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# Association of Angiotensin-Converting Enzyme Insertion-Deletion Polymorphism with Preeclampsia

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## ABSTRACT

*The aim of this study was to determine if insertion-deletion polymorphism of angiotensin-converting enzyme is a risk factor for the development of preeclampsia. Sixty women with preeclampsia and 50 normotensive pregnant women were included in this study. Preeclampsia was defined as blood pressure > 140/90 mmHg in a previously normotensive women with proteinuria >300 mg/L in a 24-hours. Twelve women also had preeclampsia in previous pregnancy. The genotyping of polymorphism in the intron 16 of the angiotensin-converting enzyme was performed by the polymerase chain reaction followed by the agarose electrophoresis. The patients were divided into three groups according to the presence (I) or absence (D) of insertional polymorphism (II, ID, and DD). Genotype distribution and allele frequencies were compared by Mantel-Haenszel  $\chi^2$  testing. The frequency of DD genotype was not significantly higher in women with preeclampsia (26/60) than in the control group (14/50,  $p=0.096$ ). The D allele frequency was significantly higher in 17 women with preeclampsias who required delivery before 34 weeks of pregnancy (0.735), than in 43 women in whom obstetric complications took place after 34 weeks of pregnancy (0.56,  $p=0.036$ ). The D allele frequency was 0.83 in women having recurrent preeclampsia, i.e. significantly higher compared with women, who were for the first time, experienced preeclampsia (0.57,  $p=0.013$ ). This study showed a significantly positive association between D allele frequency and risk of recurrent preeclampsia and preterm delivery before 34 weeks of pregnancy. The deletion genotype could be an important contributing factor for an early onset and recurrent preeclampsia.*

**Key words:** preeclampsia, Caucasians, renin-angiotensin system, gene, polymorphism

## Introduction

Preeclampsia (PE) is a heterogeneous disorder which is characterized by hypertension and proteinuria after 20 weeks of gestation. The PE is the major cause of maternal and perinatal morbidity with an estimated incidence between 5–7% in pregnant population<sup>1</sup>. The cause of PE remains unknown although genetic factors, placenta, diet, parity, race, maternal weight and environment all play a role<sup>2,3</sup>.

The renin-angiotensin-aldosterone system (RAS) plays the central role in blood pressure control<sup>4</sup>. All components of RAS, angiotensinogen, angiotensin II, aldosterone and plasma renin activity are increased during the normal pregnancy. Despite the increase, hypertension does not develop in normal pregnancy because blood ves-

sels do not respond to angiotensin II<sup>5</sup>. Angiotensin-converting enzyme (ACE), which is an important component of RAS, plays a key role in the regulation of vascular tone and circulatory homeostasis by converting angiotensin I to angiotensin II and by inactivating bradykinin<sup>2,6</sup>. It has been shown that PE can be caused by polymorphism-induced altered expression in renin-angiotensin-aldosterone system genes<sup>7</sup>. The ACE I/D polymorphism contributed for about 50% of total phenotypic variance of the serum levels, with a D allele highest effect<sup>2</sup>. Several genetic studies showed that insertion-deletion polymorphism of ACE has an important role in pathogenesis of experimental and human hypertension<sup>2,8</sup>. The recently published studies have investigated the association between inser-

tion-deletion angiotensin-converting gene polymorphism and preeclampsia, showing conflicting results between investigated population of the different ethnic origin<sup>9–13</sup>.

The aim of our study was to investigate the association of ACE insertion-deletion gene polymorphism with preeclampsia and adverse pregnancy outcome in our study population.

## Patients and Methods

The study included 60 women with singleton pregnancy and preeclampsia recruited at the Department of Perinatal Medicine, »Sveti Duh« Hospital in Zagreb, Croatia between January 2000 and December 2005. The control group consisted of 50 age- and parity-matched normotensive women who had at least one normal delivery beyond 37 weeks of pregnancy. Control participants were age and parity matched. Informed consent was obtained from all study subjects and Hospital Ethics Committee approved the study.

Preeclampsia was defined as an increase in blood pressure of  $\geq 140/90$  mmHg on two separate occasions 6 hours apart in previously normotensive women with proteinuria of  $> 300$  mg/L per 24 hours after 20 weeks of gestation<sup>1</sup>. Women with history of chronic hypertension, diabetes mellitus, hyperthyroidism, chronic renal disease, collagen disorders, and cardiovascular disease were excluded from the study.

### Angiotensin-converting enzyme insertion-deletion genotyping

The whole blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA). DNA was isolated by a standard NaCl extraction method<sup>14</sup>. Amplification was carried out in a total volume of 50  $\mu$ L containing 0.3  $\mu$ g of genomic DNA, 0.2 mmol/L dNTPs, 0.2  $\mu$ mol/L of each primer in polymerase chain reaction (PCR) buffer and 1 U DNA Taq-polymerase. Detection of insertion-deletion polymorphism of the ACE gene was carried out by PCR amplification using sense primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT -3', and antisense primer

5'-GAT GTG GCC ATC ACA TTC GTC AGA -3'. PCR products from D alleles were 190bp long and from I alleles were 490 bp long. Additional amplification using insertional primers was used to avoid misgenotyping of the ID genotype due to preferential amplification of the D allele 5'-TGG GAC CAG AGC GCC CGC CAC TAC-3' and 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3', respectively. This PCR product was 335 bp long. Then, the PCR products were electrophoresed in 1.5% agarose gel, and visualised by ethidium bromide staining. The patients were divided into three groups according to the combination of D and I alleles: genotype II, ID, and DD.

### Statistical methods

The  $\chi^2$  test was used to compare allele frequencies and genotype distribution between women with preeclampsia and control group. Mantel-Haenszel  $\chi^2$  test was used to correlate the influence of the deletion allele and genotype on clinical parameters. Fisher's exact test was applied when a small group was expected. The variance between groups was expressed as odds ratio (OD) with 95% confidence interval (CI). Clinical variables were expressed as mean  $\pm$  standard deviation (SD), and were compared by use of Student's t-test. Differences were considered statistically significant when  $P < 0.05$ . SAS software was used for statistical analysis (V8 SAS Institute, Cary, NC).

## Results

There were significant differences in blood pressure values, birth weight and weeks of delivery between women with preeclampsia and normotensive control group (Table 1). As expected, women with preeclampsia had significantly higher blood pressure. Also, in the group of women with preeclampsia, the babies were born at significantly earlier gestational age and with significantly lower birth weight. The D and I allele frequency between preeclamptic and control group was similar (Table 2). The frequency of D allele was 0.633 in preeclamptic and 0.54 in control group ( $\chi^2 = 1.8378$ ;  $p = 0.1752$ ;  $df = 1$ ).

TABLE 1  
CLINICAL CHARACTERISTICS OF WOMEN WITH PREECLAMPSIA AND CONTROL GROUP (MEAN VALUE  $\pm$ SD)

Characteristics	Preeclampsia (n=60)	Control (n=50)	p
Maternal age (years)	$\pm 6.1$ (19–45)	$31.4 \pm 5.6$ (19–45)	0.524
Gestational age at delivery (weeks)	$36.5 \pm 3.4$ (26–42)	$39.22 \pm 1.04$ (36–41)	$< 0.001$
Birth weight (g)	$2500 \pm 0.93$	$3590 \pm 0.56$	$< 0.001$
Blood pressure on admission (mm Hg)			
Systolic	$153.05 \pm 9.45$	$121.63 \pm 4.18$	$< 0.001$
Diastolic	$99.14 \pm 8.05$	$77.13 \pm 4.77$	$< 0.001$
Proteinuria on admission(g/L)	$1.28 \pm 0.94$	—	

**TABLE 2**  
ANGIOTENSIN-CONVERTING ENZYME GENOTYPE DISTRIBUTION AND ALLELE FREQUENCIES

Group	Genotype Distribution				Allele Frequency	
	N	DD(%)	ID (%)	II (%)	D	I
Preeclampsia	60	26	24	10	0.636	0.364
Control	50	14	26	10	0.54	0.46

DD – deletion homozygote, ID – heterozygote, II – insertional homozygote  
 $\chi^2 = 1.8378$ ,  $p = 0.1752$ ,  $df = 1$

Genotype distribution was quite similar in preeclamptic and control group. The rate of DD genotype was slightly, but not significantly higher in women with preeclampsia (43.3%) than in 28% in controls (OR 1.97, 95%CI 0.88–4.38,  $p = 0.096$ ). The D allele frequency was 0.735 in 17 women with preeclampsia who had delivery before 34 weeks of pregnancy because of obstetric complications (OR 2.5 (95%CI 1.03–6.05), compared to 43 women delivered after 34 weeks of pregnancy (0.56,  $p = 0.036$ ). Twelve women also had preeclampsia in previous pregnancy. The D allele prevalence was 0.83 in women who had preeclampsia in two consecutive pregnancies (OR 7.11 (95%CI 1.4–36.117), Figure 1), which was significantly higher compared with women, who were for the first time, experienced preeclampsia (0.57,  $p = 0.013$ ). In the group of women with previous and successive pregnancy with preeclampsia, about 41% (5/12) had delivery before 34. weeks of pregnancy. Four women had DD genotype, whereas one woman had ID genotype.

There was no significant difference between genotype distribution and observed fetal growth restriction in women with preeclampsia (OR=1.57 95%CI 0.5534–4.4623). In women with preeclampsia, intrauterine fetal death was detected in three cases. Two patients had II genotype, while one had DD genotype. Abruption of placenta was diagnosed in two women with preeclampsia with II genotype.

## Discussion and Conclusion

This study failed to confirm the association between the angiotensin-converting enzyme deletion gene polymorphism and preeclampsia in our study group. Several studies conducted among Chinese population suggested that DD genotype was a risk factor, whereas insertion genotype was a molecular marker of reduced risk for preeclampsia<sup>10,15,16</sup>. Kobashi et al.<sup>17</sup> found no significant differences in the frequency of DD genotype between Japanese women with preeclampsia and control group, although the authors confirmed the higher frequency of DD genotype in the patients with positive history of preeclampsia (25%) compared with the control group (8%). Choi et al.<sup>9</sup> showed that I/D polymorphism of the ACE gene (rather than angiotensinogen T174M and M235T genotypes) was associated with the higher incidence of preeclampsia in Korean woman. Several groups investigated the association of insertion-deletion polymorphism with pree-

clampsia in Caucasian population, but failed to prove significant difference in allelic distribution between women with preeclampsia and healthy controls<sup>12,18,19</sup>. According to the results of previously published studies, it could be concluded that association between the ACE gene insertion/deletion polymorphism and preeclampsia depends on the ethnic origin of investigated population<sup>20</sup>.

We found increased D allele frequency in women with recurrent preeclampsia and those who required delivery before 34 weeks of pregnancy. Several groups have investigated the frequency of D allele in carefully selected groups of patients with early onset and recurrent forms of preeclampsia. Mello et al.<sup>21</sup> showed a significant association between DD genotype in women with history of preeclampsia and the recurrence of an adverse obstetric outcome such as preeclampsia and fetal growth restriction affecting umbilical and uteroplacental flows. They also showed that the treatment of women with DD genotype and history of preeclampsia with low-molecular-weight heparin reduced the recurrence of adverse clinical outcomes, improving uteroplacental flow and maternal blood pressure<sup>22</sup>. Another study implicated the influence of DD genotype on haemostasis balance in patients with untreated hypertension, reflecting as an impairment of endothelial function<sup>23</sup>. Moreover, ACE DD geno-

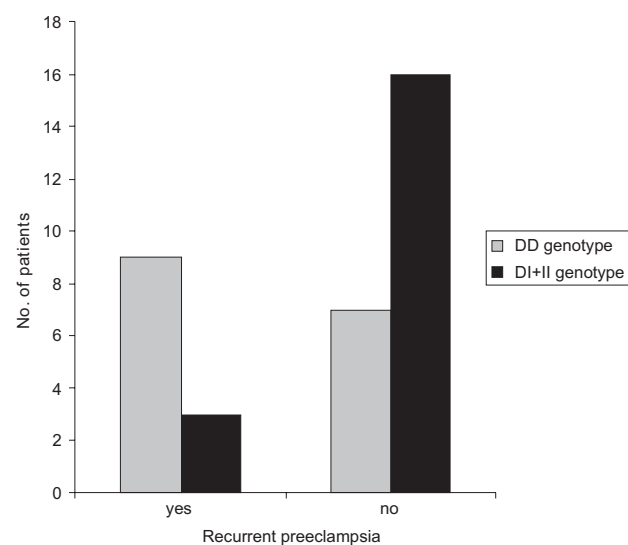


Fig. 1. Distribution of deletion genotype in patients with previous and successive pregnancy with preeclampsia.

type could be also a risk factor for fetal first trimester loss and for venous thromboembolism<sup>24,25</sup>.

Our study did not show that ACE gene insertion-deletion polymorphism had a direct impact of the pathophysiologic condition of preeclampsia, although moderate positive associations cannot be ruled out. The results of our study imply that deletion genotype in the group of patients with positive history for preeclampsia could have some importance. In such cases, the deletion genotype could be an additional risk factor, acting synergistically with other gene polymorphisms to increase the risk of preeclampsia. A Croatia-wide study of women with re-

current preeclampsia and preeclampsia requiring delivery before 34 weeks of pregnancy should be performed in order to explain the exact role of this and other genetic polymorphisms as contributing factors for the development of preeclampsia.

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### ZNAČAJ GENOTIPIZACIJE POLIMORFIZMA ANGIOTENZIN I-KONVERTIRAJUĆEG ENZIMA KAO MOLEKULARNOG BILJEGA TRUDNOĆOM POTAKNUTE HIPERTENZIJE

### SAŽETAK

Abnormalni procesi nastajanja i modeliranja krvnih žila vode razvoju preeklampsije. Insercijsko/delecijski polimorfizam angiotenzin I-konvertirajućeg enzima, ima značajnu ulogu u patogenezi velikog broja poremećaja funkcije endotelnih žila i patogenezi nekoliko oblika eksperimentalne i humane hipertenzije. Cilj istraživanja bio je utvrditi probirni značaj insercijsko/delecijskog polimorfizma angiotenzin I-konvertirajućeg enzima kao mogućeg čimbenika rizika razvoja hipertenzije u trudnoći; odrediti pojavnost I i D alela u preeklampsičnoj i usporednoj skupini trudnica i usporediti ove dvije skupine prema ishodu, trajanju trudnoće, paritetu, dobi, indeksu tjelesne težine, porodnoj gestacijskoj dobi i porodnoj težini djeteta. Genomska DNA izolirana je iz pune krvi 60 preeklampsičnih trudnica i 50 normotenzivnih trudnica. Preeklampsija se definira kao krvni tlak viši od 140/90 mmHg u odnosu na vrijednosti prije trudnoće i protei-nurija >300 mg/L tijekom 24 sata nakon 20 tjedana trudnoće. Genotipizacija insercijsko/delecijskog polimorfizma unutar introna 16 ACE gena provela se metodom lančane reakcije polimeraze i vizualizacijom elektroforezom u gelu agaroze. Usporedba genotipova i pojavnosti alela provela se Mantel-Haenszelovim  $\chi^2$  testom. Raspodjela I i D alela ACE



polimorfizma između usporedne i preeklampsijske skupine nije se statistički razlikovala. Zastupljenost trudnica s DD genotipom u preeklampsijskoj skupini je veća, ali ne statistički značajna, i iznosi 43,3%, dok 28% normotenzivnih trudnica ima isti genotip ( $p=0,096$ ). Kod preeklampsijskih trudnica koje su rodile prije 34. tjedna trudnoće, raspodjela D alela bila je statistički značajno viša i iznosila je 0.735 u odnosu na ostale ( $p=0,036$ ). U preeklampsijskoj skupini trudnica koje su i u prethodnoj trudnoći imale preeklampsiju udio D alela iznosio je 0,83 ( $p=0,01$ ). Nismo našli statistički značajnu razliku između pojavnosti određenog genotipa i intrauterinog zastoja rasta ploda u preeklampsijskoj skupini. Delecijski polimorfizam nema direktnu ulogu u razvoju preeklampsije, međutim određene pozitivne sveze se ne mogu u potpunosti isključiti. Naše istraživanje je pokazalo statistički značajnu pozitivnu svezu između pojavnosti D alela i rizika razvoja preeklampsije u sljedećoj trudnoći kod trudnica koje su u prethodnoj trudnoći imale preeklampsiju i poroda prije 34. tjedna trudnoće kod preeklampsijskih trudnica. U ovakvim slučajevima delecijski genotip mogao bi biti dodatni čimbenik rizika, koji djeluje sinergistički s drugim genskim polimorfizmima, povećavajući rizik razvoja preeklampsije.