The Genetic Polymorphism of Angiotensin-Converting Enzyme in a Korean Population

Dong-Ryul Sohn, Jun-Tack Kwon, Hyung-Kee Kim

Department of Clinical Pharmacology, Soonchunhyang University College of Medicine, Chonan, Korea

= Abstract =

Background : Plasma angiotensin-converting enzyme (ACE) level shows a marked interindividual variability in a population and the variability is attributable to an insertion/deletion (I/D) polymorphism of the ACE gene. We have therefore studied the frequency distribution of the I/D ACE genotype and the relationship between the polymorphism and the plasma ACE concentration in a healthy young Korean population.

Methods : Eighty native, unrelated and healthy Korean subjects were selected and inclusion was based both on clinical characteristics and routine laboratory test performed. Plasma ACE levels were measured with the HPLC system. The genotype of the ACE gene was determined by the polymerase chain reaction.

Results : Of the 80 subjects, 37 were homozygous for the I allele, 10 were homozygous for the D allele and 33 were heterozygotes. The allele frequencies in the subjects studied were 0.68 and 0.32 for I and D allele, respectively. The mean plasma ACE activity revealed a significant relationship with the genotype.

Conclusions : The findings indicate that the Korean subjects have a greater incidence of I allele of ACE gene compared with that reported from Caucasians, and the plasma activity was associated with the ACE gene type.

KEY WORDS : ACE · Genetic · Polymorphism · Koreans.

The angiotensin-converting enzyme (ACE) is a zinc metallopeptidase which plays an important role in vascular physiology^{1,2)}. It is mainly involved in the circulatory homeostasis by removing the carboxyterminal dipeptide of angiotensin I, thereby activating it into the pressor and aldosterone-stimulating peptide, angiotensin II^{1,2)}. ACE is mainly localized on the endothelium of blood vessels, especially in the

pulmonary circulation but it is also found in epithelial cells, in macrophages, in male germinal cells and in a circulating form in several biological fluids³⁾. Circulating ACE probably originates from the vascular endothelial cells⁴⁾ by a mechanism which is still unclear, but could involve proteolytic cleavage of the membrane anchor⁵⁾. 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 syndrome⁶⁾. Clinical interest in plasma ACE level measurements also results from its

순천향대학교 의과대학 임상약리학교실
교신저자 : 손동렬
충남 천안시 쌍용동 366-1
TEL (0417)559-2450 Facsimile (0417)551-8995

abnormal elevation in granulomatous diseases, such as sarcoidosis, in which activated macrophages synthesize and release large amounts of the enzyme^{7,8)}.

Plasma ACE level shows a pronounced interindividual variability in a population⁹⁻¹¹⁾. The variability of plasma ACE level was largely independent of any known environmental or hormonal factor. Recent family studies suggest that approximately half of the interindividual variability of plasma ACE is attributable to a major gene polymorphism^{12,13)}. 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 16¹⁴⁾. 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^{13,14)}. These observations have been extended to the membrane-bound form of ACE by using circulating T-lymphocytes, where ACE levels have also been shown to be genetically determined and associated with the *I/D* polymorphism¹⁵⁾. The ACE *I/D* polymorphism is probably only a neutral marker in linkage disequilibrium with an as yet unidentified causal variant that alters ACE transcription¹²⁻¹⁵⁾.

The frequency distribution of the *I/D* ACE genotype studied in a number of different ethnic groups, including Caucasians, Nigerian Blacks, Samoans and Yanomami Indians, demonstrated inter-racial differences in the ratio of the frequencies of the *II*, *ID* and *DD* genotypes^{16,17)}. Furthermore, it has recently been reported that the pharmacogenetic characteristics of debrisoquine oxidation and *S*-mephenytoin hydroxylation would differ within the similar ethnic populations residing in the same geographic region¹⁸⁻²⁰⁾. In Korean population, there are a few studies for the ACE *I/D* genetic polymorphism, particularly

in patients with ischemic heart disease²¹⁾ and myocardial infarction²²⁾. However, no information on the frequency distribution of the ACE genotype and the relationship between the genotype and ACE activity in a healthy young subjects has been available from a Korean population.

We therefore explored the frequency distribution of the *I/D* ACE genotype and the relationship between this polymorphism and the plasma ACE concentration in a healthy young Korean population.

Materials and Methods

1. Chemicals

All chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

2. Subjects

Eighty healthy volunteers (49 males and 31 females) were selected for the study and inclusion was based both on clinical characteristics and routine laboratory tests performed. Inclusion criteria were a body mass index < 28 kg/m², normal blood pressure on WHO criteria (systolic blood pressure < 160 mmHg and diastolic blood pressure < 90 mmHg measured in the sitting position after a 5-min rest), glycemia < 6 mmol/liter, plasma γ -glutamyl transpeptidase < 30 IU/liter, normal sedimentation rate, normal chest X-ray, and absence of acute or chronic disease and drug intake. They were members of laboratory personnel or medical school students, and were informed both verbally and in writing about the experimental procedure and the purpose of the study. Each subject gave his written consent before the study, the protocol of which was approved by the Ethics Committee of the Soonchunhyang University Hospital (Chonan, Korea).

3. Measurement of plasma ACE levels:

Blood was drawn from the antecubital vein into heparinized Vacutainer tube between 8 and 10 a.m. and centrifuged within 2 hour, and the plasma was stored at -20°C and assayed within a month after collection.

Plasma ACE concentration was measured in duplicate in all subjects by the method as described by Neels et al.²³⁾ with minor modification. In brief, incubate 10 μ L of plasma and 100 μ L of substrate solution (50 mmol/L HEPES buffer, pH 8.0, containing, per liter, 30 mmol of Hip-Gly-Gly, 300 mmol of NaCl, and 400 mmol of Na₂SO₄) for 15 min at 37°C. Stop the reaction by adding 10 μ L of 2.4 mol/L HCl. After adding 10 μ L of internal standard (*o*-methyl hippuric acid), extract the hippuric acid and internal standard from the acidified solution into 1 mL of ethyl acetate. After centrifugation and evaporation, dissolve the residue in 200 μ L of the mobile phase, and inject 30 μ L into the chromatograph. The mobile phase used for analysis was a 3/17 (by vol) mixture of potassium phosphate buffer (10 mmol/L, pH 3.5) and acetonitrile, at a flow rate of 5.0 mL/min. The HPLC system consisted of a model 305 pump, a model 118 UV/VIS detector set at a wavelength of 228 nm, a model 234 autoinjector (Gilson Inc., Middleton, WI), a model HP 3395 integrator (Hewlett-Packard Co., Palo Alto, CA) and an octyldecylsilane stainlesssteel column [5 μ m, 4.6 mm $\times$ 150 or 250 mm I.D., Ultrasphere, Beckman (Allendale, NJ)]. The intra-assay and inter-assay variability coefficients were 6% and 11.5%, respectively.

One unit (U) of ACE activity is defined as the amount of enzyme required to release 1 nmol of hippuric acid per minute per mL of plasma at 37°C under the assay conditions described (i.e., U/mL = kU/L = nmol/mL per minute).

4. Determination of ACE genotypes

Genomic DNA was isolated from peripheral blood leukocytes as described by Iwai et al.²⁴⁾. The genotype of the ACE gene was determined by the polymerase chain reaction (PCR) according to Tiret et al.¹³⁾. The sequence of the same 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 μ L which contained 100 ng of genomic DNA, 20 pmol of each primers, 250 μ M each of the four dNTP, 1.5 mM MgCl₂, 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 an 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 product were electrophoresed in 2% agarose gels, and DNA was visualized directly with ethidium bromide staining and two alleles were identified: a 190 bp fragment *D* (in the absence of the insertion) and a 490 bp fragment *I* (in the presence of the insertion)

5. Statistical analyses:

Statistical analyses, including analysis of variance and computation of Pearson's correlation coefficient, were performed with the aid of the SAS statistical package. Results are presented as mean $\pm$ standard deviation.

Results

The values for age, body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP) for men and women are listed in Table 1. BMI,

which was slightly greater for men than for women, was the only parameter that differed significantly between gender ($p < 0.01$).

Serum ACE concentrations did not differ significantly between men and women and were not correlated to age, SBP, DBP, or BMI (Table 1). Consequently, these variables were not adjusted for subsequent analysis (Table 2).

ACE genotypes were determined for all subjects (Table 2). Of the 80 subjects, 37 were homozygous for the *I* allele, 10 were homozygous for the *D* allele and 33 were heterozygotes. Derived allele frequencies for *I* and *D* allele were 0.68 and 0.32 in the Korean subjects studied. The observed genotype distribution was in agreement with the Hardy-Weinberg proportion.

The mean plasma ACE activity, defined by the *I/D* polymorphism in the subjects was 10.1 ± 3.7 , 13.2 ± 4.8 and 14.9 ± 5.1 IU/L for homozygotes *II*, heterozygotes *ID* and homozygotes *DD*, respectively, showing a significant relationship between polymorphism and plasma ACE activity ($p < 0.01$) (Table 2, Fig 1).

Discussion

Recent work on the *I/D* ACE polymorphism has demonstrated that the *DD* genotype is associated with increased risk of cardiovascular disease, especially in those without other risk factors²⁵, excess deaths from ischemic heart disease in parents of those with the *DD* genotype²⁶, dilated or hypertrophic cardiomyopathy and sudden death^{27,28}, and restenosis²⁹. However, 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 essential hypertension has been reported by Harrap et al³⁰. It is important to note that only Caucasian subjects were included in their study. Because essential hypertension 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 blood pressure regulation are of interest. Thus, in the present study we performed a preliminary study of the *I/D*

Table 1. Clinical data and serum ACE concentrations of 80 Korean subjects

	Men (n = 49)	Women (n = 31)
Age (yr)	23.5 ± 7.1	22.5 ± 6.2
BMI (kg/m^2)	23.6 ± 2.5	$21.9 \pm 2.4^*$
SBP (mmHg)	124.8 ± 8.2	122.7 ± 10.5
DBP (mmHg)	78.5 ± 7.1	74.2 ± 6.9
ACE concentration (IU/L)	12.8 ± 3.1	13.0 ± 3.4

The data given as mean $\pm$ SD; * $p < 0.01$; men versus women

Table 2. Clinical parameters and serum ACE values in groups with different ACE genotypes

	<i>II</i> (n = 37)	<i>ID</i> (n = 33)	<i>DD</i> (n = 10)
Age (yr)	22.9 ± 6.9	23.9 ± 7.0	23.2 ± 6.2
BMI (kg/m^2)	22.5 ± 3.1	23.2 ± 3.0	22.1 ± 2.9
SBP (mmHg)	123.5 ± 14.1	124.2 ± 10.1	122.4 ± 10.9
DBP (mmHg)	77.2 ± 9.2	76.9 ± 6.9	75.1 ± 7.3
ACE concentration (IU/L)	$10.1 \pm 3.7^*$	$13.2 \pm 4.8^*$	$14.9 \pm 5.1^*$

The data are given as mean $\pm$ SD; *Comparison between genotype groups; $p < 0.01$

Fig. 1. Plasma ACE activity in individuals with II, ID, and DD genotypes in 80 Korean population.

polymorphism of ACE gene with the plasma activity for assessing the association study of with ACE gene polymorphism and coronary heart disease or essential hypertension in a Korean subjects.

All subjects included in the study were tested for ACE gene polymorphisms by DNA gel-blot hybridization. It is noteworthy that the frequency of *I* allele in this subjects, 0.68, was higher than the previously reported values of 0.41 by Zee et al³¹⁾ in Australian Caucasian and 0.43 by Tired et al¹³⁾ in French Caucasian subjects, and was very similar to the value of 0.60 and 0.7 in Japanese subjects³²⁾ and Chinese subjects¹¹⁾, respectively. And also, the data are consistent with those of the previous studies in Korean patients with ischemic heart disease²¹⁾ and myocardial infarction²²⁾. These findings are indicating that the frequencies of the insertion allele among Oriental subjects are much higher prevalence than had been reported various Caucasians. Thus our observation suggests a possibility than an interethnic difference in the gene frequencies of ACE may exist within the populations like as the debrisoquine

type¹⁸⁾ and *S*-mephenytoin type³³⁾ oxidation polymorphism. Furthermore, it may be the factor to explain the interracial difference in the prevalence of the coronary heart disease and hypertension.

When the level of serum ACE was compared in subjects of the three genotype classes (*II*, *DD*, and *ID*), a marked difference was found between the three groups in the subjects studied. A group with the deletion polymorphism had higher ACE levels than those with the insertion polymorphism. Intermediate ACE levels were observed in heterozygote group (Table 2). Cambien et al.¹²⁾ recently proposed a model for the genetic control of plasma ACE levels, based on the results of a family study. In this study, the genetic analysis of familial phenotypes suggested that these levels are affected by a major gene, which was estimated to account for 29% of the total phenotypic variance of plasma ACE in adults. The present results confirm this major gene's effect as a clear difference in serum ACE levels was observed between the three genotype classes and the ACE gene was found to be the major gene responsible for this effect. Other genetic or environmental factors may be involved in the interindividual variations in circulating ACE. However, in a study of several candidate parameters, no clear hormonal or environmental influence was detected.

The observation of a genetic polymorphism that explains much of the interindividual variability in plasma ACE levels has clinical implications, particularly for the diagnosis of granulomatous disease, such as sarcoidosis in which circulating ACE levels are high because of increased ACE secretion by monocyte-derived cells^{7,34)}. By determining the genotype of patients, it may be possible to reduce the size of the reference interval to which a given measurement of the plasma ACE level should be compared. This would allow more accurate conclusions

to be drawn about the significance of plasma ACE levels in individual patients. As ACE is implicated in vasoactive peptides metabolism, the ACE gene is a candidate gene for essential hypertension.

However, more studies are required to see if this is translated into altered risks of coronary artery disease and hypertension.

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= 국 문 초 록 =

한국인에서의 Angiotensin 전환효소의 활성화도 및 유전자형과의 상관성

손동렬 · 권준택 · 김형기

순천향대학교 의과대학 임상약리학교실

배 경 : 혈장 내의 angiotensin 전환효소(ACE)는 개체에 따라 다양한 활성화도를 나타내며 이는 ACE 유전자의 insertion/deletion(I/D) 다형성과 관련이 깊다. 따라서 한국인에서의 ACE의 I/D 유전자형의 분포를 검색하고 혈장내 ACE농도와 ACE 유전자형의 관련성 여부를 확인하고자 본 연구를 시도하였다.

방 법 : 80명의 건강한 자원자에서 혈액을 채취하여 고속액체 크로마토그래피로 혈장 내 ACE 활성화도를 측정하였다. 또한 ACE 유전자형은 Taret 등의 방법으로 PCR을 이용하여 확인하였다.

결 과 : 본 연구에 참여한 80명 중 37명은 I allele의 homozygote, 10명은 D allele의 homozygote이었으며 33명은 heterozygote이었다. 따라서 I allele의 빈도는 0.68이었고 D allele의 빈도는 0.32이었다. 혈장 ACE 활성화도는 PCR로 확인한 유전자형과 통계적으로 유의하게 상관관계가 있었다.

결 론 : 한국인에서의 ACE 유전자형은 I allele가 백인 보다는 높은 빈도를 보였고 D allele는 낮은 빈도를 보였으며 혈장 ACE 활성화도는 각각의 유전자형과 관련이 있었다.