The Angiotensin Converting Enzyme Genetic Polymorphism in Acute Coronary Syndrome - ACE polymorphism as a risk factor of acute coronary syndrome -

The deletion polymorphism of angiotensin converting enzyme (ACE) genotype has been reported as an independent risk factor for the development of myocardial infarction (MI). However there are conflicting data showing no relationship between the ACE genotype and coronary artery disease. The present study was performed to investigate the correlation between ACE genetic polymorphism and acute coronary syndrome by comparing the distribution of ACE genotypes and ACE activities in patients with acute MI and unstable angina with those in control group. The frequency of genotype DD was significantly higher in patients with acute coronary syndrome than in controls. Logistic regression analysis showed that ACE polymorphism affected the development of acute coronary syndrome in recessive pattern of D allele. When we divided the patients into MI and unstable angina groups, the frequencies of genotype DD and D allele were significantly higher in unstable angina group than in MI or control groups. In the patients with MI, the frequency of D allele was significantly higher in patients without previous angina than in those with previous angina. There was no significant difference in ACE genotype or allelic frequency according to the severity of coronary lesions. The ACE genotype was associated with marked differences of ACE activity, but there was no difference between the patient and control groups for each genotype. In conclusion, the genotype DD of ACE gene associated with acute coronary syndrome, but not with the severity of coronary artery lesion. These results showed that the genotype DD of ACE gene might be associated with acute coronary syndrome by another mechanism rather than the coronary atherosclerosis.

(JKMS 1997; 12: 391~7)

Key Words : *Peptidyl-dipeptidase A; Angiotensin converting enzyme; Polymorphism (Genetics); Coronary disease*

Hyun-Young Park, Hyuck-Moon Kwon, Dongsoo Kim, Yangsoo Jang, Won Heum Shim, Seung-Yeon Cho, Hyun-Seung Kim

Department of Internal Medicine, Yonsei University, College of Medicine, Seoul, Korea

Received : January 30, 1997
Accepted : June 4, 1997

Address for correspondence
Dong Soo Kim, M.D., Cardiology divsion of Internal Medicine, Yongdong Severance Hospital College of Medicine, Yonsei University, 146-92 Dogok-dong, Kangnam-gu, Seoul, Korea
Tel : (02) 3450-3310, Fax : (02) 561-3887
E-mail : Kim0426@yumc.yonsei.ac.kr

INTRODUCTION

Angiotensin I converting enzyme (ACE), which is one of the two major enzymes of the renin-angiotensin system, converts the angiotensin I into angiotensin II and inactivate vasodilator peptide bradykinin (1). The deletion polymorphism of angiotensin converting enzyme (ACE) which results from the absence of the alu repeat in intron 16 of the ACE gene, has been studied extensively concerning its relationships with myocardial infarction (MI) (2~4), hypertension (5), myocardial hypertrophy (6) and cardiomyopathy (7, 8). Many studies have reported that deletion polymorphism of ACE is an independent risk factor of coronary artery disease, however recently some studies reported that the atherosclerotic change of vasculature was not associated with ACE polymorphism (9, 10), even though there are several reports which showed marked reduction in atherosclerosis with treatment of ACE inhibitors in animal studies (11, 12). The mechanisms of positive correlation between ACE polymorphism and IHD have not been known yet.

The pathophysiology of acute coronary syndrome including unstable angina, non-Q wave MI and Q-wave MI is somewhat different from chronic stable angina. The atheromatous plaque rupture and thrombus formation are the major causes of acute coronary syndrome (13). The plaque rupture is influenced by the factors including intraluminal pressure, sympathetic activity and vasoconstriction. Renin-angiotensin system controls the systemic and local vascular tone through the angiotensin II receptor and bradykinin. So there is high possibility that renin-angiotensin system, especially angiotensin-converting enzyme is related with the acute coronary syndrome.

Recently, Lee et al. reported that there was no dif-

ference of ACE genotype frequency between the patient group of acute coronary syndrome and normal control group in Korean (14). In our previous report, the genotype DD was weakly related with development of MI in low risk group, but the frequency of D allele of MI group was not significantly different from that of control group (15). The effect of ACE polymorphism on the development of acute coronary syndrome is still controversial in Korean population. In the present study, we explored the distribution of ACE genotype and ACE activity in patients with acute MI and unstable angina to investigate the correlation of ACE polymorphism with acute coronary syndrome.

MATERIALS AND METHODS

Subjects

This study included 189 patients with acute coronary syndrome (140 MI, 49 unstable angina) and 73 healthy controls (who visited the Severance Hospital in Seoul, Korea). The MI was clinically verified by electrocardiography and echocardiography, and both acute and old MI were included. The MI patient with previous angina was defined the patient who had the exertional chest pain at least two months before attack of MI, and the MI patient without previous angina was defined who did not experience the exertional chest pain before attack of MI. All those patients who were classified as having unstable angina had an increase in pain frequency or rest pain. Patient with diabetes, hypertension or personal history of ischemic heart disease was not included in the control group. Patients with sarcoidosis, tuberculosis or liver disease were excluded for the analysis of ACE activity because those disease are known to increase the ACE activity. In patient group coronary angiography was performed in 160 patients among 189 patients, and the severity of coronary artery stenosis was determined by the number of significantly stenosed coronary artery. The coronary artery lesion was defined as stenosed if more than 50% diameter narrowing in any major epicardial vessel which was demonstrated by angiography. Total cholesterol, triglyceride and HDL cholesterol were measured by routine chemical methods.

Plasma ACE activity measurement

Prior to the sampling of blood, patients stopped medication of ACE inhibitor for at least two weeks, and plasma was stored in a freezer (-70℃) for a mean period of 2 months. The activity of plasma ACE was measured by automatic kinetic method using N-(3-(2-furyl)acryloyl)-L-phenylalanylglycylglycine (FAPGG, Sigma, St. Louis, USA) as a substrate.

Genotype determination

DNA was extracted from 10 ml of whole blood with a DNA extraction matrix (Instagene, Biorad, UK). Polymerase chain reaction (PCR) to detect insertion/deletion (I/D) polymorphism of ACE was carried out with 200 ng genomic DNA as a template. The sense oligonucleotide primer was 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and the antisense primer was 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3'. Reactions were performed with 4 pmole of each primer, 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.5 mM of each dNTP, 0.5 unit of *Taq* polymerase (Perkin Elmer, Foster, USA) in a final volume of 20 μl. The DNA was amplified for 30 cycles with denaturation at 94℃ for 15 seconds, annealing at 58℃ for 15 seconds, and extension at 72℃ for 2 min using a GeneAmp PCR system 9600 (Perkin Elmer, Foster, USA). PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. Each sample revealed one of three patterns after electrophoresis: a 490-bp band (genotype II), a 190-bp band (genotype DD), or both 490 and 190-bp band (genotype ID) (Fig. 1).

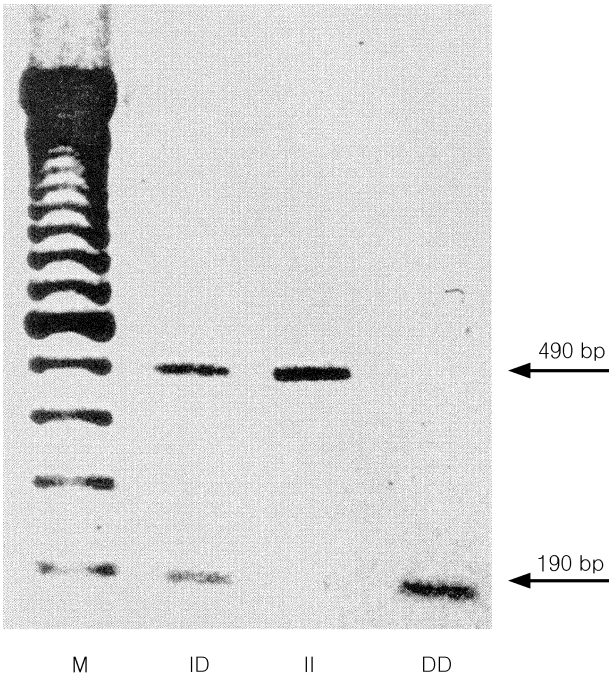

Fig. 1. Identification of the three ACE genotypes by the PCR products stained with ethidium bromide on 1.5% agarose electrophoresis. M : molecular marker (100 bp ladder marker, BRL, United States), ID : genotype ID, II : genotype II, DD : genotype DD

Statistical analysis

The differences for age, body mass index (BMI), total cholesterol, triglyceride, and HDL cholesterol between patient group and control group were analyzed by ANOVAR. The ACE activities among three genotypes were analyzed by ANOVA. The genotype and allele frequencies were determined from observed genotypic counts and evaluated by χ^2 analysis. Logistic regression comparing patients and control subjects was used to assess the independent effects of the ACE I/D polymorphism on MI risk after adjustment on covariates. A value of $p<0.05$ was considered significant.

RESULTS

Clinical parameters

The mean age was higher in the patient groups than in the control group ($p<0.001$). There was no significant differences in BMI, total cholesterol, HDL cholesterol between two groups, but concentration of triglyceride was significantly low in the control group in comparison with patient group ($p=0.008$) (Table 1).

Table 1. Clinical characteristics of study population

	Controls(n=73)	Patients(n=189)
Age (y)	45.4±10.4	55.5±11.6*
Sex (M:F)	63 : 10	145 : 44
Smoker (n)	-	89
Diabetes (n)	-	36
Hypertension (n)	-	86
BMI (kg/m^2)	23.5±2.9	24.2±2.9
Total cholesterol (mg/dl)	181.3±28.0	196.0±43.3
Triglyceride (mg/dl)	130.0±53.7	171.1±117.3*
HDL cholesterol (mg/dl)	40.2±9.4	38.1±19.0

Results are given as mean±SD; n=number of subjects in each group.
* $p<0.05$

Distribution of ACE genotype in patients and controls

Genotype and allele frequencies in the control and patient groups were shown in Table 2, and there was no detectable distortion from Hardy-Weinberg's equilibrium in any data set ($\chi^2=1.073$, $df=2$, $p>0.05$ for control group, $\chi^2=3.948$, $df=2$, $p>0.05$ for all patients, $\chi^2=0.282$, $df=2$, $p>0.05$ for patients with MI, and $\chi^2=5.479$, $df=2$, $p>0.05$ for patients with unstable angina).

As shown in Table 2, the frequency of genotype DD was significantly higher in the patient group including MI and unstable angina than in the control group. When the patient group was divided into two subgroups, MI group and unstable angina group, the frequency of genotype DD in patients with unstable angina were significantly higher than in the control group and in patients with MI ($p<0.001$ and $p=0.002$, respectively). The frequency of D allele showed the same results ($p<0.001$ and $p=0.004$, respectively). When the patients with MI were divided into two groups according to the presence or absence of angina before the attack of MI, the frequency of genotype DD was higher in patients without previous angina than in patients with previous angina even if there was no significant difference ($p=0.076$). The frequency of D allele was significantly higher in patients without previous angina than in patients with previous angina ($p=0.047$) (Table 3).

Effect of the ACE polymorphism on the risk of acute coronary syndrome

The effects of the ACE polymorphism on the risk of acute coronary syndrome was assessed by logistic regression analysis adjusted on control group (Table 4). The ACE I/D polymorphism was entered in the models as an ordered variable (0, 1, and 2 was assigned for genotype

Table 2. ACE genotype and allele frequencies in patients and controls

	Controls n=73	All patients n=189	MI patients n=140	UA patients n=49
ACE genotype				
II	22(0.301)	47(0.249)	37(0.264)	10(0.204)
ID	40(0.548)	88(0.466)	73(0.521)	15(0.306)
DD	11(0.151)	54(0.286)*	30(0.214)	24(0.490)**
Allele				
I	0.575	0.481	0.525	0.357
D	0.425	0.519	0.475	0.643**

Number of subjects with frequency in parenthesis is for ACE genotype.
* $P<0.05$ when compared with controls
** $p<0.05$ when compared with controls and MI patients

Table 3. ACE genotype and allele frequencies in MI patients according to presence of previous angina

	Previous angina(+) n=47	Previous angina(−) n=80
ACE genotype		
II	15(0.319)	16(0.200)
ID	25(0.532)	41(0.512)
DD	7(0.149)	23(0.288)
Allele		
I	0.585	0.456
D	0.415	0.544*

Number of subjects with frequency in parenthesis is for ACE genotype.
* $p<0.05$ when compared with the patients with previous angina

Table 4. Analysis of the risk of acute coronary syndrome according to ACE genotype

	Logistic Regression Coefficient(SE)	p value
All patients		
DD vs. ID vs. II†	1.036(0.507)/0.007(0.409)	0.041/0.999
DD vs. ID and II‡	1.076(0.497)	0.030
Patients with low cholesterol level*		
DD vs. ID vs. II	0.995(0.610)/-0.154(0.496)	0.103/0.756
DD vs. ID and II	1.036(0.432)	0.016

* Patients in this group had the total cholesterol level less than 200 mg/dL.
† Additive effect of the D allele
‡ Recessive effect of the D allele

Table 5. Relationship between ACE genotypes and coronary artery lesions

	Minimal n=15	1 VD n=61	2 VD n=45	3 VD n=39
ACE genotype				
II	2(0.133)	18(0.295)	11(0.244)	8(0.205)
ID	10(0.667)	24(0.393)	20(0.444)	18(0.462)
DD	3(0.200)	19(0.311)	14(0.311)	13(0.333)
Allele				
I	0.467	0.492	0.467	0.436
D	0.533	0.508	0.533	0.564

Number of subjects with frequency in parenthesis is for ACE genotype.

II, ID, and DD, respectively). The ACE polymorphism was only weakly associated with acute coronary syndrome. To see the recessive effect of D allele, we changed the ordered variable 0 for combined genotypes of II and ID, and 1 for genotype DD. When the genotype DD was compared with the genotype ID and II (assuming recessive effect of D allele), the genotype DD was associated with acute coronary syndrome (0=0.030). When the patients with low choleterol level were included for the logistic analysis, the genotype DD was also associated with acute coronary syndrome ($p=0.016$).

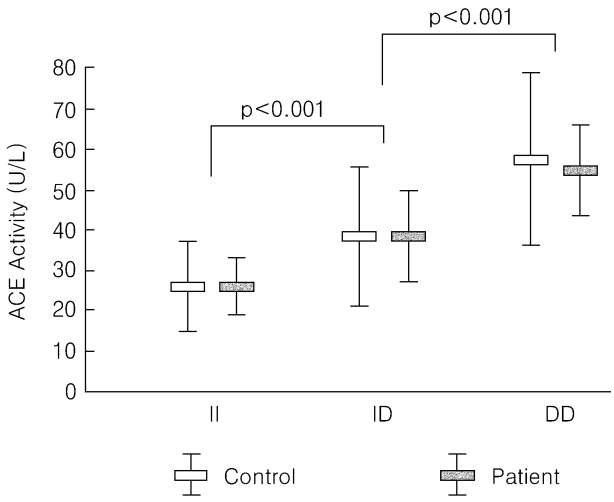

Fig. 2. Serum ACE activities among three different genotypes. Results are mean±SD. II : genotype II, ID : genotype ID, DD : genotype DD

Relationship between the ACE genotypes and coronary artery lesions

As shown in Table 5, there was no significant difference in the frequencies of genotype DD or allele D according to the number of major coronary artery narrowing ($p=0.550$ and $p=0.896$, respectively).

Serum ACE activity among three different genotypes

Serum ACE activity levels were 26.5±10.3, 38.2±16.1, and 54.3±21.3 U/L in patient group and 25.4±6.3, 37.3±10.9, and 57.9±11.1 U/L in control groups for genotypes II, ID, and DD, respectively (Fig. 2). There were significant differences in ACE activity among each genotypes ($p<0.001$), but there were no differences between the patient group and control group for each genotypes ($p>0.05$).

DISCUSSION

The results of Etude Cas-Temoin de I'infarctus de Myocarde (ECTIM) study suggested that a deletion polymorphism of ACE gene was an independent risk factor for myocardial infarction in the low risk Caucasian population (2). After that, there were many reports which showed the relationship between the ACE polymorphism and coronary artery disease. But recently, there is much conflicting data on the relation between ACE genotype

and the risk for myocardial infarction or coronary artery disease (3, 4, 7, 16). In the present study, the frequency of genotype DD was associated with acute coronary syndrome especially with unstable angina, but there was no relationship between ACE genotype and development of coronary atherosclerosis. These are consistent with the report of Ludwig et al. (9). This result shows the possibility that the ACE genotype is related with acute coronary syndrome through the thrombotic modulation or vasoconstriction rather than the degree of coronary atherosclerosis.

The rupture of atheromatous plaque is considered to be the common pathophysiological substrate of acute coronary syndrome, and the process of plaque rupture is related to the intrinsic properties of individual plaque and the extrinsic factors acting on plaque including sympathetic activity, intraluminal pressure and vasoconstriction (13). In patients with unstable angina, quantitative angiography showed hyperreactive vasomotor tone localized to the regions of preexisting coronary atheroma (17). After plaque rupture, coronary artery lumen is obstructed by thrombus which produce the acute coronary syndrome. Thrombosis can occur on preexisting disrupted plaque or intact plaques when thrombotic tendency is high due to platelet hyperaggregability or impaired fibrinolysis (18, 19). The pathophysiological mechanisms linking the ACE genotype and cardiovascular events have not been proven yet. There are several hypotheses. One of them is a relation between ACE genotype and ACE activity. The plasma and cellular levels of ACE activity are associated with an ACE I/D polymorphism, and the presence of the D allele is associated with a higher level of plasma ACE activity (20, 21). In this study, we also observed higher serum ACE activity in the subjects with genotype DD than in those with genotype ID or II, which agreed with other reports. However, no apparent relation was noted between the plasma ACE activity and the risk of MI (16, 22). In addition, ACE activity has no limiting influence on systemic angiotensin II generation and its effects (23). In this report, we also could not find any difference of ACE activity in one genotype between patients with acute coronary syndrome and control, and the ACE activity did not effect the development of acute coronary syndrome by logistic regression analysis (data not shown). But, this does not exclude the possibility that ACE polymorphism affect the plaque vulnerability through the increasing the tissue ACE activity. Recently Diet et al. reported marked ACE accumulation in region of inflammatory cells, especially in areas of clustered macrophage and T lymphocyte, which is considered to be vulnerable part of plaque (24). They speculated that the increased ACE expression in the coronary vessel may promote the induction of acute coronary events through the effect of angiotensin II. Angiotensin II can contribute to the acute coronary events by inducing vasoconstriction, stimulating plasminogen activator inhibitor (PAI-1) production and increasing platelet aggregation (25~27). Decreased bradykinin due to increasd ACE activity can also potentiate the acute coronary events. But, there is no confirmative data which show the increased ACE activity in DD genotype compared to ID and II genotype in atherosclerotic coronary lesions to date.

The other hypothesis is a linkage disequilibrium of ACE gene with another gene. The ACE I/D polymorphism, located in intron 16, can act as a marker in tight linkage to a functional variant yet to be identified (28). Our results did not show the mechanism of relationship between the acute coronary syndrome and ACE genotype, even if there was significant association with genotype DD and acute coronary syndrome.

The genotype frequencies were 30%, 55%, and 15% for genotype II, ID and DD, respectively for control subjects. This result showed that the frequencies of genotype DD and D allele were lower than those of Caucasians, as reported by our previous study (15), but similar to those of Japanese (29). Lee et al. also reported similar genotype frequency for control subjects, and they speculated that D allele is associated with the development of acute coronary syndrome in patients with normal lipid profile when compared with patients with high lipid profile (14). We also analyzed distribution of genotypes according to lipid profile (data not shown), but there was no any significant difference of genotype frequencies. Interestingly, in patients with acute coronary syndrome, the subgroup with unstable angina showed higher frequency of genotype DD than patients with MI, and when we divided the patient group with MI according to the presence of previous angina, the allele and genotype frequencies of patients without angina revealed high frequencies of D allele and genotype DD than those of patients with previous angina. Since the pathophysiologic mechanisms of unstable angina and MI is similar, we cannot explain the different distributions of ACE genotypes between two groups exactly. When we compared the clinical features and other risk factors, the number of women was higher in unstable angina group than in MI group ($p<0.05$). And the frequency of genotype DD was higher in women with acute coronary syndrome compared with the men (0.364 vs. 0.262, $p>0.05$). This can be an one explanation for higher frequency of genotype DD in unstable angina group than in MI group, and implies that the ACE polymorphism is a risk factor of acute coronary syndrome independent of other traditional risk factors. In addition, there was a tendency of less severity of coronary artery lesions in unstable

angina group and patients without previous angina, but there was no significant difference.

Due to the time discrepancy between the ischemic attack and performing coronary angiogram, we may underestimate the role of ACE genotype for the severity of coronary artery lesions. The other limitation is the age distribution of control group is younger than that of patient group. Therefore, in control group, there is possibility that the subjects who might exhibit the acute coronary syndrome within several years were included.

In conclusion, the genotype DD was an independent risk factor of acute coronary syndrome in our study, and ACE polymorphism may be related with acute coronary syndrome through the other pathophysiological mechanism than the progression of underlying atherosclerotic lesion.

REFERENCES

1. Erdos EG. *Angiotensin I converting enzyme and the changes in our concepts through the years. Hypertension 1990; 16: 363-70.*
2. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Richard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F. *Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359 : 641-4.*
3. Bohn M, Berge KE, Bakken A, Erikssen J, Berg K. *Insertion/deletion (I/D) polymorphism at the locus for angiotensin I-converting enzyme and myocardial infarction. Clin Genet 1993 ; 44 : 292-7.*
4. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH. *A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995 ; 332 : 706-11.*
5. Marrop SB, Davidson HR, Connor JM. *The angiotensin I-converting enzyme gene and predisposition to high blood pressure. Hypertension 1993; 21 : 455-60.*
6. Shunkert H, Hense HW, Holmer SR, Stender M, Perz S, Keil H, Lorell BH, Riegger GAJ. *Association between a deletion polymorphism of the angiotensin-converting enzyme gene and left ventricular hypertrophy. N Eng J Med 1994 ; 330 : 1634-8.*
7. Raynolds MV, Bristow MR, Bush EW, Abraham WT, Lowes BD, Zisman LS, Taft CS, Perryman MB. *Angiotensin-converting enzyme DD genotype in patients with ischaemic or idiopathic dilated cardiomyopathy. Lancet 1993; 342 : 1073-5.*
8. Montgomery HE, Keeling PJ, Goldman JH, Humphries SE, Talmud PJ, McKenna WJ. *Lack of association between the Insertion/Deletion polymorphism of the angiotensin-converting enzyme gene and idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1995; 25 : 1627-31.*
9. Ludwig E, Corneli 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.*
10. Markus HS, Barley J, Lunt R, Bland J, Jeffery S, Carter ND, Brown MM. *Angiotensin-converting enzyme gene deletion polymorphism: a new risk factor for lacunar stroke but not carotid atheroma. Stroke 1995; 26 : 1329-33.*
11. Schuh JR, Blehm DJ, Frierdich GE, McMahon EG, Blaine EH. *Differential effects of renin-angiotensin system blockade on atherogenesis in cholesterol-fed rabbits. J Clin Invest 1993; 91 : 1453-8.*
12. Chobanian AV, Haudenschild CC, Nickerson C, Drago R. *Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. Hypertension 1990; 15 : 327-31.*
13. Falk E, Shah PK, Fuster V. *Coronary plaque disruption. Circulation 1995; 92 : 657-71.*
14. Lee MM, Kim HS, Song JM, Choi YJ, Kim DK, Sohn DW, Oh BH, Park YB, Choi YS, Seo JD, Lee YW. *Insertion/Deletion polymorphism of angiotensin I-converting enzyme gene in Korean patients with ischemic heart disease. The Korean Circulation Journal 1996; 26 : 5-13.*
15. Park HY, Kwon HM, Kim HS, Song KS, Kim CH. *An I/D polymorphism in angiotensin-converting enzyme gene in myocardial infarction. The Korean Circulation Journal 1996; 26 : 465-72.*
16. Winkelmann BR, Nauck M, Klein B, Russ AP, Bohm BO, Siekmeier R, Ihnken K, Verho M, Gross W, Marz W. *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; 125 : 19-25.*
17. Brown BG, Bolson EL, Dodge HT. *Dynamic mechanisms in human coronary stenosis. Circulation 1984; 70 : 917-22.*
18. Angleton P, Chandler WL, Schmer G. *Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). Circulation 1989; 79 : 101-6.*
19. Tofler GH, Brezinski D, Schafer AI, Czeisler CA, Rutherford JD, Willich SN, Gleason RE, Williams GH, Muller JE. *Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. N Engl J Med 1987; 316 : 1514-8.*
20. Jan Danser AH, Schalekamp MADH, Bax WA, van den Brink AM, Saxena PR, Riegger GAJ, Schunkert H. *Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995; 92 : 1387-8.*
21. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvel P, Soubrier F. *An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme level. J Clin Invest 1990; 86 : 1343-6.*
22. Gardemann A, Weiss T, Schwartz O, Eberbach A, Katz N,

Hehrlein FW, Tillmans H, Waas W, Haberbosch W. *Gene polymorphism but not catalytic activity of angiotensin I-converting enzyme gene is associated with coronary artery disease and myocardial infarction in low-risk patients. Circulation 1995 ; 92 : 2796-9.*

23. Lachurie ML, Azizi M, Guyene TT, Alhenc-Gelas F, Menard J. *Angiotensin-converting enzyme gene polymorphism has no influence on the circulating renin-angiotensin-aldosterone system or blood pressure in normotensive subjects. Circulation 1995 ; 91 : 2933-42.*
24. Diet F, Pratt RE, Berry GJ, Momose N, Gibbon GH, Dzau VJ. *Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease. Circulation 1996 ; 94 : 2756-67.*
25. Ridker PM, Gaboury CL, Conlin PR, Seely EW, Williams GH, Vaughan DE. *Stimulation of plasminogen activator inhibitor in vivo by infusion of angiotensin II. Circulation 1993 ; 87 : 1969-73.*
26. Van Leeuwen RTJ, Kol A, Andreotti F, Kluft C, Maseri A, Sperti G. *Angiotensin II increases plasminogen activator inhibitor type 1 and tissue-type plasminogen activator mRNA in cultured rat aortic smooth muscle cells. Circulation 1994 ; 90 : 362-8.*
27. Touyz RM, Schiffrin EL. *Effect of angiotensin II and endothelin-1 on platelet aggregation and cytosolic pH and free Ca^{2+} concentrations in essential hypertension. Hypertension 1993 ; 22 : 853-62.*
28. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, Soubrier F. *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.*
29. Higashimori K, Zhao Y, Higaki J, Kamitani A, Katsuya T, Nakura J, Miki T, Mikami H, Ogihara T. *Association analysis of a polymorphism of the angiotensin converting enzyme gene with essential hypertension in the Japanese population. Biochem Biophys Res Commun 1993 ; 191 : 399-404.*