampsia group (n = 51)	Control group (n = 71)	*P* value*
ACE genotype (n)			
Deletion-allele homozygotes (n)	16 (31%)	21 (30%)	
Heterozygotes (n)	23 (45%)	33 (46%)	.977
Insertion-allele homozygotes (n)	12 (24%)	17 (24%)	
Allelic frequency (%)			
I ACE alleles	46%	47%	.865
D ACE alleles	54%	53%	

* Chi-squared test.

Comment

The present study found no association between the ACE gene insertion/deletion polymorphism and the occurrence of preeclampsia in this sample from southern Brazil.

In contrast to our results, studies that were conducted in a Chinese population sample suggested that the ACE insertion-I genotype is a marker for a reduced risk for preeclampsia and that DD is a risk genotype.[9,10] A difference in genotype distribution and allele frequency and an association between DD genotype and risk of recurrent preeclampsia were reported in Italian women.[16]

On the other hand, this study described no association between the DD genotype or allelic frequency with preeclampsia. Corroborating with us, in another study that examined maternal and fetal genotypes in 72 women with preeclampsia and 83 control subjects, no evidence of ID polymorphism association with preeclampsia was found.[12] In an indigent African-American women population, no significant impact of ACE gene polymorphism on pregnancy outcomes or changes in blood pressure during the course of pregnancy could be detected. In addition, the incidence of pregnancy-induced hypertension and/or preeclampsia was not different among the 3 ACE genotypes.[11]

Our study included only nulliparous preeclamptic women, because there is a consensus among authors in both epidemiologic and clinical studies that recognize nulliparity as a risk factor for the development of preeclampsia.[1,17-19] This selection criterion may have contributed to the lack of association between D allele frequency and the risk of preeclampsia. Recently, Mello et al[16] found an association between the ACE DD genotype and risk of recurrent preeclampsia or fetal growth restriction.

We have also confirmed that ACE genotyping with the use of standard PCR can result in the mistyping of ID heterozygotes as deletion-D homozygotes. It has been suggested that the use of the triple primer method allows the correct genotyping to be accomplished accurately.[15] Eventually, this may account for some contra-

dictions in published reports, although only approximately 10% of the DD genotype were mistyped in agreement with Ueda et al.[15]

Our study does not support the hypothesis that ACE gene insertion/deletion polymorphism contributes to the pathophysiologic condition of preeclampsia. The involvement of other gene polymorphisms (such as angiotensinogen, angiotensin-receptor gene, methylene tetrahydrofolate reductase gene) has been investigated as predisposing factors to preeclampsia. These studies may contribute to elucidate the complex cause of preeclampsia.

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