Insertion/Deletion Polymorphism in the Angiotensin-Converting Enzyme Gene Affects Heart Weight

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Background—Angiotensin (Ang) II, a major regulatory factor for left ventricular mass, is generated from Ang I by ACE. ACE levels are associated with an insertion/deletion (I/D) polymorphism in the ACE gene. The ACE polymorphism should result in varied Ang II concentrations and hence affect left ventricular mass. We therefore investigated whether ACE genotype is a predictor of heart weight.

Methods and Results—From 693 consecutive patients autopsied between 1994 and 1998 in our hospital, patients with valvular disease, myocardial infarction, or cardiomyopathy were excluded. The remaining 443 autopsy patients were the subjects of our study. The heart weight at autopsy was corrected for body surface area. Genomic DNA was purified from the kidney, and ACE genotype was determined by polymerase chain reaction. Heart weight in the DD genotype (249.9±49.9 g/m²) was significantly higher than that in the ID (230.0±51.2 g/m²; P<0.05) and II (226.8±49.8 g/m²; P<0.01) genotypes. Heart weight was also positively related to age (r=0.145, P<0.0001) and coronary stenosis index (r=0.147, P=0.0019). Multiple regression analysis showed that a history of hypertension (P<0.0001), age (P=0.0001), and DD genotype (P=0.0154) were independent predictors of heart weight.

Conclusions—ACE genotype predicts cardiac mass; however, it was less effective than epigenetic factors such as hypertension or age. (Circulation. 2000;101:148-151.)

Key Words: hypertrophy ■ myocardium ■ polymerase chain reaction ■ genes ■ risk factors

Angiotensin II (Ang II) is a potent vasoconstrictor and stimulator for cell growth. Ang II also plays a major role in the pathogenesis of hypertension, intimal hyperplasia, and cardiac hypertrophy.1–4 Ang II is generated from Ang I by ACE. An insertion/deletion (I/D) polymorphism of intron 16 of the ACE gene is related to plasma and tissue ACE levels.5 Individuals who are homozygous for the deletion (DD) exhibit higher circulating and tissue levels of ACE than individuals who are homozygous for the insertion (II) or who are heterozygous (ID). The variance of ACE density among the 3 genotypes may generate diverse Ang II production.

In the present study, we examined the possible influences of the ACE genotype on heart weight and found it was a predictor of heart weight; however, it was less effective than epigenetic factors such as hypertension or age.

Methods

Subjects
From 693 consecutive patients autopsied between 1994 and 1998 in Tokyo Metropolitan Geriatric Hospital with written consent by the patient and/or families under the Act of Postmortem Examination, 226 patients with myocardial infarction, valvular heart disease, or cardiomyopathy and 24 patients whose data were incomplete were excluded. The remaining 443 patients (208 females and 235 males) were the subjects of the present study. A history of hypertension, diabetes mellitus, or hypercholesterolemia (>220 mg/dL) was sought in the medical records.

DNA Purification and Determination of ACE Genotype
Genomic DNA purification from frozen kidney tissue and determination of ACE genotypes by polymerase chain reaction were performed according to standard methods.6 Amplification products were analyzed on a 2% agarose gel.

Heart Weight
Body weight, height, and heart weight were obtained at autopsy. To standardize the individual heart, the heart weight was corrected for body surface area (BSA). The BSA was calculated according to DuBois’ formula: weight0.425×height0.725×71.84. The corrected heart weight was expressed as g/m².

Coronary Stenosis Index
Coronary arteries were cut every 5 mm, and each section was examined and its degree of stenosis scored. The stenosis was expressed as a range from 0 to 5 as follows: 5, 100% or 99% stenosis; 4.5, 90% stenosis; 4, 75% stenosis; 3, 50% stenosis; 2, 25%
TABLE 1. Patient Characteristics According to ACE Genotype

<table>
<thead>
<tr>
<th>ACE Genotype, n</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>DD (n=51)</td>
<td>ID (n=184)</td>
<td>II (n=208)</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>79.4±10.1</td>
<td>79.6±8.6</td>
<td>80.3±9.4</td>
</tr>
<tr>
<td><strong>Sex, % female (n)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49.0% (25)</td>
<td>45.1% (83)</td>
<td>48.1% (100)</td>
</tr>
<tr>
<td><strong>BSA, m²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.36±0.18</td>
<td>1.35±0.18</td>
<td>1.32±0.18</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52.9% (27)</td>
<td>47.3% (87)</td>
<td>42.8% (89)</td>
</tr>
<tr>
<td><strong>Diabetes mellitus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.4% (9)</td>
<td>18.1% (33)</td>
<td>10.1% (21)*</td>
</tr>
<tr>
<td><strong>Hypercholesterolemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.4% (11)</td>
<td>16.5% (27)</td>
<td>19.5% (36)</td>
</tr>
<tr>
<td><strong>CSI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.6±4.0</td>
<td>6.8±4.2</td>
<td>6.9±4.2</td>
</tr>
</tbody>
</table>

*P=0.023 vs ID. Values are mean±SD or percent (n).

Results

Among 443 patients, 51 were homozygous for the deletion (DD), 184 were heterozygous (ID), and 208 were homozygous for the insertion (II). The observed frequency distribution of ACE genotypes did not deviate from that predicted (46.2, 193.7, and 203.2, respectively) by Hardy-Weinberg equilibrium (χ²=1.107, P=0.20). There were no significant differences in sex, BSA, history of hypertension, or history of hypercholesterolemia among the 3 ACE genotype groups. Diabetes mellitus was significantly less frequent in the II genotype. The CSI was greater for the DD genotype, but this difference was not statistically significant (Table 1).

Heart weight was 249.9±49.9 g/m² for patients with the DD genotype, 230.0±51.2 g/m² for the ID genotype, and 226.8±49.8 g/m² for the II genotype. Hearts in the DD group were significantly heavier than those of patients with ID (P<0.05) or II (P<0.01) genotype.

Figure 1. Heart weights of each ACE genotype. Heart weights in DD, ID, and II genotypes were 249.9±49.9 (n=51), 230.0±51.2 (n=184), and 226.8±49.8 (n=208) g/m², respectively. Hearts of patients with DD genotype were heavier than those of patients with ID (P<0.05) or II (P<0.01) genotype.

That in the non-DD (DI and II) group was 228.3±50.5 g/m². Hearts of patients in the DD group were 20 g/m² heavier than those of patients in the ID and II groups (P<0.01) (Table 2). There was no relation between heart weight and sex, diabetes mellitus, or hypercholesterolemia (Table 2).

Simple regression analysis was performed between heart weight, CSI, and age (Table 3). These 3 factors are interrelated, with some difference in correlation coefficients.

To analyze the relative contribution of each of these factors to heart weight, we performed multiple regression analysis.

Figure 2. Heart weight of each ACE genotype was analyzed in subgroups with and without history of hypertension. In normotensive subgroup, there was no significant heart weight difference among 3 genotypes. In hypertensive subgroup, difference of heart weight was prominent. Heart weights were as follows: normotensive subgroup, DD 227.1±37.5 (n=24), ID 214.1±44.6 (n=102), II 218.3±46.5 g/m² (n=119). Hypertensive subgroup, DD 266.3±52.1 (n=27), ID 249.9±52.3 (n=82), II 238.0±52.2 g/m² (n=89). Predicted frequencies of ACE genotypes from Hardy-Weinberg equilibrium were 23.0, 104.1, and 118.0 in normotensive subgroup and 23.4, 89.3, and 85.4 in hypertensive subgroup, respectively. Observed genotype frequencies did not differ from Hardy-Weinberg expectations (normotensive subgroup: χ²=0.098, P>0.20; hypertensive subgroup: χ²=1.321, P>0.20).
using the factors that were positively related in simple regression analysis: history of hypertension, DD genotype, age, and CSI. History of hypertension \((P<0.0001)\) and age \((P=0.0001)\) were strong predictors of heart weight. The DD genotype was also an independent predictor of heart weight \((P=0.0154)\). There was no relation between CSI and heart weight \((P=0.1389)\).

### Discussion

These studies indicate that there is a relationship between ACE genotype and heart weight. Hearts of patients with the DD genotype were significantly heavier than those of patients with the ID and II genotypes. For cardiac muscle mass, the ACE genotype was a less effective determinant than epigenetic factors such as hypertension or age.

Our subjects were taken from consecutive autopsies in a general hospital for the aged and were not limited to certain medical specialities. Hence, these patients reflect the general population in Japan. The frequencies of the DD, ID, and II genotypes were 11.5%, 41.5%, and 47.0%, respectively. The prevalence of the D allele was 32.3%. These values are comparable to those of previous reports on Asian people.\(^7\)\(^-\)\(^9\) The renin-angiotensin system plays a major role in mediating left ventricular hypertrophy and cell growth.\(^2\)\(^-\)\(^4\) This effect is thought to be caused by Ang II generated from Ang I. Although Ang II is also produced by enzymes other than ACE, such as chymase,\(^10\)\(^-\)\(^11\) ACE is thought to be an important enzyme for the production of Ang II.

Circulating ACE activity has been reported to differ in the 3 ACE genotypes by unknown mechanisms.\(^5\) This diverse ACE activity may result in diverse Ang II concentrations and hence variations in left ventricular mass.\(^12\) However, there is a lack of agreement about the relation of the ACE polymorphism and left ventricular hypertrophy.\(^13\)\(^-\)\(^16\) There are several reasons for this disagreement. First, the relationship between ACE genotypes and heart weight may not be conspicuous enough to be specified above the background noise level. Second, there is no way to precisely measure heart weight or left ventricular weight/mass except at autopsy. Left ventricular mass is usually estimated indirectly by echocardiography or angiography.\(^13\)\(^,\)\(^14\)

A history of hypertension was the most effective predictor of heart weight (Figure 2), which suggests that mechanical stress may be a major factor in the regulation of cardiac muscle mass. Hemodynamic stimulation causes autocrine release of Ang II from cardiac myocytes,\(^2\)\(^-\)\(^4\) activates signal transduction through receptors in myocytes, and triggers myocardial hypertrophy.\(^17\)\(^,\)\(^18\)

There was a prominent difference in heart weight between the DD and II genotypes in the hypertensive group, whereas there was no statistical difference between genotypes in the normotensive group (Figure 2). The observed heart weight difference by genotype is apparently triggered by high blood pressure.\(^19\) The effect of ACE genotype on left ventricular muscle mass seems to be enhanced by stimulation, such as physical training,\(^20\) hemodialysis,\(^21\) and myocardial ischemia.\(^22\) Another less plausible explanation is that ACE genotype is related to the severity of hypertension. Unfortunately, data regarding the severity of hypertension in our patient population were not available. In most studies, the relation between hypertension and ACE genotype has been negative.\(^23\)\(^-\)\(^26\)

Although the relation between CSI and heart weight was negative on multiple regression analysis, it is difficult to reject such a relation, because the 3 factors examined (age, history of hypertension, and CSI) were closely correlated. Because patients with myocardial infarction were not included in our study, the role of ischemia in heart weight remains to be elucidated.

Diabetes mellitus was less frequent in the II genotype (Table 1). There is a report that patients with non–insulin-dependent diabetes mellitus who have the DD genotype have higher blood glucose levels and are more glucose intolerant.\(^27\) Our results may be comparable to this.

In conclusion, these studies demonstrate that the ACE genotype was a predictor of heart weight, although it was less effective than epigenetic factors such as hypertension or age. The association between ACE genotype and left ventricular mass had been conflicting. Our findings provide new evidence that the ACE gene polymorphism plays a detectable role in the structure of heart mass.

### References


#### Table 2. Comparison of Heart Weight in Presence or Absence of Various Factors

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Heart Weight/BSA, g/m²</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension, no vs yes</td>
<td>245/198</td>
<td>217.4±44.9 vs 246.8±52.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DD vs ID+II</td>
<td>51/392</td>
<td>247.9±49.5 vs 228.3±50.5</td>
<td>0.0093</td>
</tr>
<tr>
<td>Male vs female</td>
<td>235/208</td>
<td>228.1±50.0 vs 233.2±51.4</td>
<td>0.2943</td>
</tr>
<tr>
<td>Hypercholesterolemia, no vs yes</td>
<td>322/74</td>
<td>228.8±50.3 vs 234.1±51.1</td>
<td>0.4143</td>
</tr>
<tr>
<td>Diabetes mellitus, no vs yes</td>
<td>375/63</td>
<td>230.9±51.6 vs 229.3±44.5</td>
<td>0.8172</td>
</tr>
</tbody>
</table>

Heart weights are means±SD.

#### Table 3. Relation Between Heart Weight, CSI, and Age

<table>
<thead>
<tr>
<th>Relationship</th>
<th>(r)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight/BSA and age</td>
<td>0.219</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CSI and age</td>
<td>0.147</td>
<td>0.0019</td>
</tr>
<tr>
<td>CSI and heart weight/BSA</td>
<td>0.145</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

\(n=443.\)


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