Brief Communications

Deletion Polymorphism of the Angiotensin I–Converting Enzyme Gene Is Associated With Serum ACE Concentration and Increased Risk for CAD in the Japanese

Kenji Nakai, MD, PhD; Chuichi Itoh, MD, PhD; Yoshinori Miura, PhD; Kazuhiro Hotta, MD; Takehiko Musha, MD; Tomonori Itoh, MD; Tomohisa Miyakawa, MD, PhD; Ryu Iwasaki, PhD; Katsuhiko Hiramori, MD, PhD

**Background** The angiotensin I–converting enzyme (ACE) is a key component of the renin-angiotensin system thought to be important in the pathogenesis of hypertension and cardiovascular disease. Deletion polymorphism in the ACE gene may be a risk factor for myocardial infarction in the Caucasian population. However, this finding has not yet been investigated in the Japanese population.

**Methods and Results** A 287-bp insertion/deletion polymorphism in intron 16 of the ACE gene was examined by polymerase chain reaction in a cross-sectional study of 100 healthy subjects and 178 patients with coronary artery disease (CAD) (70 angina pectoris, 108 myocardial infarction), whose serum ACE levels were concomitantly measured. Polymorphism of the ACE gene was characterized by three genotypes: two deletion alleles (genotype DD), two insertion alleles (genotype II), and heterozygous alleles (genotype ID). No differences could be detected among the three genotypes for total cholesterol, HDL cholesterol, and body mass index. Serum ACE levels were 11.4±2.7, 14.5±3.5, and 16.6±4.6 IU/mL for genotypes II, ID, and DD, respectively. In the study population, the genotype DD was more closely associated with CAD than the other two genotypes (ID and II). The frequency of deletion alleles was higher (0.58) in the CAD group than in healthy control subjects (0.42) (P<.05). Furthermore, multivessel disease was more strongly associated with deletion alleles than with insertion alleles (P<.05).

**Conclusions** A deletion polymorphism of the ACE gene is associated with serum ACE activity and increased risk for CAD in the Japanese. (*Circulation, 1994;90:2199-2202.)*

**Key Words** | angiotensin | enzymes | coronary disease | genes

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Hypercholesterolemia, hypertension, diabetes, tobacco usage, and obesity are well-known risk factors for cardiovascular disease. However, there is a population of patients without such classic risk factors who develop coronary artery disease (CAD). Angiotensin I–converting enzyme (ACE) is an effective target for antihypertensive medication in patients with hypertension. Recently, Pfeffer and colleagues found that administration of an ACE inhibitor not only lowered the risk for developing heart failure but also reduced the risk for recurrent myocardial infarction. ACE is a membrane-bound dipeptidyl carboxypeptidase ectoenzyme located in the endothelial lining of blood vessels throughout the body. Cloning of the ACE gene revealed a 287-bp insertion/deletion polymorphism in intron 16 (ACE/ID polymorphism). In the Etude Cas-Temoine de l'Infarctus du Myocarde (ECTIM) study, designed to identify genetic determinants of myocardial infarction, Cambien and colleagues found that the frequency of this ACE deletion polymorphism was significantly greater in patients with myocardial infarction than in control subjects in a Caucasian population. This finding suggested that genetic variation in or near the ACE locus may be involved in the pathogenesis of cardiovascular disease.

The purpose of this study was to investigate the correlation of the 287-bp ACE/ID polymorphism with serum ACE activity and CAD in the Japanese.

**Methods**

**Subjects**

This study consisted of 100 healthy subjects and 178 patients with CAD (70 angina pectoris, 108 myocardial infarction) in Japan. Mean age was 42±10 years in normal subjects and 59±9 years in patients with CAD. Diagnoses of angina pectoris and acute myocardial infarction were made according to conventional criteria. Resting blood pressure was measured by standard cuff technique. Total cholesterol and HDL cholesterol were measured by routine chemical methods. Coronary angiography was performed by the Judkins method, and coronary vessels were defined as diseased if at least 75% stenosis was demonstrated.

**Serum ACE Measurement**

Serum ACE activity was measured in duplicate by colorimetric method based on the quinineimine dye produced from the substrate of p-hydroxyhippuryl-L-histidyl-L-leucine.

**DNA Extraction**

Peripheral venous blood samples (8 mL) were drawn, and white blood cells were separated. High-molecular-weight DNA was extracted.

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From the Second Department of Internal Medicine (K.N., K. Hotta, T. Musha, T.I., T. Miyakawa, K. Hiramori) and Clinical Pathology (C.I., Y.M.), Iwate Medical University, and Mitsubishi-Yuka Bio-Clinical Laboratories, Inc (R.I.), Morioka, Japan.

Correspondence to Kenji Nakai, MD, PhD, The Second Department of Internal Medicine, Iwate Medical University, 19-1 Uchimaru, Morioka, Japan 020.
DNA was isolated from peripheral blood leukocytes by use of a DNA extraction kit (Stratagene).

### Southern Blot Analysis of Human Genomic DNA

High-molecular-weight DNA samples (5 μg) were incubated with restriction enzyme Sac I (Boehringer Mannheim). Digested DNAs were electrophoresed through a 1% agarose gel, transferred to nylon membrane (Pall), and hybridized with a 32P-labeled 247-bp Genomic DNA 1 genomic fragment that contained 247 bp of intron 16 of ACE gene. The filter was washed with 2× SSC, 0.1% SDS, and 0.2× SSC, 0.1% SDS at 52°C and exposed to x-ray film (Kodak).

### Polymerase Chain Reaction for Detection of ACE Insertion/Deletion Polymorphism

Template DNA (100 ng) was used in a polymerase chain reaction (PCR) under stringent conditions to avoid the possibility of false positives. The sense oligonucleotide primer was 5′ CAG ACC ACT CCC ATC TTT TCT 3′, and the antisense primer was 5′ GAT GTG GCC ATC TAC TTC 3′. Reactions were performed in a final volume of 100 μL containing 25 pmol of primer, 1.5 mmol/L MgCl2, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 0.1 mg/mL gelatin, 0.5 mmol/L of each dNTP, and 2.5 U of Taq polymerase (Perkin-Elmer/Cetus). Amplification was carried out in a DNA Thermal Cycler (PC-700, Sci-Media Ltd) for 30 cycles with steps of denaturation at 93°C for 1.5 minutes, annealing at 58°C for 2 minutes, and extension at 72°C for 2 minutes. PCR products were electrophoresed on 1.2% agarose gels, and DNA was visualized by ethidium bromide staining.

### Statistical Analysis

Data of total cholesterol, HDL cholesterol, body mass index (BMI), and serum ACE activity were presented as mean±SD. The differences between groups were analyzed by unpaired Student’s t test. Data were compiled according to genotype, and allele frequencies were calculated. Statistical analysis required calculation of the observed number of alleles from genotype data in each group. The difference between groups was then tested by multiple group contingency table analysis. A value of P<.05 was considered significant.

### Results

Clinical parameters—age, BMI, total cholesterol, and HDL cholesterol—are listed in Table 1. No differences were detected among the three genotype populations in terms of age, total cholesterol, HDL cholesterol, and BMI.

The polymorphism detected by PCR was evident as a 490-bp product in the presence of the insertion (I allele) and as a 190-bp fragment in the absence of the insertion (D allele). Thus, each DNA sample revealed one of three possible patterns after electrophoresis: a 490-bp band ( genotype II), a 190-bp band ( genotype DD), or both a 490- and a 190-bp band ( genotype ID). Southern blot analysis of high-molecular-weight DNA digested by Sac I demonstrated the same polymorphism as PCR (Figure).

Serum ACE levels were 11.4±2.7, 14.5±3.5, and 16.6±4.6 IU/mL for genotypes II, ID, and DD, respectively, as shown in Table 2. The results showed a relation between the ACE gene polymorphism and the serum ACE level.

ACE genotypes and derived allele frequencies are shown in Table 3. The genotype DD characterized by two short alleles was more strongly associated with CAD compared with the two other genotypes (ID and II). The frequency of deletion alleles (D) was higher (0.58) in the CAD group than in normal subjects (0.42). Statistical analysis (Table 3) showed that values for the CAD group differed significantly from those for the healthy control ( $\chi^2$=13, P<.01).

The relation between coronary lesions and the ACE/ID polymorphism is shown in Table 4. Multivessel disease was more strongly associated with deletion alleles (D) than single-vessel disease ( $\chi^2$=5.3, P<.05).

### Table 1. Clinical Parameters of the Patient Population

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61±9</td>
<td>58±8</td>
<td>61±8</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>192±42</td>
<td>195±40</td>
<td>191±35</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>41±14</td>
<td>39±11</td>
<td>40±12</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>137±15</td>
<td>135±23</td>
<td>133±19</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82±8</td>
<td>83±8</td>
<td>83±10</td>
</tr>
<tr>
<td>Body mass index, kg · m⁻²</td>
<td>24.7±3.2</td>
<td>24.1±3</td>
<td>24.3±3.1</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin I-converting enzyme.

### Table 2. Mean Serum ACE Levels Among the Three Groups Defining the ACE Gene Polymorphism

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>26</td>
<td>44</td>
<td>41</td>
</tr>
<tr>
<td>Serum ACE concentration, IU/mL</td>
<td>11.4±2.7</td>
<td>14.5±3.4</td>
<td>16.6±4.6</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin I-converting enzyme.
TABLE 3. Coronary Artery Disease and ACE/ID Genotype and Allele Frequencies

<table>
<thead>
<tr>
<th>Frequencies</th>
<th>ACE Genotype</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>II</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>100</td>
<td>41</td>
</tr>
<tr>
<td>Myocardial infarction and angina pectoris</td>
<td>178</td>
<td>44</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>108</td>
<td>26</td>
</tr>
<tr>
<td>Angina pectoris</td>
<td>70</td>
<td>18</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin I-converting enzyme.
*P<.01 (χ²=13), †P<.01 (χ²=11), ‡P<.01 (χ²=7.7) vs healthy control subjects.

Discussion

The ACE deletion polymorphism detected by PCR analysis appears to be associated with serum ACE level and increased risk for CAD in the Japanese. It is a common observation that there is a patient population without classic risk factors who develop myocardial infarction. It is very important to identify this population for the purpose of primary or secondary prevention of myocardial infarction. Most genetic studies on CAD have focused on genes involved in lipid metabolism.12,13 Cambien and colleagues8 showed that the ACE gene may affect myocardial infarction independently of lipid metabolism. Recently, Pfeffer and colleagues9 found that administration of an ACE inhibitor not only decreased the risk for developing heart failure but also reduced the risk of recurrent myocardial infarction.

ACE is a key component of the renin-angiotensin system that has long been considered to play a role in the pathogenesis of hypertension and cardiovascular disease.3,4 The ACE gene, which spans 21 kb and consists of 26 exons, was originally cloned by Soubrier et al.15 Two different mRNAs, endothelial and testicular ACE, are transcribed from the ACE gene and have been assigned to their respective exons.15 An earlier experimental study16 had shown that endothelial-type ACE gene expression was increased in myocardial tissue after coronary artery occlusion. Inhibitors of ACE were shown to prevent myocardial proliferation after vascular injury.16 Circulating ACE probably originates from the vascular endothelial cells, although the biochemical mechanism of ACE secretion is not clear.17 Several hypotheses have been proposed, including proteolytic cleavage of the hydrophobic anchor and passive leakage of the membrane-bound enzyme.

Rigat et al17 and Tiret et al17 originally detected the insertion/deletion polymorphism in the ACE gene locus using an ACE cDNA probe that spanned the complete endothelial ACE mRNA sequence. The polymorphism was detected with the restriction enzyme Hind III. The insertion allele was designated I and the deletion allele, D. They reported that marked differences in serum ACE levels of healthy subjects were found among the three genotypes. Subjects with the deletion polymorphism had higher serum ACE levels than those with the insertion polymorphism. Since the major effect of this polymorphism was reflected in different serum ACE levels, they concluded that the ACE gene played a significant role in the pathogenesis of essential hypertension.

In the ECTIM study, the data of Cambien et al9 suggested that a deletion polymorphism in the gene encoding ACE was a risk factor for myocardial infarction in the Caucasian population, particularly in those otherwise thought to be at low risk for cardiovascular disease. In the present study, the frequency of genotype DD was more strongly associated with CAD in Japanese patients. The frequencies were 25%, 34%, and 41% for genotypes II, ID, and DD, respectively, for Japanese patients with CAD, whereas they were 17%, 51%, and 32%, respectively, for Caucasian patients with CAD. The frequency of deletion allele (D) was higher (0.58) in the CAD group than in normal subjects (0.42). Furthermore, multivessel disease was more significantly associated with genotype DD than with genotype II. These findings suggest that the ACE/ID polymorphism plays a significant role in development of CAD in the Japanese as well as in Caucasian populations.

Since the insertion/deletion polymorphism in the ACE gene lies within an intron and there is no evidence

TABLE 4. Relation Between Coronary Artery Lesions and the ACE/ID Gene Polymorphism

<table>
<thead>
<tr>
<th>Frequencies</th>
<th>ACE Genotype</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>II</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>95</td>
<td>25</td>
</tr>
<tr>
<td>Double-vessel disease</td>
<td>54</td>
<td>13</td>
</tr>
<tr>
<td>Triple-vessel disease</td>
<td>29</td>
<td>5</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin I-converting enzyme.
*P=NS (χ²=1.9), †P<.01 (χ²=5.3) vs single-vessel disease.
for differential splicing that might incorporate this sequence into a primary transcript, the polymorphism would be unlikely to influence the dipeptidyl carboxypeptidase activity of the encoded enzyme. It also remains to be shown whether this DNA might influence the splicing of the primary transcript that leads to mature mRNA. Another possibility is that the target gene responsible for CAD is actually another gene located not far from the ACE gene. The ACE locus (17q23) is near the growth hormone gene (17q22-q24), raising the possibility that the ACE polymorphism is in linkage disequilibrium with a growth hormone variant. Growth hormone enhances overall somatic growth, so that a genetic variation in growth hormone production could increase the rate and extent of growth of cardiomyocytes and vascular smooth muscle cells or regional vascularization at an early stage.

In this study, an ACE deletion polymorphism is shown to be associated with increased risk for CAD and thereby may be a potential tool for genetic studies of cardiovascular disease. However, the mechanisms in the pathogenesis of myocardial infarction are very complex. Further works are needed to identify other loci that may be important in the pathogenesis of CAD.

Acknowledgments

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