Association Between Angiotensin Converting Enzyme Gene Polymorphism and Coronary Artery Disease in Individuals of the South-Eastern Anatolian Population

Güneydoğu Anadolu Populasyonunda Anjiyotensin Konverting Enzim Gen Polimorfizmi ve Koroner Arter Hastalığı İlişkisi

**Muradiye Nacak*, MD, Vedat Davutoğlu, MD, Serdar Soydinç, MD, Hakan Dinçkal, MD
Serdar Türkmen**, MD, Binnur Erbağcı***, MD, Murat Akçay, MD, Şükrü Aynacıoğlu****, MD**

University of Gaziantep, Department of Pharmacology,
Department of Cardiology* and Department of Biochemistry**, *Gaziantep*
***Adıyaman State Hospital, *Adıyaman*
****Pharmacogenetics Laboratory, Epidauros Biotechnology AG, *Bernried-Germany*

Abstract

Objective: The deletion (D) allele of the angiotensin-converting enzyme (ACE) gene has been proposed as a genetic marker of the risk of coronary artery disease (CAD). In this study we aimed to determine the relevance of ACE gene polymorphism for coronary artery disease in the South-Eastern Anatolian population.
Methods: Angiotensin converting enzyme genotypes were determined in 133 CAD patients who underwent coronary angiography. Severity of CAD was subgrouped according to the number of stenotic vessels on coronary angiography. The control group was selected from 154 healthy volunteers. Angiotensin converting enzyme genotypes were determined by agarose gel sizing after polymerase chain reaction (PCR) amplification.
Results: Frequency of ACE DD genotype did not differ between patients with CAD and control subjects. However the ACE II genotype in CAD group was significantly less frequent than in control group (p=0.02). The relative risks were 0.9 (95% CI=0.56-1.43) for the DD genotypes, and 2.2 (95% CI=1.09 – 4.11) for the II genotype. In the 2-vessel CAD subgroups, the II genotypes were significantly different from control group.
Conclusion: Our study did not confirm the possibility that the ACE DD genotypes may be associated with predisposition to CAD in this certain population but there is a relationship between the least frequencies of the II genotype and CAD. The II genotype seems to be an independent protective factor for CAD in the South-Eastern Anatolian population. *(Anadolu Kardiyol Derg 2004; 4: 45-51)*
Key Words: ACE gene polymorphism, coronary artery disease, South-Eastern Anatolia

Özet

Amaç: Anjiyotensin konverting enzim (ACE) delesyon (D) alelinin koroner arter hastalığı riski için genetik bir belirteç olduğu ileri sürülmüştür. Bu çalışmada, Güneydoğu Anadolu populasyonunda ACE gen polimorfizminin koroner arter hastalığı ile olan ilişkisinin belirlenmesi amaçlanmıştır.
Yöntem: Koroner anjiyografileri yapılmış olan 133 koroner arter hastasında ACE genotipleri incelenmiştir. Koroner arter hastalığı ciddiyeti koroner anjiyografide stenotik damar sayısına göre gruplandırılmıştır. Kontrol grubu 154 sağlıklı gönüllüden oluşturulmuştur. Anjiyotensin konverting enzim genotipleri agaroz jel boyutlama ve polimeraz zincir reaksiyonu amplifikasyonu yöntemiyle incelenmiştir.
Bulgular: Anjiyotensin konverting enzim DD genotip sıklığı koroner arter hastaları ve kontrol grubu arasında farklı bulunmadı. Bununla beraber, koroner arter hastaları grubunda ACE II genotipi kontrol grubuna göre anlamlı olarak daha az sıklıktaydı (p=0.02). Rölatif risk DD genotipi için 0.9 (95% CI=0.56-1.43) ve II genotipi için 2.2 (95% CI=1.09 – 4.11) olarak saptandı. İki koroner damar hastası grubunda II genotipi kontrol grubundan anlamlı olarak farklıydı.
Sonuçlar: Çalışmamız ACE DD genotipinin koroner arter hastalığına yatkınlık sağlayabileceği ile ilgili düşünceyi desteklememiştir, ancak ACE II genotipinin az sıklığı ile koroner arter hastalığı arasında anlamlı ilişki saptanmıştır. Güneydoğu Anadolu hastalarında ACE II genotipi koroner arter hastalığı için bağımsız bir koruyucu faktör olarak gözükmektedir. *(Anadolu Kardiyol Derg 2004; 4: 45-51)*
Anahtar Kelimeler: ACE gen polimorfizmi, koroner arter hastalığı, Güneydoğu Anadolu

Correspondence adress: Yrd.Doç.Dr.Vedat Davutoğlu, Güneykent mah.Beşyüzevler sitesi 7.Blok Daire 10   27310 Şahinbey/Gaziantep
Tlf:0 342 3606060/7507   Cep tlf: 0532 4212546, Fax: 0342 3603928, E-mail: vedatdavutoglu@hotmail.com

Introduction

The genetic factors that contribute to the development of coronary artery disease (CAD) are poorly understood. It is likely that multiple genes that act independently or synergistically contribute to the development of CAD and the outcome. Probably, the most intensively studied one has been the angiotensin-converting enzyme (ACE) gene, and more specifically the insertion/deletion polymorphism. The role of renin-angiotensin system polymorphisms as risk factors for coronary heart disease (CHD) is controversial.

The ACE gene, located at 17q23 (1), contains a polymorphism in intron 16 resulting in the 3 genotypes of insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD) (2). An insertion/deletion (I/D) polymorphism of the ACE gene has been proposed as a genetic marker of risk of coronary heart disease (3). This polymorphism has been recognized to be a major determinant of plasma ACE activity, with the highest values found in subjects homozygous for the D allele and the lowest in subjects homozygous for the I allele; ID heterozygotes show intermediate values (4). Angiotensin converting enzyme converts angiotensin I to the angiotensin II and inactivates bradykinin, two peptides that may play role in the pathogenesis of myocardial ischaemia (5). It can influence several important components of atherosclerosis, such as endothelial vasomotor function (6), smooth muscle cell migration and proliferation (7). In addition, it has been suggested that angiotensin II may directly influence the development of atheroma by promoting the growth of vascular smooth muscle cells (5). Although some studies on the association between ACE genotypes and the risk of CAD have provided controversial results (8,9), some well designed studies revealed the importance of this marker. Ethnic features of the population may affect and explain these controversial results (10). Thus it is still possible that in subjects lacking other risk factors, the D/I polymorphism may have a predictive role for CAD in different ethnic region.

Although there are a few studies about distribution of ACE gene polymorphism in the Middle and Western part of Turkey (11,12) on correlation between CAD and the DD genotypes, the distribution and importance of the ACE gene polymorphism in South-Eastern Anatolian populations is not known. Because CAD is common and is associated with potentially high mortality and morbidity, we thought it

is wortwhile to evaluate its potential relevance for CAD in the South-Eastern Anatolian population. We achived this objective by determining the distribution of the ACE genotypes in the general healthy population and comparing it with angiographically documented CAD patients.

Material and Methods

Study population: Our case-control study population consisted of 133 CAD patients (93 men, 40 women) with ages ranging from 40 to 78 years (mean 60.3 ± 8.3 years) and 154 healthy subjects (73 women, 81 men) with ages ranging from 18 to 78 years (mean 40.4 ± 14.8 years). Angiotensin converting enzyme was identified in patients who underwent coronary angiography between June 2002- December 2002 at the Department of Cardiology of the Gaziantep University Faculty of Medicine. All patients signed an informed consent allowing genetic analysis for cardiovascular research. All of them were Turkish men and women living in the South-Eastern Anatolia. Indication for coronary angiography were stable angina in 70 patients (52%), unstable angina in 50 patients (37%), myocardial infarction (MI) in 6 patients (4.5%) and miscellaneous causes in the remaining 7 patients. With use of a standardized questionnaire, the medical history of patients and medications used at the time of the coronary angiography were carefully recorded. Diabetes and hypertension were determined as fast blood sugar measurements of ≥140 mg/dl and blood pressure measurements of ≥140/90 mmHg, respectively. Cholesterol, triglycerides, and high density lipoprotein (HDL-C) concentrations were determined using an Olympus AU800 automated analyser (Olympus Diagnostica GmbH, Hamburg, Germany) with Randox reagents according to the manufacturer's instructions (Randox Laboratories Ltd. Ardmore, UK). Low density lipoprotein (LDL-C) was estimated using the Friedewald equation. Control groups consisted of subjects free from CAD, based on physical examination, history of cardiovascular disease and electrocardiogram. The presence of hypertension, diabetes mellitus, smoking status were exclusion criteria from the control group.

Coronary angiography was performed using standard techniques. The severity of obstruction was estimated visually by two cardiologists and CAD was defined as the presence of one or more stenoses >50% in at least one major coronary vessel. The size

of obstruction and number of diseased coronary vessels were recorded.

Determination of ACE I/D polymorphism: Genomic DNA was extracted from leucocytes by standard methods from peripheral blood collected in tubes containing EDTA (13). Angiotensin converting enzyme (ACE) genotypes were classified as II, ID, DD. Angiotensin converting enzyme gene I/D polymorphism was determined by polymerase chain reaction (PCR) using a primer pair flanking the polymorphic region of intron 16 that produces either an amplified 490 bp (I allele) or a 190 bp product (D allele), or both. The reactions were performed according to the method of Rigat et al. (14). The sense nucleotide primer was 5'-CTGGAGACCACTCCCATCCTTTCT-3' and the antisense primer was 5'-GATGTGGCCATCA-CATTCGTCAGAT-3'. Polymesare chain reactions were performed in 50 ml reaction volumes with 50 pmoles each primer, 100 ng genomic DNA, 1.5 mmol/L of $MgCl_2$, 50 mmol/L of KCl, 10 mmol/L of Tris-HCl (pH 8.3), 200 ml/L for ach dNTP, and 2.5 U Amplitaq DNA polymerase (Fermentas). Amplification was performed as follows: initial denaturation at 95°C for 2 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60 °C for 2 min, and extension at 72° for 3 min. The PCR products were visualized by electrophoresis in a 2 % agarose gel with ethidium bromide and documented with a gel documentation system (Vilber-Lourmar) (Figure 1).

Statistical Analysis

Differences in the genotype distribution between patients with CAD and control patients were tested by the Chi-square test. Expected genotype frequencies were derived by the Hardy-Weinberg equation from single allele frequencies. The odds ratios for different association models were calculated with 95 % confidence interval (CI) and p-values were calculated with Fisher exact test. A value of $P < 0.05$ was considered as statistically significant. Calculations were done by GraphPad InStat, version 3.05 programe..

Results

The baseline characteristics of the study groups are presented in the Table 1. The age and gender distributions of patients with CAD and controls were not similar. To prevent affection of these bias on our study we selected control patients as free of coronary risk factors (smoking, hypertension, diabetes). But despite we minimize these bias, age and gender differences are remained to be the limitations of our study. Significant differences were noted between study groups in respect of total cholesterol, triglycerides and HDL-C levels. All of them were higher in the CAD group than in control group. Descriptive statistics of the coronary angiography study population are presented in Table 2. Coronary
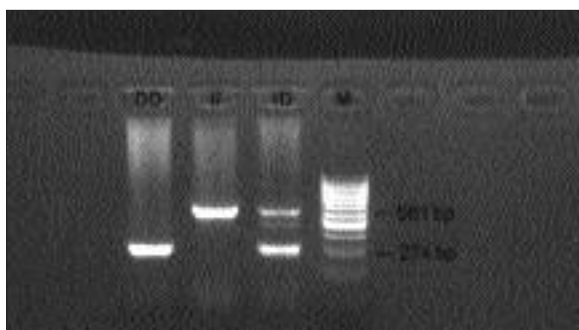

Figure 1. Sample of an Agarose gel.

Table 1. Characteristics of the study groups

	CAD group N= 133	Control group N= 154	P
Age	60.3 ± 8.34	40,4 ± 14.8	<0.05
Smoker	-	-	
Diabetes	26.3 %	-	
Hypertension	8.2 %	-	
DM plus HT	4.5 %	-	
T.Cholesterol (mg/dl)	199.2 ± 4.3	174.6 ±4,5	< 0.001
Triglyceride (mg/dl)	198.2 ± 12.3	121 ± 1	<0.001
HDL-C (mg/dl)	38.5 ± 1.7	45.6 ± 1	<0.005
LDL-C (mg/dl)	115.1 ± 3.8	112 ± 5.2	NS
DM: diabetes mellitus, HDL-C: hig density lipoprotein cholesterol, HT: hypertension, LDL-C: low density lipoprotein cholesterol, NS: nonsignificant T: total			

artery disease patients were grouped according to the severity. Hypertension, diabetes and hypertension plus diabetes have a rate of 8.2%, 26.3% and 4.5% respectively in CAD group. The frequency of the DD genotype in the hypertension, diabetes and hypertension plus diabetes subgroups did not differ significantly from that of the control group. No significant differences between stable, unstable and MI patients and controls were found for any of the investigated polymorphisms, neither in the distribution of the genotypes nor in the allele frequency. No

significant difference was found in D and I allele frequency between CAD and control groups (Table 2 B). And also no statistical differences in frequencies of the DD genotypes were noted between two groups. But the prevalence of II genotype was significantly lower than in control group (p=0,0195). The relative risks were 0.9 (95% CI=0.56-1.43) for the DD genotypes and 2.2 (95% CI=1.09 –4.11) for the II genotype. In the 2-vessel CAD subgroups the II genotypes were significantly least frequent than in control group (Table 2 D).

Table 2 A. Distribution of ACE gene genotypes in CAD and control group (overall)

Genotype	CAD n(%)	Control n(%)	Odds ratio(CL%)	P
II	12 (9.0)	28 (18.2)	2.2 (1.09-4.11)	0.0195*
ID	68 (51.10)	69 (44.8)	0.8 (0.49-1.24)	0.1708
DD	53 (39.9)	57 (37.0)	0.9 (0.56-1.43)	0.3553
ACE: angiotensin converting enzyme, CAD: coronary artery disease				

Table 2 B. D and I allele frequency in CAD and control group

Allele	CAD n(%)	Contol n(%)	Odds ratio(CL%)	P
I	92 (34.9)	125 (40.6)	1.3 (0.92-1.82)	0.820
D	174 (65.4)	183 (59.4)	0.8 (0.56-1.09)	0.820
ACE: angiotensin converting enzyme, CAD: coronary artery disease				

Table 2 C. Comparison of the distribution of ACE gene genotypes in 1 vessel CAD group and control group

Genotype	1 vessel n(%)	Control n(%)	Odds ratio(CL%)	P
II	4 (11.8)	28 (18.2)	1.7 (0.54 – 5.11)	0.2582
ID	13 (38.2)	69 (44.8)	1.3 (0.61 – 2.81)	0.3057
DD	17 (50.0)	57 (37.0)	0.6 (0.28 - 1.24)	0.1133
ACE: angiotensin converting enzyme, CAD: coronary artery disease				

Table 2 D. Comparison of the distribution of ACE gene genotypes in 2 vessel CAD group and control group

Genotype	2 vessel n(%)	Control n(%)	Odds ratio(CL%)	P
II	2 (4.1)	28 (18.2)	5.2 (1.20 – 22.80)	0.0142*
ID	28 (57.1)	69 (44.8)	0.6 (0.32 - 1.17)	0.0898
DD	19 (38.8)	57 (37.0)	0.9 (0.48 - 1.80)	0.4790
ACE: angiotensin converting enzyme, CAD: coronary artery disease				

Table 2 E. Comparison of the distribution of ACE gene genotypes in 3 vessel CAD group and control group

Genotype	3 vessel n(%)	Control n(%)	Odds ratio(CL%)	P
II	6 (12.0)	28 (18.2)	1.6 (0.63 – 5.00)	0.2116
ID	27 (54.0)	69 (44.8)	0.7 (0.36 - 1.31)	0.1663
DD	17 (34.0)	57 (37.0)	1.1 (0.58 - 2.23)	0.4146
ACE: angiotensin converting enzyme, CAD: coronary artery disease				

Discussion

Cardiovascular disease is the major cause of morbidity and mortality in Westernised societies. It is well known that the etiology of this devastating disorder involves both genetic and environmental factors. Sequence variants of the components of the renin-angiotensin-aldosterone system and the kallikrein-kinin system are suggested to have significant influences on cardiovascular homeostasis. Thus, the ACE gene has been recognized as a top candidate gene for cardiovascular research. While a number of studies have implicated the role of the ACE polymorphism in cardiac disorders, such as myocardial infarction (3, 15-17), CAD (18, 19), left ventricular hypertrophy (20, 21) and hypertension (22) others have argued that it may be associated with increase in plasma ACE activity without being a risk factor for coronary heart disease (9, 23, 24). These inconsistent results may depend on the gender differences and ethnic traits of the individual populations (10, 25-27).

Two studies those have been performed in Turkish population including Middle and Western part of Turkey, reported significant association of ACE gene DD polymorphism and CAD (11, 12). In this study, we analyzed the association between polymorphism in the ACE gene and CAD relation in South-Eastern Anatolian individuals. Age and gender had no significant effects on the frequencies of the three studied polymorphisms. No significant difference in gene polymorphism was observed between hypertension and diabetes subgroups in the CAD group.

When interpreting these results possible sources of bias should be considered. It is well known that angiography is only a crude measure of the presence or absence of coronary atherosclerosis. Thus, to limit selection bias, we compared patients with marked differences in the degree of coronary stenosis. However differences in age between patient and control groups limit interpretation of the results of our study. Thus, we can not exclude development of CAD in our control group in the future despite we select control group as free of coronary risk factors. This bias may be accepted as a limitation of our study.

The mechanism by which the ACE I/D genotype may predispose an individual to the development of CAD remains unclear. Angiotensin converting enzyme is responsible for the conversion of angiotensin I to the angiotensin II. Angiotensin II has been implicated in the pathogenesis of atherosclerosis through induction of hyperplasia and hypertrophy of smooth muscle cells and increased expression of platelet-derived growth factor and protooncogenes (7, 28). Angiotensin converting enzyme inhibitors are reported to provide a protective effect in animals on a long-term atherogenic diet, possibly because of reducing angiotensin II level (29-31). Association of the DD genotypes of the ACE gene and CAD has been reported (3,16). However, these reports have been challenged by series of negative studies (9, 32, 33). In our study, there was no significant increase in the frequency of the DD genotype in CAD patients. The II genotypes have been associated with low plasma levels of ACE and in our study we found least frequently II genotypes in CAD group that support and confirm indirectly the relationship of angiotensin II and atherosclerosis (34). This distribution pattern differs slightly from that of reported in the meta-analysis by Samani et al (19). However, these reports have been challenged by series of negative studies (9, 23, 24, 32, 33). The inconsistency of these results may have several explanations, including differences in the study populations or the failure to separate accurately cases from controls and widely different ethnic group. In addition, differences in the wide range risk status between the patient series could influence the effect of ACE gene polymorphism on the prediction of clinical manifestation of CAD. Although it's precursors may be genetically determined, CAD event rates and population differences appear to be largely determined by environment and lifestyle (35). Some studies have suggested that ACE polymorphism may be relevant for those factors that predispose individuals to development atherosclerosis, rather than being a direct cause of CAD itself. Further efforts should be made to elucidate the specific nature of these genotype/phenotype interactions. Overall, since ACE I/D is only an intronic marker, the true locus that controls the ACE enzyme activity remains to be identified, and could be located within either the ACE gene (36-39) or another nearby gene such as the human growth hormone gene. We note that the II genotype of the angiotensin converting enzyme may protect against or delays the onset of atherosclerosis (40) and since associations tend to vary across different gender (25) or ethnic groups (26, 27), or across different socio-ecological settings, consideration of potential gene-gene and gene-environment interactions should be made.

Conclusion

Our study does not confirm the possibility that the DD genotypes may be associated with predisposition to CAD in certain population. We found II genotypes with least frequencies in our CAD group. The II genotypes may have protective effect because of its a marker of low angiotensin II level. The II genotype seemed to be an independent protective factor for CAD in the South-Eastern Anatolian population. However, further investigation of protective role of the genotype II for atherosclerosis may be indicated.

References

1. Mattei MG, Hubert C, Alhenc-Gelas F, Roeckel N, Corvol P, Soubrier F. Angiotensin I-converting enzyme gene is on chromosome 17. Cytogenet Cell Genet 1989; 51: 1041.
2. Fogarty DG, Maxwell AP, Doherty CC, Hughes AE, Nevine NC. ACE gene typing. Lancet 1994; 343: 851.
3. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-4.
4. Tiret L, Rigat B, Visvikis S, 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.
5. Brown NJ, Vaughan DE. Angiotensin converting enzyme inhibitors. Circulation 1998; 97: 1411-20.
6. Kupari M, Perola M, Koskinen P, Virolainen J, Karhunen PJ. Left ventricular size, mass, and function in relation to angiotensin-converting enzyme gene polymorphism in humans. Am J Physiol. 1994; 267: 1107-11.
7. Campbell-Boswell M, Robertson AL. Effects of angiotensin II and vasopressin on human smooth muscle cells in vitro. Exp Mol Pathol. 1981; 35: 265-76.
8. Butler R, Morris AD, Struthers AD. Angiotensin-converting enzyme gene polymorphism and cardiovascular disease. Clin Sci 1997; 93: 391-400.
9. Lindpainter K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995; 332: 706-11.
10. Barley J, Blackwood A, Carter ND, et al. Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. J Hypertens. 1994; 12: 955-7.
11. Akar N, Aras O, Omurlu K, Cin S. Deletion polymorphism at the angiotensin converting enzyme gene in Turkish patients with coronary artery disease. Scand J Clin Lab Invest 1998; 58: 491-5.
12. Isbir T, Yilmaz H, Agachan B, Aydin M, Isbir CS. Association between angiotensin converting enzyme gene polymorphism and coronary artery disease. IUBMB Life 1999; 48: 205-7.
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extraction DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.
14. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase1). Nucleic Acids Res 1992; 20: 1433.
15. Bohn M, Berge KE, Bakken A, Erikssen J, Berg K. Insertion/deletion (I/D) polymorphism at the locus for angiotensin I-converting enzyme and parental history of myocardial infarction. Clin Genet 1993; 44: 292-301.
16. Ludwig E, Comeli PS, Anderson JL, Marshall HW, Lalouel JM, Ward RH. Angiotensin-converting enzyme gene polymorphism is associated with myocardial infarction but not with development of coronary stenosis. Circulation 1995; 91: 2120-4.
17. Schuster H, Wienker TF, Stremler U, Noll B, Steinmetz A, Luft FC. An angiotensin-converting enzyme gene variant is associated with acute myocardial infarction. Am J Cardiol 1995; 76: 601-3.
18. Marenberg ME, Risch N, Berkman LF, Floderus B, de Faire U. Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 1994; 330: 1041-6.
19. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 1996; 94: 708-12.
20. Marian AJ, Yu QT, Workman R, Roberts R. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. Lancet. 1993; 342: 1085-6.
21. Iwai N, Ohmichi N, Nakamura Y, Kinoshita M. DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. Circulation. 1994; 90: 2622-8.
22. Harrap SP, Davidson HR, Connor JM, et al. The angiotensin I-converting enzyme gene and predisposition to high blood pressure. Hypertension 1993; 21: 455-60
23. Winkelmann BR, Nauck M, Klein B, et al. Deletion polymorphism of the angiotensin I-converting enzyme gene is associated with increased plasma angiotensin-converting enzyme activity but not with increased risk for myocardial infarction and coronary artery disease. Ann Intern Med 1996; 12: 519-25.
24. Friedl W, Krempler F, Paulweber B, Pichler M, Sandhofer F. A deletion polymorphism in angiotensin conver-

ting enzyme gene is not associated with coronary artery disease in an Austrian population. Atherosclerosis 1995; 112: 137-43.

25. O'Donnell CJ, Lindpaintner K, Larson MG et al. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. Circulation 1998; 97:1766-72.

26. McKenzie CA, Abecasis GR, Keavney B, et al. Trans-ethnic fine mapping of a quantitative trait locus for circulating angiotensin I –converting enzyme (ACE). Hum Mol Genet 2001; 10: 1077-84.

27. Zhu X, McKenzie CA, Forrester T, et al. Localization of a small genomic region associated with elevated ACE. Am J Hum Genet 2000; 67: 1144-53.

28. Naftilan AJ, Pratt RE, Dzau VJ. Induction of platelet-derived growth factor A-chain and c-myc gene expressions by angiotensin II in cultured rat vascular smooth muscle cells. J Clin Invest 1989; 83: 1419-24.

29. Schuh JR, Belhm DJ, Frierdich GE, McMahon EG, Blaine EH. Differential effects of renin-angiotensin system blockade on atherogenesis in colesterol-fed rabbits. J Clin Invest 1993; 91: 1453-8.

30. Itoh K, Yoshido S, Fukuda M. The ACE inhibitor alacepril suppresses atherogenesis independent of serum lipids in cholesterol-fed rabbits-critical analysis with new ultrasound technique. Jpn Circ J 1994; 58: 844-54.

31. Becker RH, Wiemer G, Linz W. Preservation of endothelial function by ramipril in rabbits on a long-term atherogenic diet. J Cardiovasc Pharmacol 1991; 18: S110-5.

32. Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Sorensen TIA. The Copenhagen City Heart Study Group. The angiotensin-converting enzyme gene polymorphism fails to predict coronary heart disease in a case-control study of 7300 individuals (abstract). Circulation 1995; (Suppl. I): 800.

33. Eichner JE, Christiansen VJ, Moore WE, Dunn ST, Schechter E. Angiotensin-converting enzyme gene polymorphism in a cohort of coronary angiography patients. Atherosclerosis 2001; 154: 673-9.

34. Danser AH, Schalekamp MA, Box WA, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995; 15: 1387-8.

35. Rose G. The Strategy of Preventive Medicine. New York: Oxford University Pres; 1992: p.138.

36. Rieder MJ, Taylor SL, Clark AG, Nickerson DA. Sequence variation in the human angiotensin converting enzyme. Nat Genet 1999;22:59-62.

37. Soubrier F, Martin S, Alonso A et al. High-resolution genetic mapping of the ACE-linked QTL influencing circulating ACE activity. Eur J Hum Genet 2002; 10: 553-61.

38. Villard E, Tiret L, Visvikis S, Rakotovao R, Cambien F, Soubrier F. Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. Am J Hum Genet 1996; 58: 1268-78.

39. Wang JG, Staessen JA, Tizzoni L, et al. Renal function in relation to three candidate genes. Am J Kidney Dis 2001; 38: 1158-68.

40. Iwai N, Tamaki S, Ohmichi N, Kinoshita M. The II genotype of the angiotensin-converting enzyme gene delays the onset of acute coronary syndromes. Arterioscler Thromb Vasc Biol 1997; 17: 1730-3.