Journal of Human Hypertension (2018) 32:825–830
https://doi.org/10.1038/s41371-018-0096-4

ARTICLE

ACE gene rs4343 polymorphism elevates the risk of preeclampsia in pregnant women

Atieh Abedin Do[1] · Emran Esmaeilzadeh[2] · Mona Amin-Beidokhti[1] · Reihaneh Pirjani[3] · Milad Gholami[4] · Reza Mirfakhraie[1,5]

Received: 18 April 2018 / Revised: 16 July 2018 / Accepted: 23 July 2018 / Published online: 20 August 2018

Abstract

The multifactorial basis of preeclampsia (PE) implies that there are several genes and risk factors that are important in the development of the disease. Therefore, the exact etiology and pathogenesis of preeclampsia remains unclear. It is suggested that inappropriate regulation of the renin–angiotensin system (RAS) is a risk factor for hypertension during pregnancy. The angiotensin I-converting enzyme (ACE) serum level, a key component of the RAS, affects the blood pressure. It is hypothesized that the ACE gene polymorphisms contribute to preeclampsia development. In a case–control study containing 296 subjects (165 PE patients and 131 normotensive controls), we aimed to examine the association of the ACE gene I/D and rs4343 polymorphisms with preeclampsia in Iranian women. Genotyping for rs4343 and ACE I/D polymorphisms was performed by using TP-ARMS-PCR and conventional PCR, respectively. The rs4343 G allele frequency was higher in the case group (OR = 1.90, 95% CI, 1.37–2.65; $P = 0.0001$). Besides, a significant difference was detected for the genotype frequencies between the studied groups under dominant (OR = 3.94, 95% CI, 2.05–7.56; $P < 0.0001$) and recessive (OR = 2.21, 95% CI, 1.22–4.01; $P = 0.009$) inheritance models. For the I/D polymorphism, no significant differences were detected in the genotype and allele frequencies or any of the inheritance models between PE patients and controls. To verify the current results and validate the significance of the studied genetic variations, additional studies in diverse ethnic populations are required.

Introduction

Preeclampsia (PE) is a common multisystem and multifactorial pregnancy disorder that affects approximately 2%–8% of all pregnancies. Despite the developments in prenatal and neonatal care, this disorder is still one of the most important reasons behind maternal and neonatal morbidity and mortality [1]. PE is characterized by systolic blood pressure (SBP) ≥ 140 mm Hg or diastolic blood pressure (DBP) ≥ 90 mm Hg with evidence of proteinuria (≥ 300 mg/24 h) after 20 weeks of pregnancy. Other symptoms include edema, vision defects, headache, abdominal pain, and thrombocytopenia [2, 3]. Moreover, women suffering from PE have an increased risk of cardiovascular disorders that suggests a common etiology [4]. Although the etiology and pathogenesis of PE have not been clearly identified, it is thought to have an essential genetic component [5, 6] Several genetic case–control studies have stated associations between PE and various gene polymorphisms [7–10]. The lack of reproducibility of these studies has led to the uncertainty about the genes that contribute to the disease pathogenesis [11]. Several studies have demonstrated that inappropriate regulation of the renin–angiotensin system (RAS) may contribute to the pathogenesis of multiple cardiovascular disorders and blood

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41371-018-0096-4) contains supplementary material, which is available to authorized users.

✉ Reza Mirfakhraie
reza_mirfakhraie@yahoo.com

1 Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Neuroscience Research Center, Iran University of Medical Sciences, Tehran, Iran

3 Obstetrics and Gynecology Department, Arash Women Hospital, Tehran University of Medical Sciences, Tehran, Iran

4 Department of Clinical Biochemistry and Genetics, Arak, Iran

5 Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

pressure regulation during pregnancy [12, 13]. Angiotensin-converting enzyme (ACE), a key component of the RAS, converts inactive angiotensin I into active angiotensin II [14]. The ACE gene (Gene ID: 1636) is located in chromosome 17q23 and comprises several polymorphisms including Insertion/Deletion (I/D) and rs4343 that alter the enzyme activity [15, 16]. The I/D polymorphism is characterized by a 287 base pair segment that is either inserted (I) or deleted (D) in the ACE gene intron 16. The consequence of rs4343 polymorphism, located in exon 17, is Thr 776 Thr synonymous substitution (NP_000780.1).

As studies on the etiology of PE have suggested an inherited susceptibility, any relation between PE and genetic polymorphisms of the components of the RAS-related genes will be of interest.

Therefore, in the present study, we assessed the association of the ACE gene I/D and rs4343 variations with PE in Iranian women. We believe that this is the first study regarding the association between rs4343 variation and PE susceptibility in Iranian population.

Methods

Subjects

In this case–control study, 296 participants were enrolled, including 165 PE patients and 131 healthy controls with similar age and ethnic background. The mean age was 33.69 ± 8.92 years (range: 17–42) and 34.5 ± 10.03 years (range: 16–40) in the patients and controls, respectively. The criteria defined for PE were as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg measured at least two times after 20 weeks of pregnancy, and the existence of proteinuria ≥ 300 mg/24 h or ≥ 1 + reading on dipstick in a random urine sample at least twice with no evidence of a urinary tract infection.

Severe PE patients had SBP ≥ 160 mm Hg, DBP ≥ 110 mm Hg, and a proteinuria level of > 5 g/24 h or > 3 +. Patients who had gestational hypertension, history of hypertension, diabetes mellitus, and autoimmune and endocrine diseases were not included in the present study. The normotensive control subjects were recruited from the same center randomly and had no history of pregnancy complications. The same exclusion criteria were considered for the control group. We obtained informed written consent from all participants. The study protocol was approved by Shahid Beheshti University of Medical Sciences ethics committee (Code No: IR.SBMU. MSP.REC.1396.792).

Genotyping of I/D polymorphism

After collecting whole blood samples from all subjects in EDTA tubes, genomic DNA was extracted by using the BioFact Genomic DNA Prep Kit (Catalog number GD261-060, South Korea) according to the manufacturer's procedure.

I/D polymorphism was detected with conventional PCR analysis according to the previous study [17]. The 25 µl amplification reaction included 12.5 µl Taq DNA Polymerase 2 × Master Mix Red (Ampliqon, Denmark), 5 pmol/l of each primer, and genomic DNA (≥ 100 ng). PCR was performed on a GeneTouch thermal cycler apparatus (BIOER, China) and the amplification program was as follows: initial denaturation for 1 min at 94 °C; 30 cycles at 94 °C for 40 s, 67 °C for 30 s, and 72 °C for 40 s; and final extension for 4 min at 72 °C. The amplified PCR products were electrophoresed on a two percent agarose gel prepared in 0.5 × Tris-Borate-EDTA buffer. The PCR amplicons were 192 and 480 bp for the alleles with deletion and insertion, respectively. The accuracy of genotyping was further confirmed with Sanger sequencing of 10% of the DNA samples using an Applied Biosystems 3730xl DNA Analyzer (Macrogen, Korea).

Genotyping of rs4343 polymorphism

To genotype rs4343, tetra primer-amplification refractory mutation system-PCR (TP-ARMS-PCR) method was carried out according to a previous study [18]. The components of the 25 µl PCR reaction solution were as follows: 12.5 µl of Taq DNA Polymerase 2 × Master Mix Red (Ampliqon), 3 pmol/l of forward outer, 5 pmol/l of reverse outer, 12 pmol/l forward inner and 10 pmol/l of reverse inner primers, and ≥ 100 ng of genomic DNA. GeneTouch thermal cycler apparatus (BIOER) was used to amplify genomic DNA and the amplification program was as follows: initial denaturation for 1 min at 94 °C; 30 cycles at 94 °C for 35 s, 61 °C for 45 s, 72 °C for 35 s, and a final extension for 4 min at 72 °C. The amplified PCR products were electrophoresed on a two percent agarose gel prepared in 0.5 × Tris-Borate-EDTA buffer. The rs4343 A and G alleles created 202 and 134 bp bands, respectively. The outer primers amplified a common 280 bp band. The accuracy of genotyping was further confirmed with Sanger sequencing of ten percent of the samples by using an ABI 3730xl DNA analyzer (Macrogen).

Statistical analysis

We used SNPStats online software for calculating allele and genotype frequencies for both variations (http://bioinfo.iconcologia.net/SNPstats) [19]. Deviation from the Hardy–Weinberg equilibrium (HWE) was estimated by using the χ^2-test. The association with PE was investigated in recessive and dominant models. Odds ratios (OR) and 95% confidence intervals (CIs) were calculated to define the

Table 1 Clinical and demographic features and relationship between risk factors and PE in the cases and controls

	Case $\bar{x} \pm$ SD/*n* (%)	Control $\bar{x} \pm$ SD/*n* (%)	OR (95% CI)	*P*-value
Body mass index (kg/m^2)	29.59 ± 10.04	28.16 ± 4.15		0.13
Gestational age at birth (weeks)	36.16 ± 3.04	38.61 ± 0.98		<0.0001
Gestational age at preeclampsia (weeks)	33.76 ± 7.68			
Fetal weight (kg)	2.6 ± 0.68	3.3 ± 0.42		<0.0001
Systolic blood pressure (mm Hg)	146.22 ± 18.25	111.2 ± 8.11		<0.0001
Diastolic blood pressure (mm Hg)	92.98 ± 15.61	73.98 ± 6.93		<0.0001
History of pregnancy loss (*n* (%))	48 (29.09)	17 (12.97)	2.75 (1.49-5.06)	0.001
Family history of hypertension (*n* (%))	65 (39.39)	29 (22.13)	2.29 (1.36-3.83)	0.002
Preeclampsia type				
Mild	105 (63.64)			
Severe	60 (36.36)			
Delivery type				
Cesarian section	149 (90.30)	84 (64.12)		
Vaginal delivery	16 (9.69)	47 (35.87)		

CI confidence interval, *OR* odds ratio

strength of association between the studied polymorphisms and susceptibility to PE. The *P*-value of < 0.05 was considered to be statistically significant.

Results

Table 1 describes the characteristics and demographic data of the study contributors. The SBP and DBP values were significantly higher in PE patients compared with controls ($P < 0.05$). No significant difference was observed in pre-pregnancy body mass index (BMI) between the studied groups ($P > 0.05$). Moreover, the patients had a significantly lower gestational age at delivery and the fetal birth weight compared with the controls (both $P < 0.05$). A higher proportion of the patients had a history of pregnancy loss (OR = 2.75, 95% CI, 1.49–5.06; $P = 0.001$) compared with normal subjects. The same situation was observed in the family history of hypertension between the studied groups (OR = 2.29, 95% CI, 1.36–3.83; $P = 0.002$). In total, 63.64% of the patients were diagnosed as mild PE, whereas 36.36% were severe PE. The distribution of all genotypes was in complete HWE in the control group for both studied polymorphisms. Table 2 displays the genotype and allele frequencies for I/D and rs4343 polymorphisms in the studied groups.

For the rs4343, the G allele frequency was significantly higher in the case group (59.1%) compared with the control group (43.1%) (OR = 1.90, 95% CI, 1.37–2.65; $P = 0.0001$). The frequency of AG (OR = 2.36, 95% CI, 1.28–4.37; $P = 0.006$) and GG (OR = 2.89, 95% CI, 1.92–4.36; $P < 0.0001$) genotypes were different between PE patients and healthy subjects. Moreover, the genotype frequencies were different in the studied groups under dominant (OR = 3.94, 95% CI, 2.05–7.56; $P < 0.0001$) and recessive (OR = 2.21, 95% CI, 1.22–4.01; $P = 0.009$) inheritance models. For the I/D polymorphism, we did not observe significant differences in the genotype and allele frequencies or any of the inheritance models between the PE patients and controls.

Discussion

PE is a multifactorial disorder with a strong genetic evidence in the development of the disease. As a major component of the RAS, ACA has a key role in salt and water homeostasis and blood pressure [20]. Polymorphisms in the ACE gene affect the enzyme serum and tissue levels and therefore may play a significant role in PE. An increased level of ACE activity is considered a key factor in blood pressure alteration because of the increase in the potent vasoconstrictor angiotensin II and inactivation of bradykinin as a vasodilator factor [21]. The present results showed that the rs4343 is associated with PE in our studied population. Previous studies have revealed that rs4343 polymorphism is associated with left ventricular hypertrophy, blood pressure, coronary artery diseases and migraine [18, 22, 23]. However, there are only two studies regarding the role of rs4343 in developing PE [24, 25]. Zhang et al.

Table 2 The I/D and rs4343 polymorphisms genotype and allele frequencies in the cases and controls

Genotype	Case *N* (%)	Control *N* (%)	OR (95% CI)	*P*-value
I/D polymorphism				
D/D	58(35.1)	42(32.1)	1.00 (reference)	-
I/D	80(48.5)	62(47.3)	0.93 (0.56–1.57)	0.78
I/I	27(16.4)	27(20.6)	0.72 (0.37–1.41)	0.34
I/I and I/D vs. D/D			0.87 (0.54–1.42)	0.58
I/I vs. D/D and I/D			0.75 (0.42–1.36)	0.35
Allele				
D	196 (59.4)	146 (55.7)	1.16 (0.84–1.61)	0.37
I	134 (40.6)	116 (44.3)	0.86 (0.62–1.19)	0.37
rs4343				
A/A	15(9.1)	37(28.2)	1.00 (reference)	-
A/G	105(63.6)	75(57.3)	3.45 (1.77–6.74)	0.0003
G/G	45(27.3)	19(14.5)	5.84 (2.61–13.06)	< 0.0001
G/G and A/G vs. A/A			3.94 (2.05–7.56)	< 0.0001
G/G vs. A/A and A/G			2.21 (1.22–4.01)	0.009
Allele				
A	135 (40.9)	149 (56.9)	0.53 (0.38–0.73)	0.0001
G	195 (59.1)	113 (43.1)	1.90 (1.37–2.65)	0.0001

CI confidence interval, *OR* odds ratio

demonstrated that fetal but not maternal rs4343 was associated with PE in Han Chinese women [24]. Procopciuc et al. [25] also revealed that the rs4343 increased the risk of PE; however, this increase was not statistically significant.

The rs4343 results in the Thr 776 Thr synonymous substitution in the ACE gene (NP_000780.1). Emerging evidence suggests that silent mutations could have functional consequences and therefore contribute to the human disease risk [26]. On the mRNA level, these types of mutations can affect stability, folding and translation rate. In addition, they can result in aberrant mRNA splicing. It is obvious that any alterations in the mRNA molecule will affect the related protein structure, expression, substrate specificity, secretion and/or enzymatic activity [26, 27]. The main reason that a silent mutation may change protein properties is the codon usage bias, which refers to the preferred use of particular codons instead of other synonymous codons during mRNA translation. The frequency of codon usage varies among species, tissues, and genes [28].

To determine the effect of rs4343 on mRNA folding and the kinetics of local translation, we performed two in silico prediction analysis. First, the effect of the rs4343 on local stem-loop mRNA structure of ACE gene was predicted by the RNAsnp Web Server online program [29]. Second, we measured the change in relative synonymous codon usage (RSCU) value that explains possible alterations in the kinetics of local translation as previously described [26]. Although the mRNA folding was not predicted to be significantly affected by the rs4343 variation; however, the vale of RSCU decreased from 1.14 to 0.46 ($\Delta RSCU = -0.68$) (For further details, please see the Supplementary File S1). This reduction might be associated with a slower local rate of translation elongation on the rare codon compared to the wild-type and therefore could affect the enzyme properties. Moreover, it is suggested that rs4343 alters regulatory motifs for several transcription factors and therefore affects the ACE levels [18]. Zhu et al. [30] showed that the rs4343 had the greatest significant effect on the ACE function and the presence of the G allele increased both SBP and DBP. It seems that the rs4343 G allele increases activity of serum ACE and therefore produces higher levels of angiotensin II. Moreover, this allele was also associated with higher ACE serum levels in a sample of depressed patients [16].

There was no association between ACE I/D alleles or genotypes and PE in our studied population, and this is consistent with several but not all previous reports. There are controversies considering the results obtained from the previous studies regarding the role of ACE I/D in PE [20, 21, 31–34]. It is suggested that the DD genotype is associated with higher levels of ACE enzyme, blood pressure, and increased risk of cardiovascular disorders [35–37]. González-Garrido et al. [21] reported that ACE II and DD genotypes are protective and risk factors for PE in Mexican women, respectively. They concluded that the D allele increases the disease risk [21]. Although the frequency of the DD genotype in the case group was higher than in

controls in the present study, however the difference was not statistically significant (Table 2). It seems that the existence of a silencer-like sequence in the I allele results in a greater ACE level in the D allele [38]. Zhang et al. [24] also confirmed the association of ACE I/D polymorphism with PE in Han Chinese population; however, the same as rs4343, this association was found in fetal but not maternal samples [24]. In a meta-analysis study, Serrano et al. suggested that the detected increase in the PE risk associated with the D allele is due to small-study bias [39]. Totally, the observed inconsistency among previous studies is due to genetic heterogeneity, ethnicity, sample volume, PE criteria, and other environmental factors.

Investigation of the role of ACE gene polymorphisms in PE maybe a reliable tool to identify and manage the women at risk. Alvi et al. [40] showed that the combination of the AA and DD genotypes results in higher than average blood pressure level, both in hypertensive and healthy people in the Punjabi population.

In conclusion, the present results suggest the association between rs4343 and susceptibility to PE. Further educations with a larger sample size and analyses of genetic and environmental factors are needed to confirm the present results. In addition, measuring the enzyme serum levels may prove the functional effects of the studied polymorphisms.

Summary

What is known about this topic?

- Deregulation of the renin–angiotensin system play an important role in the pathogenesis of preeclampsia.
- Polymorphisms in the ACE gene affect the enzyme serum and tissue levels.
- Previous studies indicated controversies considering the association between ACE I/D gene polymorphism and susceptibility to preeclampsia.

What this study adds?

- To the best of our knowledge, this is the first study that reports maternal ACE rs4343is associated with preeclampsia.
- The rs4343 alters regulatory motifs for several transcription factors and therefore affects the ACE level.
- No association was detected between ACE I/D alleles or genotypes and PE in the studied population.

Acknowledgements We are grateful to Arash Women Hospital and all participants for their kind collaboration.

Conflict of Interest The authors declare that they have no conflict of interest

References

1. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005;365:785–99.
2. Redman CW, Sargent IL. Latest advances in understanding pre-eclampsia. Science. 2005;308:1592–94.
3. Obstetricians ACo, Gynecologists. Diagnosis and management of preeclampsia and eclampsia. Obstet Gynecol. 2002;99:159–67.
4. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA. Cardiovascular health after maternal placental syndromes (CHAMPS): population-based retrospective cohort study. Lancet. 2005;366:1797–803.
5. Salonen Ros H, Lichtenstein P, Lipworth L, Cnattingius S. Genetic effects on the liability of developing pre-eclampsia and gestational hypertension. Am J Med Genet A. 2000;91:256–60.
6. Krotz S, Fajardo J, Ghandi S, Patel A, Keith LG. Hypertensive disease in twin pregnancies: a review. Twin Res Hum Genet. 2002;5:8–14.
7. Lachmeijer AM, Arngrímsson R, Bastiaans EJ, Pals G, Leo P, de Vries JI, et al. Mutations in the gene for methylenetetrahydrofolate reductase, homocysteine levels, and vitamin status in women with a history of preeclampsia. Am J Obstet Gynecol. 2001;184:394–402.
8. Christina K, Casas JP, Savvidou MD, Sahemey MK, Nicolaides KH, Hingorani AD. Endothelial nitric oxide synthase gene polymorphism (Glu298Asp) and development of pre-eclampsia: a case-control study and a meta-analysis. BMC Pregnancy Childbirth. 2006;6:7.
9. Kosmas IP, Tatsioni A, Ioannidis JP. Association of Leiden mutation in factor V gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis. J Hypertens. 2003;21:1221–28.
10. Gholami M, Mirfakhraie R, Pirjani R, Taheripanah R, Bayat S, Daryabari SA et al. Association study of FOXP3 gene and the risk of 0020 pre-eclampsia. Clin Exp Hypertens. 2017;5:1–4.
11. Colhoun HM, McKeigue PM, Smith GD. Problems of reporting genetic associations with complex outcomes. Lancet. 2003;361:865–72.
12. Zaman MA, Oparil S, Calhoun DA. Drugs targeting the renin–angiotensin–aldosterone system. Nat Rev Drug Discov. 2002;1:621.
13. Shah DM. The role of RAS in the pathogenesis of preeclampsia. Curr HypertensRrep. 2006;8:144–52.
14. Eisenmann JC, Sarzynski MA, Glenn K, Rothschild M, Heelan KA. ACE I/D genotype, adiposity, and blood pressure in children. Cardiovasc Diabetol. 2009;8:14.
15. Chung C, Wang R, Chen J, Fann C, Leu H, Ho H, et al. genome-wide association study identifies new loci for ACE activity: potential implications for response to ACE inhibitor. Pharm J. 2010;10:537.
16. Firouzabadi N, Shafiei M, Bahramali E, Ebrahimi SA, Bakhshandeh H, Tajik N. Association of angiotensin-converting enzyme (ACE) gene polymorphism with elevated serum ACE activity and major depression in an Iranian population. Psychiat Res. 2012;200:336–42.
17. Hühne K, Leis S, Schmelz M, Rautenstrauss B, Birklein F. A polymorphic locus in the intron 16 of the human angiotensin-converting enzyme (ACE) gene is not correlated with complex regional pain syndrome I (CRPS I). Eur J Pain. 2004;8:221–25.
18. Abedin-Do A, Pouriamanesh S, Kamaliyan Z, Mirfakhraie R. Angiotensin-converting enzyme gene rs4343 polymorphism increases susceptibility to migraine. CNS Neurosci Ther. 2017;23:698–99.

19. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics. 2006;22:1928–29.
20. Choi H, Kang JY, Yoon HS, Han SS, Whang CS, Moon IG, et al. Association of angiotensin-converting enzyme and angiotensinogen gene polymorphisms with preeclampsia. J Korean Med Sci. 2004;19:253–57.
21. González-Garrido JA, García-Sánchez JR, Tovar-Rodríguez JM, Olivares-Corichi IM. Preeclampsia is associated with ACE I/D polymorphism, obesity and oxidative damage in Mexican women. Pregnancy Hypertens. 2017;10:22–27.
22. Yang Y-L, Mo Y-P, He Y-S, Yang F, Xu Y, Li C-C, et al. Correlation between renin-angiotensin system gene polymorphisms and essential hypertension in the Chinese Yi ethnic group. J Renin Angiotensin Aldosterone Syst. 2015;16:975–81.
23. Freitas AI, Mendonca I, Brion M, et al. RAS gene polymorphisms, classical risk factors and the advent of coronary artery disease in the Portuguese population. BMC Cardiovasc Disord. 2008;8:15.
24. Zhang H, Li YX, Peng WJ, Li ZW, Zhang CH, Di HH, et al. The gene variants of maternal/fetal renina-angiotensin system in preeclampsia: a hybrid case-parent/mother-control study. Sci Rep. 2017;7:5087.
25. Procopciuc LM, Caracostea G, Zaharie G, Puscas M, Iordache G, Popa M, et al. Maternal/newborn genotype contribution of the renin-angiotensin system (Met235Thr, Thr174Met, I/D-ACE, A2350G-ACE, A1166C-AT2R1, C3123A- AT2R2, 83A/G-REN) to the risk of pre-eclampsia: a Romanian study. J Renin Angiotensin Aldosterone Syst. 2011;12:539–48.
26. Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet. 2011;12:683–91.
27. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science. 2007;315:525–8.
28. Fernandez-Calero T, Cabrera-Cabrera F, Ehrlich R, Marin M. Silent polymorphisms: can the tRNA population explain changes in protein properties? Life. 2016;6:9.
29. Sabarinathan R, Tafer H, Seemann SE, Hofacker IL, Stadler PF, Gorodkin J. RNAsnp: efficient detection of local RNA secondary structure changes induced by SNPs. Hum Mutat. 2013;34:546–56.
30. Zhu X, Bouzekri N, Southam L, Cooper RS, Adeyemo A, McKenzie CA, et al. Linkage and association analysis of angiotensin I–converting enzyme (ACE)–gene polymorphisms with ACE concentration and blood pressure. Am J Hum Genet. 2001;68:1139–48.
31. 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. Biomed Res Int. 2011; 2011:941515. https://doi.org/10.1155/2011/941515.
32. 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.
33. Li H, Ma Y, Fu Q, Wang L. Angiotensin-converting enzyme insertion/deletion (ACE I/D) and angiotensin II type 1 receptor (AT1R) gene polymorphism and its association with preeclampsia in Chinese women. Hypertens Pregnancy. 2007;26:293–301.
34. Bereketoglu C, Kasap M, Pazarbasi A. Studies on angiotensin-converting enzyme insertion/deletion polymorphism and genotype distributions in Turkish preeclampsia patients. J Pregnancy. 2012;2012:108–206.
35. 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;86:1343–46.
36. Uemura K, Nakura J, Kohara K, Miki T. Association of ACE I/D polymorphism with cardiovascular risk factors. Human Genet. 2000;107:239–42.
37. Rice T, Rankinen T, Province MA, Chagnon YC, Pérusse L, Borecki IB, et al. Genome-wide linkage analysis of systolic and diastolic blood pressure: the Quebec Family Study. Circulation. 2000;102:1956–63.
38. Jackson A, Brown K, Langdown J, Luddington R, Baglin T. Effect of the angiotensin-converting enzyme gene deletion polymorphism on the risk of venous thromboembolism. Br J Haematol. 2000;111:562–64.
39. Serrano NC, Diaz LA, Paez 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.
40. Alvi FM, Hasnain S. ACE I/D and G2350A polymorphisms in Pakistani hypertensive population of Punjab. Clin Exp Hypertens. 2009;31:471–80.