

Associations of Insertion-deletion Polymorphism of Angiotensin-converting Enzyme with the Risk of Preeclampsia Development among Pregnant Women in Central Russia

Evgeny A. Reshetnikov, Tatjana I. Yakunchenko, Inna K. Aristova, Alexey V. Polonikov, Mikhail I. Churnosov

Department of Medical Biological Disciplines, Belgorod National Research University, 85 Pobeda St., Belgorod, 308015, Russia

Abstract

Objectives: The associations of the insertion-deletion angiotensin-converting enzyme (I/D ACE) genetic polymorphism with the risk of preeclampsia development were studied. **Materials and Methods:** The study group included 350 women: 100 women with a physiological pregnancy and 250 pregnant women with preeclampsia. All women underwent the typing of I/D polymorphism of the ACE gene. **Results:** The pregnant women with the preeclampsia of the 2nd degree of severity showed the lowest concentration of genotype II ACE (11.00%) as compared with pregnant women without preeclampsia (25.00%; odds ratio [OR] = 0.37; $P = 0.017$; $P_{\text{cor}} = 0.051$). With the preeclampsia of the 3rd degree of severity, the minimum frequency of the genotype II ACE (7.27%) and the maximum frequency of the allele D ACE (62.73%) are recorded among pregnant women as compared with the pregnant women without preeclampsia (25.00%; OR = 0.24; $P = 0.01$; $P_{\text{cor}} = 0.03$ and 49.50%, OR = 1.72, $P = 0.03$, respectively). **Conclusions:** Thus, the D ACE allele is associated with the risk of preeclampsia development of the 3rd degree of severity, and the ACE genotype II is a protective factor of preeclampsia development of the 2nd and the 3rd degree of severity.

Key words: Genetic polymorphism, preeclampsia, pregnancy, renin-angiotensin-aldosterone system

INTRODUCTION

Preeclampsia is the pregnancy complication that occurs in the second half of it, and characterized by the appearance of edema, proteinuria, hypertension, and profound disabilities of the vascular system function, hemostasis, immunity, hemodynamics, and microcirculation, the development of fetoplacental insufficiency, renal, liver, and lung dysfunction.^[1,2] According to the literature data, the frequency of preeclampsia makes 4-18% among all pregnant women.^[3] Preeclampsia is the major cause of preterm labor onset, the premature detachment of properly located placenta, intrauterine growth retardation, and the low birth weight among infants.^[4]

In the preeclampsia pathogenesis, an important role is provided to vascular reactions, which are based on generalized vasospasm, conditioning

the ischemic, and hypoxic changes in the tissues with the violation of their functions.^[5] Renin-angiotensin-aldosterone system plays an important role in the functioning of a cardiovascular system, particularly angiotensin-converting enzyme (ACE) hydrolyzing angiotensin I into angiotensin II which is a potent vasoconstrictor.^[6,7]

One of ACE gene well-studied polymorphisms is the presence or absence (insertion/deletion [I/D] ACE) of 287 bp fragment in the 16th gene intron.^[8] This polymorphism is not

Address for correspondence:

Evgeny A. Reshetnikov, Department of Medical Biological Disciplines, Belgorod National Research University, 85 Pobeda St., Belgorod, 308015, Russia.
E-mail: reshetnikov@bsu.edu.ru

Received: 31-08-2017

Revised: 13-09-2017

Accepted: 18-09-2017

structural, but apparently it affects the degree of this gene expression. This is confirmed by several studies in which it was shown that healthy individuals with DD genotype have maximum level of ACE in blood, people with the II genotype have decreased ACE level (in 2 times) and heterozygotes have intermediate blood enzyme level.^[8-10]

The available data on the relationship between the polymorphism of ACE gene and the risk of preeclampsia development are contradictory.^[11-20]

In this regard, further research is needed to study the role of I/D ACE locus in the development of this complication of pregnancy.

MATERIALS AND METHODS

Object of study

The study group included 350 women: 250 pregnant women with preeclampsia and 100 women with normal pregnancy. The samples under study included the individuals of Russian nationality who were born in the Central Black Earth region of Russia and did not have any kinship. The average age of pregnant women with preeclampsia was 27.11 ± 6.42 years (it varied from 18 to 44 years), the control group age was 26.50 ± 6.36 years (the age varied from 18 to 42 years) ($P > 0.05$). Clinical and laboratory examination of pregnant women was carried out at the time of delivery on the basis of the Perinatal Center of the Belgorod Regional Clinical Hospital of St. Joasaph.

Among 350 pregnant women, 100 patients had a physiological course of gestation and 250 women with the pregnancy complicated by preeclampsia: 95 pregnant women with preeclampsia of the 1st degree of severity, 100 with the preeclampsia of the 2nd degree of severity, and 55 with the preeclampsia of the 3rd degree of severity. Preeclampsia was defined as the presence of hypertension, accompanied by proteinuria, as defined by a 24 h urine protein excretion more than 300 mg (ACOG Committee on Practice Bulletins-Obstetrics, 2002).^[1] The degree of preeclampsia severity was assessed by Goecke scale in the modification by Savelieva.^[21]

Molecular and genetic methods

All women underwent the typing of I/D polymorphism of the ACE gene.

The material for the study was venous blood in the volume of 8-9 ml, taken from the ulnar vein of the proband. The isolation of genomic DNA from peripheral blood was carried out by the phenol-chloroform extraction method.^[22] The locus analysis was performed by polymerase chain

reaction of DNA synthesis. The genotyping of the DNA marker was performed by the methods of polymorphism analysis concerning the lengths of amplified fragments (I/D ACE).

Statistical methods

The development of the database and the statistical calculations were carried out using the program "STATISTICA 6.0." To assess the compliance of the observed distribution of genotypes with an expected one, the criterion χ^2 was used, based on the Hardy-Weinberg equilibrium. The associations of the alleles and the genotypes of the studied DNA markers with preeclampsia were evaluated by the analysis of 2×2 contingency tables with the calculation of the χ^2 criterion using the Yates correction for continuity and the odds ratio (OR) with 95% confidence interval. Bonferroni amendment was used during the performance of multiple comparisons.^[23]

RESULTS

The study showed that there is the tendency to increase the concentration of the D allele of the I/D ACE locus (56.80%) among the pregnant women with preeclampsia in comparison with the pregnant ones of the control group (49.50%), but these differences do not reach a statistically significant level ($P = 0.10$) [Table 1].

The analysis of genotype and allele frequency distribution of I/D ACE in the groups of pregnant women with preeclampsia with various severity and in the control group showed that the pregnant women with the preeclampsia of the 2nd severity level had the lowest concentration of genotype II ACE (11.00%) (25.00%; $\chi^2 = 5.73$; OR = 0.37; 95% CI: 1.18-6.29; $P = 0.017$; $P_{\text{cor}} = 0.051$) [Table 2].

At the preeclampsia of the 3rd degree of severity, the minimum frequency of genotype II ACE (7.27%) [Table 2] and the maximum diffusion of D ACE allele (62.73%) are recorded among pregnant women, as compared with pregnant women without preeclampsia (25.00%; $\chi^2 = 6.21$; OR = 0.24, 95% CI: 1.29-15.39, $P = 0.01$, $P_{\text{cor}} = 0.03$ and 49.50%, $\chi^2 = 4.48$, OR = 1.72, 95% CI: 1.04-2.84, $P = 0.03$, respectively).

DISCUSSION

The data obtained from the study indicate that the genetic polymorphism of the I/D ACE is associated with the severity of preeclampsia. In this work, they found that the formation of preeclampsia of the 2nd degree of severity is associated with the genetic variants of II ACE (OR = 0.37), and preeclampsia of the 3rd degree is associated with D ACE (OR = 1.72), and II ACE (OR = 0.24).

Table 1: Comparative analysis of frequencies of alleles and genotypes of I/D ACE in the studied groups of women

Gene	Allele, genotype	Pregnant women with preeclampsia		Pregnant women without preeclampsia		OR (95% CI) χ^2, P
		<i>n</i> =250 (%)		<i>n</i> =100 (%)		
		<i>n</i>	%	<i>n</i>	%	
I/D ACE	D	284	56,80	99	49.50	0.75 (0.53-1.05) 2.79, 0.10
	I	216	43.20	101	50.50	
	DD	76	30.40	24	24.00	0.72 (0.41-1.27) 1.14, 0.29
	ID	132	52.80	51	51.00	0.93 (0.57-1.52) 0.04, 0.85
	II	42	16.80	25	25.00	1.65 (0.91-2.30) 2.60, 0.11

I/D ACE: Insertion deletion angiotensin converting enzyme, OR: Odds ratio, CI: Confidence interval

Table 2: Comparative analysis of genotype frequencies of I/D ACE among pregnant women with preeclampsia of varying severity and in the control group

Polymorphism Locus	Genotype	Pregnant women without preeclampsia (<i>n</i> =100)		Pregnant women with preeclampsia (<i>n</i> =250)								
		<i>n</i>	%	Preeclampsia of the 1 st degree of severity (<i>n</i> =95)			Preeclampsia of the 2 nd degree of severity (<i>n</i> =100)			Preeclampsia of the 3 rd degree of severity (<i>n</i> =55)		
				<i>n</i>	%	χ^2, P	<i>n</i>	%	χ^2, P	<i>n</i>	%	χ^2, P
I/D ACE	DD	24	24.00	29	30.53	$\chi^2=0.75$ $P=0.39$	29	29.00	$\chi^2=0.41$ $P=0.52$	18	32.73	$\chi^2=0.96$ $P=0.33$
	ID	51	51.00	39	41.05	$\chi^2=1.56$ $P=0.21$	60	60.00	$\chi^2=1.30$ $P=0.26$	33	60.00	$\chi^2=0.82$ $P=0.36$
	II	25	25.00	27	28.42	$\chi^2=0.14$ $P=0.71$	11	11.00	$\chi^2=5.73$ $P=0.017$	4	7.27	$\chi^2=6.21$ $P=0.01$

I/D ACE: Insertion deletion angiotensin converting enzyme

According to the published data, the individuals with allele D of ACE gene have a higher circulating enzyme level in blood as compared to the carriers of the ACE genotype II, heterozygotes ID ACE have an intermediate level of enzyme.^[8-10] Increased ACE activity among the people with DD ACE genotype leads to the increase of angiotensin II level in plasma, which may increase the risk of preeclampsia development.

The studies on the search for polymorphism associations I/D ACE with the risk of preeclampsia development were conducted in various populations of the world.

The studies performed in Iranian and Indian populations showed that the risk of preeclampsia development is higher among pregnant women with the D ACE allele.^[13,15,17] In addition, the populations of Romania and the United States revealed the associations of DD ACE genotype with the development of preeclampsia.^[12,14] Besides, the studies conducted in the populations of India, China, and Colombia, revealed no significant effect of I/D ACE on the development of preeclampsia.^[11,16,19]

The observed inconsistency in the above results can be explained by interpopulation and interethnic differences in the distribution of the I/D polymorphism: allele D is predominant in Caucasians from Europe, Australia, and the USA, whereas allele I is more prevalent among Chinese and Indians.^[24-27]

CONCLUSIONS

Thus, it was established that the allele D ACE is associated with the risk of preeclampsia development of the 3rd degree of severity, and the genotype II of ACE is a protective factor in the development of preeclampsia of the 2nd and the 3rd degree of severity.

SUMMARY

The results of the performed study broaden the understanding of the molecular genetic determinants for the development of preeclampsia. The obtained data can be used in the work

of women's clinics and obstetric-gynecological hospitals to identify the groups with pregravid preparation and early pregnancy groups with an increased risk of preeclampsia development.

REFERENCES

1. ACOG Committee on Practice Bulletins-Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol* 2002;99(1):159-67.
2. Hayashi M, Inoue T, Hoshimoto K, Negishi H, Ohkura T, Inaba N. Characterization of five marker levels of the hemostatic system and endothelial status in normotensive pregnancy and pre-eclampsia. *Eur J Haematol* 2007;69:297-302.
3. Clark SL, Belfort MA, Dildy GA, Herbst MA, Meyers JA, Hankins GD. Maternal death in the 21st century: Causes, prevention, and relationship to cesarean delivery. *Am J Obstet Gynecol* 2008;199:36.e1-5.
4. Benyo DF, Miles TM, Conrad KP. Hypoxia stimulates cytokine production by villous explants from the human placenta. *J Clin Endocrinol Metab* 2008;82:1582-8.
5. Hubel CA. Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med* 2007;222:222-35.
6. Díez J. Profibrotic effects of angiotensin II in the heart: A matter of mediators. *Hypertension* 2004;43:1164-5.
7. Reshetnikov EA, Akulova LY, Batlutskaya IV. Molecular genetic mechanisms of the cardiovascular system functioning and the role of the renin-angiotensin system for cardiovascular reaction provision in a body. *Scientific bulletins of BelSU. Belgorod state university scientific bulletin. Ser Med Pharm* 2013;11:179-84.
8. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86:1343-6.
9. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, *et al.* Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 1992;51:197-205.
10. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, *et al.* Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. *Circulation* 1995;92:1387-8.
11. Serrano NC, Díaz LA, Páez MC, Mesa CM, Cifuentes R, Monterrosa A, *et al.* Angiotensin-converting enzyme I/D polymorphism and preeclampsia risk: Evidence of small-study bias. *PLoS Med* 2006;3:e520.
12. Uma R, Forsyth SJ, Struthers AD, Fraser CG, Godfrey V, Murphy DJ. Polymorphisms of the angiotensin converting enzyme gene in early-onset and late-onset pre-eclampsia. *J Matern Fetal Neonatal Med* 2010;23:874-9.
13. Aggarwal S, Dimri N, Tandon I, Agarwal S. Preeclampsia in North Indian women: The contribution of genetic polymorphisms. *J Obstet Gynaecol Res* 2011;37:1335-41.
14. Procopciuc LM, Hazi GM, Caracostea G, Nemeti G, Olteanu I, Stamatian F. Apolipoprotein E polymorphism-a risk factor in Romanian pregnant women with preeclampsia. *Gineco Ro* 2011;7:134-40.
15. Salimi S, Mokhtari M, Yaghmaei M, Jamshidi M, Naghavi A. Association of angiotensin-converting enzyme intron 16 insertion/deletion and angiotensin II Type 1 receptor A1166C gene polymorphisms with preeclampsia in South East of Iran. *J Biomed Biotechnol* 2011;2011:941515.
16. Shaik AP, Sultana A, Bammidi VK, Sampathirao K, Jamil K. A meta-analysis of eNOS and ACE gene polymorphisms and risk of pre-eclampsia in women. *J Obstet Gynaecol* 2011;31:603-7.
17. Rahimi Z, Rahimi Z, Mozafari H, Parsian A. Preeclampsia and angiotensin converting enzyme (ACE) I/D and angiotensin II type-1 receptor (AT1R) A1166C polymorphisms: Association with ACE I/D polymorphism. *J Renin Angiotensin Aldosterone Syst* 2013;14:174-80.
18. Ovcharova VS. The study of polymorphism relation-1306C/T MMP-2 c with the development of arterial hypertension among the women with preeclampsia. *Scientific bulletins of BelSU. Series medicine. Pharmacia* 2014;11:210-3.
19. Ma L, Fan P, Liu XH, He GL, Liu R, Ren RM, *et al.* Interaction between GNB3 C825T and ACE I/D polymorphisms in pre-eclampsia. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2015;46:118-22.
20. Reshetnikov EA, Akulova LY, Dobrodomova IS, Dvornyk VY, Polonikov AV, Churnosov MI. The insertion-deletion polymorphism of the ACE gene is associated with increased blood pressure in women at the end of pregnancy. *J Renin Angiotensin Aldosterone Syst* 2015;16:623-32.
21. Savelyeva GM, Shalina RI. Modern problems of etiology, pathogenesis, therapy and gestosis prevention. *Obstet Gynecol* 1998;5:6-9.
22. Mathew CG. The isolation of high molecular weight eukaryotic DNA. *Methods Mol Biol* 1985;2:31-4.
23. Yu RO. *Statistical Analysis of Medical Data Application, The Use of the Statistica Software Package.* Moscow: StatSoft, Inc.; 2006.
24. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, *et al.* Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641-4.
25. Lee EJ. Population genetics of the angiotensin-converting enzyme in Chinese. *Br J Clin Pharmacol* 1994;37:212-4.
26. Sagnella GA, Rothwell MJ, Onipinla AK, Wicks PD,

Cook DG, Cappuccio FP. A population study of ethnic variations in the angiotensin-converting enzyme I/D polymorphism: Relationships with gender, hypertension and impaired glucose metabolism. *J Hypertens* 1999;17:657-64.

27. Sabbagh AS, Otrock ZK, Mahfoud ZR, Zaatari GS,

Mahfouz RA. Angiotensin-converting enzyme gene polymorphism and allele frequencies in the Lebanese population: Prevalence and review of the literature. *Mol Biol Rep* 2007;34:47-52.

Source of Support: Nil. **Conflict of Interest:** None declared.