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# Angiotensin-Converting Enzyme Gene Polymorphism is not Associated with Myocardial Infarction in Koreans

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To assess the relationship between angiotensin-converting enzyme (ACE) gene polymorphism and myocardial infarction in Koreans, we recruited 112 healthy, unrelated subjects (mean age 53.4 years) and 104 myocardial infarction survivors (mean age 54.2 years) of both sexes. An insertion/deletion (*I/D*) polymorphism of the ACE gene was typed by polymerase chain reaction. The *I* allelic frequency of ACE gene in Korean subjects was irrelavant to myocardial infarction (patients, 65%; control subjects 66%), as was true with the *D* allele. When compared with other populations, the frequency of *D* allele in Koreans (0.34) was lower than that in Caucasians, and was close to that of other Oriental populations. The data suggest that the ACE gene polymorphism is not an independent genetic risk factor for myocardial infarction in Koreans.

Key Words: Angiotensin-converting enzyme, Gene, Polymorphism, Myocardial infarction, Koreans

## INTRODUCTION

The angiotensin-converting enzyme (ACE), an endothelial ectoenzyme secreted in plasma, is mainly involved in the metabolism of two major vasoactive peptides, converting angiotensin I into the pressor peptide angiotensin II and inactivating the vasodilatory peptide bradykinin (Skeggs et al, 1956; Erdös and Skidgel, 1987). ACE is mainly localized in the endothelium of blood vessels, and plasma ACE level is a potential marker of endothelial cell injury and has been found in decreased levels in diseases with extensive pulmonary involvement such as adult respiratory distress syndrome (Oparil et al, 1976). Clinical interest in plasma ACE level measurements also lies in its abnormal elevation in granulomatous diseases, such as sarcoidosis, in which activated macrophages synthesize and release large amounts of ACE (Hinman et al, 1979).

Plasma ACE level shows a pronounced interindividual variability in a population. Recently, the gene encoding human ACE has been cloned and assigned to chromosome 17q23 (Mattei et al, 1989). Cloning of human ACE cDNA and restriction fragment length polymorphism analysis led to the description of an insertion/deletion (*I/D*) polymorphism of the ACE gene that consists of the presence or absence of a 287-bp DNA fragment located in intron 16 (Tiret et al, 1992). This ACE gene *I/D* polymorphism is strongly associated with serum ACE levels and accounts for a large part of the total serum ACE variance (Tiret et al, 1992). The frequency distribution of the *I/D* ACE genotype studied in a number of different ethnic groups, including Koreans, Caucasians, Nigerian Blacks, Samoans and Yanomami Indians, demonstrated inter-racial differences in the ratio of the frequencies of the *II*, *ID* and *DD* genotypes (Barley et al, 1994; Sohn et al, 1997).

Myocardial infarction is a major health problem in many industrialized countries, contributing significantly to morbidity and mortality in these countries (Higgins et al, 1989). Because a complex interplay of multiple etiologic factors is involved in the pathogenesis of myocardial infarction, this disease entity is better understood in terms of its risk factors. Re-

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cently, Cambien et al. (1992) reported that homozygosity for the *D* polymorphism of the ACE gene is significantly more frequent in male myocardial infarction survivors than in controls. An elevation in the frequencies of the *DD* genotype has been reported in patients with acute myocardial infarction, idiopathic dilated cardiomyopathy and coronary artery spasm (Cambien et al, 1992; Raynolds et al, 1993; Oike et al, 1995). However, in a recent large prospective study, the presence of the *D* allele of the ACE gene was not associated with any appreciable increase in the risk of ischemic heart disease or myocardial infarction (Lindpaintner et al, 1995). Thus the role of the *I/D* polymorphism in myocardial infarction is unclear. In addition, the difference in findings may reflect ethnic differences. Therfore, further study is necessary to clarify the importance of the ACE gene polymorphism as a genetic risk factor for myocardial infarction in Koreans. The aim of this study was to explore *I/D* polymorphism of the ACE genotype in Korean patients with myocardial infarction.

## METHODS

### *Subjects*

One hundred and twelve control subjects (66 males and 46 females) and 104 myocardial infarction survivors (62 males and 42 females) who have been admitted in the Department of Medicine, Soonchunhyang University Hospital were attended. Coronary angiography identified myocardial infarction patients (defined by >50% stenosis in major coronary artery). The patients have experienced myocardial infarctions that were clinically verified by electrocardiogram and the development of left ventricular regional wall motion abnormalities on left ventriculography. All control subjects had no clinical evidences of myocardial infarction with: (1) no history of typical angina pectoris, (2) no abnormal Q wave or ST-T changes in the resting electrocardiogram, and (3) a negative mater exercise test. Mean age was 54.2±0.5 years (mean±SEM) for the patients and 53.4±0.4 years for the control group. The presence of hypertension, diabetes mellitus, hypercholesterolemia (defined as a serum cholesterol level of 250 mg/dl or more) or smoking was determined by history taking, previous medical records, current medications or by the results of examination during hospitalization. The biometrical features of the study population are shown in Table 1. They were informed both verbally and in writing about the experimental procedure and the purpose of the study. Each subject gave his or her written consent before the study, the protocol of which was approved by the Ethics Committees of the Soonchunhyang University Hospital (Chonan, Korea). Blood was drawn from the antecubital vein into heparinized Vacutainer® tube between 8 and 10 a.m. and centrifuged within 2 hours, and the plasma was stored at −20°C and assayed within a month after collection.

### *Determination of ACE genotypes*

Genomic DNA was isolated from peripheral blood leukocytes as described by Iwai et al (1991). The genotype of the ACE gene was determined by the polymerase chain reaction (PCR) according to Tiret et al (1992). The sequences of the sense primer and the antisense primer were 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGTA-3', respectively. PCR was performed in a final volume of 50 μl which contained 100 ng of genomic DNA, 20 pmol of each primers, 250 mM each of the four dNTP, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.4, and 0.4 unit of *Taq* polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). Amplification was carried out in a SANTHERM PCR WS-145 (Sankyo Junyaku Co. Ltd., Osaka, Japan) for 30 cycles with steps of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. The PCR products were electrophoresed in 2% agarose gels, and DNA fragments were visualized directly with ethidium bromide staining. As a result, two alleles were identified: a

**Table 1.** Biometrical features of the study population

| | Controls | Patients |
|---|---|---|
| Number | 112 | 104 |
| Sex (M/F) | 82/44 | 58/46 |
| Age (years) | 53.4±0.4 | 54.2±0.5 |
| Hypertension | 21% | 45% |
| Diabetes mellitus | 5% | 12% |
| Hypercholesterolemia | 9% | 17% |
| Smoking | 42% | 60% |
| Body mass index | 21.2±0.6 | 24.2±0.5 |

190 bp fragment *D* (in the absence of the insertion) and a 490 bp fragment *I* (in the presence of the insertion).

*Statistical analyses*

For comparison of allelic and genotype frequencies, the data were analyzed by using the $\chi^2$ test and multivariate logistic regression analysis using the SPSS program. The clinical characteristics of the two groups were expressed as mean±SEM and were compared by unpaired Student's *t* test. $P<0.05$ was considered statistically significant.

## RESULTS

ACE genotypes were determined for all subjects (Table 2). Out of the total 216 subjects, 91 were homozygous for the *I* allele, and 24 were homozygous for the *D* allele and 101 were heterozygotes. Derived allele frequencies for *I* and *D* allele were 0.66 and 0.34, respectively, in all subjects studied. The observed genotype distribution was in agreement with the Hardy-Weinberg proportion. The frequency of the *DD* genotype in the control group did not differ significantly from that of the myocardial infarction patient group (12% *versus* 11%, P=0.3), as was the frequency of the *D* allele (34% *versus* 35%, P=0.4). When patients were grouped according to sex or age (younger or older than 55 years), no significant difference was found in *DD* genotype frequency between patients and control subjects.

Multivariate logistic regression analysis using the SPSS computer program showed that hypertension (P=0.01), diabetes mellitus ($P<0.001$), hypercholesterolemia ($P<0.001$), smoking ($P<0.01$), and obesity ($P<0.01$) were all independent risk factors for myocardial infarction. The results were similar regardless of the inclusion or the exclusion of age and sex in the multivariate analysis. In contrast, *DD* genotype (P=0.38) was not associated with myocardial infarction in the study population (relative risk, 1.10; 95 confidence interval, 0.87～1.52).

**Table 2.** Genotypic and allelic frequencies of the insertion/deletion (*I/D*) polymorphism of the angiotensin-converting enzyme (ACE) gene in the study population

| | Controls (n=112) | Patients (n=104) | Total (n=216) |
|---|---|---|---|
| Genotype | | | |
| *II* | 48 (43%) | 43 (41%) | 91 (42%) |
| *ID* | 51 (46%) | 50 (48%) | 101 (47%) |
| *DD* | 13 (12%) | 11 (11%) | 24 (11%) |
| Allelic frequency | | | |
| *I* | 66% | 65% | 66% |
| *D* | 34% | 35% | 34% |

## DISCUSSION

A number of studies on the *I/D* ACE polymorphism have demonstrated that the *DD* genotype is associated with the increased risk of cardiovascular diseases (Cambien et al, 1992; Tiret et al, 1993). Although the associations between ACE polymorphism and cardiovascular diseases have been elucidated, they are neither strong nor consistent in all ethnic groups. Moreover, little attention has been given to the impact of race or ethnic origin on this polymorphism. Recently, a significant association of an *I/D* polymorphism of the ACE gene with myocardial infarction has been reported by Cambien (1994). It is important to note that only Caucasian subjects were included in their study. Because myocardial infarction has many underlying causes that may be specific to the genetic and cultural background of the patients, racial differences in genetic and etiological mechanisms which can influence the pathogenesis of myocardial infarction are the interests of this study. Thus, the association between *I/D* polymorphism of the ACE gene and myocardial infarction in Korean patients was investigated.

All subjects included in the study were tested for ACE gene polymorphisms by PCR. Allelic frequencies of Korean myocardial infarction patients were not significantly different compared with those found in controls. Furthermore, the *DD* genotype of the ACE *I/D* polymorphism were not associated with myocardial infarction risk. These observations correspond well with the reports of Lindpainter et al (1995) and Wang et al (1996), studied with American and Australian whites, respectively. While controversial results have been reported in other populations, myocardial infarction in French patients showed a significant association (Ruiz et al, 1994), and Nakai et al (1994) demonstrated a close association between

the ACE polymorphism and the risk for coronary artery diseases in Japanese population.

The frequency of *D* allele in all of the subjects studied, 0.33, was lower than the previously reported value of 0.59 by Zee et al (1992) in Australian Caucasians and 0.57 by Tiret et al (1992) in French Caucasian subjects, but it was very close to the value of 0.40 and 0.3 in Japanese subjects (Higashimori et al, 1993) and Chinese subjects (Lee, 1994), respectively. These findings indicate that the frequencies of the deletion allele among Oriental subjects are much lower than those reported in various Caucasian populations. These findings are of note in that they suggest an existence of interethnic difference in the frequency of gene allele within the various ethnic populations just as other drug metabolizing enzymes do (Muhn et al, 1997; Sohn et al, 1992; 1994).

Recently, genetic risk factors for myocardial infarction have been gradually elucidated. These include polymorphisms on the genes of the renin-angiotensin system, polymorphisms on the genes associated with lipid metabolism and apolipoproteins, and polymorphisms on the genes involved in coagulation and fibrinolytic pathway (Ahn et al, 1993; Behague et al, 1996; Katsuya et al, 1995). However, study results varied according to the types of polymorphisms. In addtion to the *DD* genotype of the ACE I/D polymorphism, conflicting results about the genetic risk for myocardial infarction have been reported for the S2 allele of the *SSt*I polymorphism on the apolipoprotein CIII gene and the B2 allele of the *Taq*I polymorphism on the cholesterol ester transfer protein gene (Bai et al, 1995; Tenkanen et al, 1991). The discrepancy between these studies may be due to ethnic differences or to different selection criteria for the control and patient groups. Thus, our observation suggests a possibility that an interethnic difference in the gene frequencies of ACE may exist within a population.

In conclusion, our data suggest that the *DD* genotype of the ACE *I/D* polymorphism are not associated with myocardial infarction risk in Koreans. Further studies are necessary to elucidate the genetic risk factors for myocardial infarction.

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