Relationship of Angiotensin Converting Enzyme Gene Polymorphism and Hypertension in Yogyakarta, Indonesia

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ABSTRACT

Aim: *to evaluate the association between ACE gene polymorphism I/D and hypertension in Yogyakarta population.*

Methods: *this study is a cross-sectional. Sample was taken by random sampling method from hypertensive, prehypertensve and normotensive subjects; from that were obtained 125 subjects, 97 subjects and 108 subjects, consecutively. ACE gene polymorphism I/D was examined by PCR. Genotype was classified as II, ID, or DD based on positive or negative insertion/delateion allele.*

Results: *this study shows significant differences of three groups (ages, body mass index (BMI), and family history of hypertension) and total cholesterol level in blood, which tends to have greater value in the hypertension group. Frequency of genotype II, ID, DD are 85 (68%), 39(31.2%), 1(0.8%) in hypertension, 66 (61.1%), 38(35.2%), 4(3.7%) in normo-tension and 56(57.7%), 37 (38.1%), 4 (4.1%) in pre-hypertension subject, consecutively.*

Chi-square analysis shows statistically significant association between ID+DD vs. II genotype and hypertension. Multiple logistic regression analysis shows four variables that significantly influence to hypertension, namely ages, family history of hypertension, BMI, and ACE gene polymorphism.

Conclusion: *ACE ID+DD genotype has significant relationship with hypertension in Melati population, Sleman, Yogyakarta, Indonesia.*

Key words: *hypertension, genetic, ACE, polymorphism, insertion/deletion.*

INTRODUCTION

Hypertension is one of major health problems in the world. The Joint National Committee on Prevention, Detection, Evaluation, and Treatment on High Blood Pressure VII (JNC-VII) reported that nearly one billion people suffered from hypertension. National Health and Nutrition Examination Survey (NHANES) III 1999-2000 in USA declared that approximately 69% patients had good awareness of this disease. There were only 58% patients who received therapy, and only 31% who had controlled blood pressure.[1]

Many factors may influence blood pressure. Renin-Angiotensin system (RAS) is one of the factors that have an important role in controlling blood pressure and sodium homeostasis. Many studies reported RAS polymorphism as a genetic determinant of essential hypertension and end-organ damage.[2] Angiotensin converting enzyme (ACE) is described as an enzyme which has a role in hypertension pathogenesis.[3]

Several authors in the world have investigated the relation of ACE gene polymorphism insertion/deletion either with hypertension or response of ACE-inhibitor. Rigat et al.[4] reported that ACE gene polymorphism insertion/ deletion affected both serum ACE concentration and blood pressure. Blood pressure was influenced by the variance of angiotensin II, aldosterone or other vasoactive agents. People with DD genotype have twice as high as ACE concentration compared to people with II genotype, while people with ID genotype has intermediate or moderate ACE

plasma concentration. RAS has a potent role to cardiovascular homeostasis in DD genotype or D allele.[4] There have been several studies in Indonesia regarding variation of ACE gene polimorphism in several major ethnic groups of South Sulawesi conducted by Bakrie et al.[5] These studies show no difference in terms of alel and genotype distribution among four groups studied. But all groups shows the high number of genotype II as compare with genotype DD. Bakrie et al.[6] in other study found that alel D of ACE gene significantly increase the risk of left ventricle hypertrophy.

In the world, the differentiation of ACE gene genotype in hypertension and normotension patients has been studied, but there are not any reports of prehypertension, while actually 40% prehypertension cases will become serious hypertension in four years. The association between ACE gene polymorphism and hypertension is controversial.[7] Genetic diversity and vary environment among different ethnic groups could lead to inconsistent results. Consequently, gene polymorphism should be investigated in a big homogenous population.[8] In Indonesia, ACE gene polymorphism study had not been performed in the community, either in normotension, prehypertension, or in hypertension subjects. Therefore, this study was performed in large community in Yogyakarta to minimize inconsistent results. The objective of this study is to evaluate the differentiation of ACE gene genotype among hypertension, prehypertension and normotension subjects, and to investigate the association between ACE gene polymorphism with hypertension, prehypertension and normotension.

METHODS

The method of this study is cross-sectional. The samples required for this investigation were 98 samples for each group. Each subject was included according to the criteria, ages 30-59 years old, agree with the rule of this study and informed consent, history of captopril-treatment for more than 2 months. This study was approved by the human research ethics committee of The Faculty of Medicine Gajah Mada University - Sardjito Hospital, and informed consent was obtained from each patient. Exclusion criteria: obesity (IMT >30), chronic kidney disease (creatinine ≥1.5 mg/dl), fasting blood glucose >160 mg/dl, serum cholesterol >240 mg/dl and ischemia ECG.

Data was obtained from 36 regions in Melati subdistrict, Sleman Yogyakarta, between year 2008 - 2009. There were a total 12 083 subjects aged ≥17 years old whose blood pressure was measured and filled in the questionnaires. The questionnaires include history of hypertension, history of antihypertension treatment and family history of hypertension. As inclusion criteria, the study has 7695 subjects whose age are 30-59 years old.

After stratified by random sampling and selected according to inclusion and exclusion criteria, 330 subjects were obtained (actually, it should be 432 subjects), consisted of 125 hypertension patients (staging I and II hypertension), 97 prehypertension subjects and 108 normotension subjects.

For every subject, 10 ml serum was drawn from anterior cubiti vein and put in sodium ethylene diamine tetra-acetic acid (Na2EDTA) tube. In 2 hours limitation time, serum was centrifuged with velocity 3000 g in 15 minutes. The plasma was stored in -20 ^{o}C, while erythrocyte was stored in 4-8^{o}C until analysis was performed. Plasma was used to analyze blood glucose concentration, ureum and creatinine, while erythrocyte was used to analyze ACE genotype. Urine was stored in -20^{o}C and used to analyze urine albumin.

Analysis of ACE II, ID, DD genotype was performed based on methods from Rigat et al.[9] and Shanmugan et al.[10] D and I allele of ACE gene was identified using conventional PCR. Fragment identification of 16 intron from ACE gene produces PCR 490 bp for insertion allele or 190 bp for deletion allele. Visualization was performed after stained by ethidium bromide. Genotype was classified as II, ID or DD. Classification was based on positive or negative of insertion allele.[8] The classification grouped 84 bp for D allele and 65 bp for I allele of ACE gene.

Statistical Analysis

In this study, allele and genotype frequencies in hypertension, prehypertension and normotension subjects were analyzed by chi-square test. Relation of other variables, such as sex, age, smoking, and family history of hypertension were also analyzed using chi-square test. Kolmogorov Smirnov's test was performed to know data distribution. Variables with numerical scale, such as blood glucose, cholesterol, creatinine, was analysed by Kruskal Wallis test, since data distribution was not normal.

Before hypothetical test was performed to allele and gene, ID and DD allele was combined first, because only a few subjects were included in DD group. Quantitative effect of covariable was remarked by multiple logistic regression. OR was count as relation between D dominant genotype (number 1 for II and number 2 for ID+DD) with hypertension. P values <0.05 were regarded as statistically significant.

All of statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 16 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of Research Population

Ages differentiation was statistically significant. Mean of normotension group has younger age 41.3 ± 7.6 years old (p = 0.000), while in 41-60 years old group, more subjects have hypertension compared to prehypertension and normotension subjects. Significant differences among three groups were reached in BMI, family history of hypertension and total serum cholesterol level, with a higher value in hypertension group. Hypertension subjects have more family history of hypertension than normotension and prehypertension subjects. Most of research subjects [197 (59.7%)] do not have any family history of hypertension.

In hypertension group, the number of overweight subjects is twice as high as that of prehypertension and normotension subjects. No obese subject was found. Serum cholesterol level in the hypertension group is higher than that of the prehypertension group, while in the prehypertension subjects it is higher than that of the normotension group.

Allele Frequency and ACE Gene Genotype

ACE gene genotyping result which was performed by PCR methods could be seen in **Figure 1**.

ACE gene genotype (II, ID, DD) and allele (I, D) distribution in hypertension, prehypertension and normotension subjects were shown in **Table 2**.

Frequencies of II, ID, DD genotype are 85 (68%), 39 (31.2%), 1 (0.8%) in hypertension, 66 (61.1%), 38 (35.2%), 4 (3.7%) in normotension and 56 (57.7%), 37 (38.1%), 4 (4.1%) in prehypertension, consecutively. Chi-square analysis result shows that ACE gene genotype was not statistically significant in hypertension, prehypertension and normotension

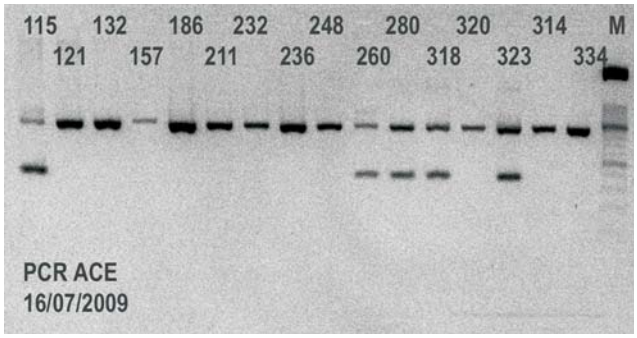

Figure 1. ACE gene genotyping result was performed by PCR methods. (M=marker)

Table 1. Characteristics of hypertension, prehypertension and normotension subjects

Variables	Hypertension	Prehypertension	Normotension	P
Sex				0.636
- Male (%)	43.2	46.4	39.8	
- Female (%)	56.8	53.6	60.2	
Ages (years old) (mean ± SD)	45,22 ± 7,453	44,03 ± 7,753	41,26 ± 7,55	**0.000***
- 30-39 years old (%)	24.8	29.9	49.1	**0.001**
- 40-49 years old (%)	42.4	40.2	35.2	
- 50-59 years old (%)	32.8	29.9	15.7	
Smoking				0.722
- Yes (%)	22.4	22.7	19.4	
- Already stopped (%)	8	12.4	13	
- No (%)	69.6	64.9	67.6	
Family history of hypertension				**0.000**
- Positive (%)	55.2	27.8	34.3	
- Negative (%)	44.8	72.2	65.7	
BMI (kg/m^2) (mean±SD)	23.0 ± 3.3	21.4 ± 3.2	21.7 ± 2.9	**0.000***
- < 20 (%)	16.8	36.1	33.3	**0.002**
- 20 - <25 (%)	53.6	46.4	52.8	
- 25-30 (%)	29.6	17.5	13.9	
Systolic (mmHg) (mean±SD)	149.8 ± 19.2	121.0 ± 6.9	105.6 ± 6.5	**0.000***
Diastolic (mmHg) (mean±SD)	95.1 ± 9.8	78. 5 ± 4.6	68.2 ± 7.1	**0.000***
GDS (mg/dl) (mean±SD)	90.0 ± 8.9	89.5 ± 10.6	87.6 ± 9.7	0.108*
Serum creatinine (mg/dl) (mean±SD)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.197*
Total cholesterol (mg/dl) (mean±SD)	192.3 ± 35.9	186.5 ± 34.5	180.3 ± 28.8	**0.001***
ACE gene genotype				
- II (%)	68	61.1	57.7	0.338
- ID+DD (%)	32	38.9	42.2	

subjects. I and D allele frequencies were 209 (83.6%) and 41 (16.4%) in hypertension, 170 (78.7%) and 46 (21.3%) in normotension and 149 (76.8%) and 45 (23.2%) in prehypertension, consecutively.

The Relation of ACE Gene Genotype and Hypertension

Relation of ACE gene polymorphism and hypertension was analyzed with assumption that D allele had dominant or recessive effects. To examine dominant effects of D allele, hypertension prevalence was compared to subjects with ID+DD genotype combination and subjects with II genotype. Chi-square 2x2 analysis result for D allele dominant effects to hypertension shows statistically significant relation. Analysis result of II vs. ID vs. DD genotype and recessive effect of D allele (II+ID vs. DD) did not show any significant relation between ACE gene polymorphism and hypertension. The prevalence ratio for DD+ID vs. II was 0.75, OR was 0.64, with confidence interval 95% 0.373 up to 0.967, so D allele was protective factor of hypertension development.

Multivariate logistic regression analysis was performed in order to know the most influencing factor to hypertension. Analysis about many affecting factors was performed, such as age, family history of hypertension, BMI, and total serum cholesterol level. Multivariate logistic regression analysis shows four variables which significantly influenced hypertension, such as age, family history of hypertension, BMI, and ACE gene polymorphism (**Table 6**).

Sex Specificity in Association with Hypertension

We compared genotype distribution according to sex. This comparison was performed to examine specificity of relationship between sex and hypertension. The result shows that in female, and not in male, ACE genotype DD+ID has significant relationship with hypertension.

Table 2. ACE gene genotype (II, ID, DD) in hypertension, prehypertension, and normotension

ACE gene genotype	Hypertension Staging			Total
	Hypertension	Normotension	Prehypertension	
II	85	66	56	207
ID	39	38	37	114
DD	1	4	4	9
Total	125	108	97	330

Table 3. ACE gene allele (I, D) distribution in hypertension, prehypertension and normotension subjects

ACE gene allele	Hypertension			Total
	Hypertension	Normotension	Prehypertension	
I	209	170	149	528
D	41	46	45	132
Total	250	216	194	660

Table 4. Distribution of ACE gene genotype (II, ID+DD) in hypertension and nonhypertension subject

ACE Gene Genotype	Hypertension Staging		Total	P
	Hypertension	Nonhypertension		
ID+DD	38	83	121	**0.042**
II	87	122	209	
Total	125	205	330	

Table 5. Distribution of ACE gene genotype (II, ID+DD) in pre-hypertension and normotension subjects

ACE gene genotype	Hypertension Staging		Total	P
	Prehypertension	Normotension		
ID+DD	70	63	133	**0,027**
II	27	45	72	
Total	97	108	205	

Table 6. Result of logistic regression analysis between variables and hypertension

Variable	B	SE	Wald	Df	Sig.	Exp (B)/ OR	95% Confidence Interval for Exp(B)	
							Lower Limit	Upper Limit
Age	0.056	0.016	11.518	1	**0.001**	1.057	1.024	1.092
BMI	0.139	0.040	12.277	1	**0.000**	1.149	1.063	1.241
Family History of Hypertension	-1.081	0.253	18.259	1	**0.000**	0.339	0.207	0.557
Serum Cholesterol Level	0.007	0.004	3.169	1	0.075	1.007	0.999	1.014
Polimorfisme Gen ACE	-0.510	0.243	4.402	1	**0.036**	0.600	0.373	0.967

DISCUSSION

This study showed there are significant difference of age statistically related to hypertension. There were many subjects with age above 40 years suffering from hypertension. That result is appropriate with other studies which report that increased age is associated with a significant increase of hypertension prevalence, such as Gunnar.[11] Study by Chobannian et al[1], and Llyod's et al.[12] compared hypertension in different age classification which is different from our study. They had inclusion criteria above 60 years old. However, the conclusion is similar to our study.

In this study, family history of hypertension had significantly positive association with hypertension (55.2%), which is appropriate with Lu et al.[13] and Al-safia et al.[14] However, there were some studies showing that blood pressure was influenced more by environment than heredity.

From the result of this study, it could be reported that there is a significant relationship between BMI and hypertension (55.2%) which is similar to Lu et al[13], whereas Hilmanti et al[15] reported that BMI influenced only hypertension prevalence in male subjects. In Yogyakarta, Indonesia, Sinorita et al[16] investigation showed no significant association between distribution of ACE I/D genotype and component of metabolic syndrome such as central obesity, hypertension, and dyslipidemia.[16]

Sposito[17], explained that there was a significant association between hypercholesterolemia and hypertension. This relationship was influenced by some mechanisms, such as decreased bioavailability of nitric oxide, enhanced activity of vasoconstrictor (angiotensin II and endothelin-1), decreased salt sensitivity, enhanced oxidative stress, etc.[17] The study informed that serum cholesterol level in hypertension subjects was higher than prehypertension subjects and serum cholesterol level in prehypertension subjects was higher than normotension ones. Nasri[18] reported no significant association between serum lipids (cholesterol and trygliseride) and mean diastolic blood pressure, but there is a significant positive correlation of serum LDL cholesterol with mean systolic blood pressure.[18]

Frequencies of II, ID and DD genotype for all subject are 207 (62.7%), 114 (34.6%), 9 (2.7%), whereas frequencies of I and D allele are 528 (80%) and 132 (20%). Frequency of DD genotype is very low compare to ID and II genotype in hypertension group, normotension group and prehypertension group. Frequency of I allele is bigger than D allele. This feature of D allele frequency also occurs in Malaysia (35%)[19], Singapore (31%)[20] and especially in Aborigin-Australian population (3%).[21] However, there are some different results from other studies that reported D allele frequency was higher than I allele, for example in Arabic population (61-73)[22,23] and African-American population (59%).[24]

In this study, D allele showed statistically significant relationship with hypertension. Analysis result of II vs. ID vs. DD genotype and the recessive effect of D allele (II+ID vs.DD) didn't show significant result between ACE gene polymorphism and hypertension. Other studies showing positive association between allele D and hypertension are Niu et al[25], etc.[26-28] In contrast, several studies reported that ACE I/D gene polymorphism was not associated with hypertension, which had been showed by Fuentes et al,[29] and Castellano.[30] Meta-analysis by Staessen et al.[31] from 23 studies concluded that there was significant association between allele D with hypertension in female and Asia population, whereas there was no association with other groups. But contrary, meta-analysis by Agerholm-Larsen et al.[32] which was limited in Caucasian population indicated that blood pressure was not affected by DD genotype compared to II. In a review about human hypertension genetic in 2005, Agarwal et al.[33] collected 26 studies consisting of 12 studies which reported positive association and 14 studies which reported negative

association. Other studies about association between ACE I/D gene polymorphism and hypertension showed inconsistent results. Some studies reported association of D allele and hypertension, but other studies did not show any association.

According to association of genotype distribution, comparing gender with hypertension, there is significant relationship of DD genotype in female, but not in male, with hypertension. This result is similar to meta-analysis by Staessen et al.[31]

CONCLUSION

This study showed significant relationship between ACE gene polymorphism I/D and hypertension in Yogyakarta, Indonesia. According to ACE genotype analysis result, it can be concluded that D allele frequency of ACE gene in Yogyakarta population is very low.

Advanced study on ACE gene polymorphism in all areas in Indonesia is required in order to know D allele of ACE gene distribution in Indonesia population. Further study will be needed to associate ACE gene polymorphism in relationship with hypertension patient's response to ACE inhibitor in Indonesia.

For people with normotension and prehypertension, it is recommended to have normoweight because BMI has significant relationship with hypertension. People whose siblings with history of hypertension should have healthy life style, such as regular aerobic exercise, healthy diet, avoidance of unhealthy life style, such as alcohol drinking, obesity, high cholesterol intake, and smoking to prevent hypertension.

REFERENCES

1. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, et al. The seventh report of joint national committee on prevention, detection, evaluation, and treatment of hypertension. The JNC 7 report. JAMA. 2003;289:2560-72.
2. Giner V, Poch E, Bragulat E, Oriola J, Gonzalez D, Coca A, et al. Renin-angiotensin system genetic polymorphism and salt sensitivity in essential hypertension. Hypertension. 2000;35:512-7.
3. Lim S. Blockade of renin-angiotensin-aldosterone system in kidney and heart disease: How much do we need? Acta Med Indones-Indones J Intern Med. 2008;40(1):34-7.
4. 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.
5. Bakri S, Yusuf I. Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in different south sulawesi ethnic groups in indonesian. J med nusantara. 2003;24(1):823-7.
6. Bakri S, Yota S, Yusuf I, Patelongi I. Synergistic effect of angiotensin-converting enzyme gene variation and blood pressure on left ventricular hypertrophy. Program & abstract book of 3rd international eijkman symposium. Jakarta; 2004. p. 1-6:103.
7. Crisan D, Carr J. Angiotensin I-converting enzyme, genotype and disease associations. J Mol Diag. 2000;2(3):105-15.
8. Al-Hinai AT, Hassan MO, Simsek M, Al-Barwani H, Bayoumi R. Genotypes and allele frequencies of angiotensin converting enzyme (ACE) insertion/deletion polymorphism among Omanis. SQU J Scientific Res: Med Sci. 2002;4(1-2):25-7.
9. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP 1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992;20:1433.
10. Shanmugan W, Sell KW, Saha BK. Mistyping ACE heterozygotes. PCR Meth Appl. 1993;3:120-1.
11. Gunnar A. Effect of age on hypertension: analysis of over 4800 referred hypertensive patients. Saudi J Kidney Dis Transplant. 1999;10:268-97.
12. Llyod-Jones DM, Evans JC, Levy D. Hypertension in adults across the age spectrum: current outcomes and control in community. JAMA. 2005;294:466-72.
13. Lu FH, Tang SJ, Yang YC, Chang CJ. Hypertension in elderly persons: its prevalence and associated cardiovascular risk factors in Tainan city, southern Taiwan. J gerontol. 2000;55A(8):M463-8.
14. Al-Safia SA, Aboul-Eneinb FH, Aboul-Eneinc BH, Manuelb B. Influence of Family history and lifestyle on blood pressure and heart rate in young adults in Jordan. Public Health Elsevier. 2006;120(11):1027-32.
15. Hilmanti D, Djais JTB, Marina H. Hubungan antara indeks massa tubuh dan peningkatan tekanan darah pada anak remaja obes dengan hipertensi. Bionatura 2008;10(1).
16. Sinorita H, Madiyan M, Pramono RB, Purnama LB, Ikhsan MR, Asdie AH. ACE gene insertion/deletion polymorphism among patients with type 2 diabetes, and its relationship with metabolic syndrome at Sardjito Hospital Yogyakarta, Indonesia. Acta Med Indones-Indones J Intern Med. 2010;42(1):12-6.
17. Sposito AC. Emerging insights into hypertension and dislipidaemia synergies. Euro heart j supple. 2004;6:G8-12.
18. Nasri H. the influence of serum LDL-cholesterol and HDL-cholesterol on systolic pressure of type II diabetic patient with various kidney functions not yet on dialysis. Pak J Physiol. 2006;2(1):p1-4.
19. Jayapalan JJ, Muniandy S, Chan SP. Angiotensin-1 converting enzyme I/D gene polymorphism: scenario in Malaysia. South-east Asian J Trop Med Public Health. 2008;39(5):917-21.
20. Lau YK, Woo KT, Choong HL, Zhao Y, Tan HB, Cheung W, et al. ACE gene polymorphism and disease progression of IgA nephropathy in Asians in Singapore. Nephron. 2002; 91:499-503.
21. Lester S, Heatley S, Bardy P, Bahnisch J, Bannister K, Faull R, et al. The DD genotype of the angiotensin-converting enzyme gene occurs in very low frequency in Australian Aborigines. Nephrol Dial Transplant. 1999;14:887-90.
22. Bayoumi RA, Simsek M, Yahya TM, Bendict S, Al-Hinai A, Al-Barwani H, et al. Insertion-deletion polymorphism in the angiotensin-converting enzyme (ACE) gene among Sudanese, Somalis, Emiratis, and Omanis. Hum Biol. 2006;78:103-8.
23. Al-Hinai AT, Hassan MO, Simsek M, Al-Barwani H, Bayoumi R. Genotypes and allele frequencies of angiotensin converting

enzyme (ACE) insertion/deletion polymorphism among Omanis. SQU J Sci Res Med Sci. 2002;4:25-7.
24. Barley J, Blackwood A, Carter ND, Crews DE, Cruickshank JK, Jeffery S, et al. Angiotensin-converting enzyme insertion/deletion polymorphism: Association with ethnic origin. J Hypertens. 1994;12:955-7.
25. Niu W, Qi Y, Hou S, Zhai X, Zhou W, Qiu C. Haplotype-based association of the renin-angiotensin-aldosterone system genes polymorphisms with essential hypertension among Han Chinese: the Fangshan study. J Hypertens. 2009; 27(7):1384-91.
26. Giner V, Poch E, Bragulat E, Oriola J, Gonzalez D, Coca A, et al. Renin-angiotensin system genetic polymorphism and salt sensitivity in essential hypertension. Hypertension. 2000;35:512-7.
27. Agachan B, Isbir T, Yilmaz H, Akoglu E. Angiotensin converting enzyme I/D, angiotensinogen T174M-M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. Exp Mol Med. 2003;35:545-9.
28. Kario K, Hoshide S, Umeda Y, Sato Y, Ikeda U, Nishiuma S, et al. Angiotensinogen and angiotensin-converting enzyme genotypes, and day and night blood pressures in elderly Japanese hypertensives. Hypertens Res. 1999;22:95-103.
29. Fuentes RM, Perola M, Nissinen A, Tuomilehto J. ACE gene and physical activity, blood pressure, and hypertension: a population study in Finland. J Appl Physio. 2002;92:2508-12.
30. Castellano M, Glorioso N, Cusi D, Sarzani R, Fabris B, Opocher G, et al. Genetic polymorphism of the renin-angiotensin-aldosterone system and arterial hypertension in the Italian population: the GENIPER Project. J Hypertens. 2003;21:1853-60.
31. Staessen JA, Wang JG, Ginocchio G, Petrov V, Saavedra AP, Soubrier F, et al. The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk. J Hypertens. 1997;15:1579-92.
32. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A. ACE gene polymorphism in cardiovascular disease: meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol. 2000;20:484-92.
33. Agarwal A, Williams GH, Fisher ND. Genetics of human hypertension. Trends Endocrinol Metab. 2005;16:127-33.