Deletion Allele of Angiotensin-Converting Enzyme Gene Increases Risk of Essential Hypertension in Japanese Men

The Suita Study

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Background—The Framingham Study recently revealed that the homozygous deletion polymorphism of the angiotensin-converting enzyme gene (ACE DD) is associated with increased risk for essential hypertension in a male-specific manner. However, this association has not been confirmed in races other than whites.

Methods and Results—Using a large number of Japanese subjects (n = 5014) that were randomly selected from the general population (the Suita Study), we examined the association between ACE DD and hypertension. The frequency of DD (17.1%) in hypertensive men was significantly higher (P < 0.0015) than that (11.8%) in other mildly hypertensive or normotensive men, and the estimated odds prevalence for hypertension (DD vs II) was 1.75 (95% CI 1.21 to 2.53). In contrast, no significant association was confirmed in women (OR 1.17, 95% CI 0.79 to 1.72).

Conclusions—Despite the lower frequency of the DD genotype in Japanese than in whites, the ACE gene polymorphism was associated with increased risk for hypertension, suggesting that this polymorphism is a mild but certain genetic risk factor for essential hypertension in men.

Key Words: renin ▪ angiotensin ▪ genetics ▪ population

Several genetic investigations, such as case-control studies, affected sib-pair analyses, and rat cross-experiments, have attempted to elucidate the genetic pathogenesis of essential hypertension. The candidate gene approach is one means of identifying which genes are responsible for hypertension.1–3 Studies of the renin-angiotensin system have shown that several genetic variants of the angiotensinogen gene (AGT) play a role in increasing risk for hypertension. A major locus for high blood pressure (BP/SP1) is located on rat chromosome 10, which contains the rat angiotensin-converting enzyme gene (ACE) locus, according to several rat crosses between a genetically hypertensive rat strain and normotensive controls.4,5 However, most human studies have failed to identify a positive association between essential hypertension and the ACE polymorphism that is mapped on human chromosome 17.6,7

Two recent studies describe a linkage between the ACE locus and hypertension in a large number of hypertensive sibs.8,9 An insertion/deletion (I/D) polymorphism in intron 16 of ACE was significantly associated with hypertension only in men. The ACE I/D polymorphism, identified in 1990 by Rigat et al.,10 is partially associated with the plasma ACE level.11 Although the ACE DD genotype increases the plasma ACE concentration and the risk for numerous cardiovascular-renal diseased states, such as myocardial infarction,12 cardiomyopathy,13 IgA nephropathy,14 and diabetic nephropathy,15 the findings from case-control studies have not been consistently positive. Genetic and environmental heterogeneity among different ethnic groups may account for the inconsistent results.16 If so, the effect of gene polymorphism should be examined within a large homogeneous population.17

The present study examines the association between hypertension and ACE I/D polymorphism as well as sex specificity in the Japanese population. This study is the first large-scale genetic epidemiologic investigation to assess the cardiovascular risk of the ACE DD genotype in Japan.

Methods

Study Population

The Suita Study18 was based on a random sample of 14 200 Japanese residents of Suita, a city located in the second largest urban area in Japan (Osaka). Participants between the ages of 30 and 79 were arbitrarily selected from the municipality population registry, stratified by sex and age groups of 10 years. The basic sampling of the population started in 1989 with a cohort study base, and 51.7% (n = 7347) of the subjects had paid their initial visit to the National

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Cardiovascular Center by February 1997. The participants have visited the National Cardiovascular Center every 2 years since then for regular health check-ups. In addition to performing routine blood examinations, we extracted DNA from an extra 5 mL of blood withdrawn from those who visited the National Cardiovascular Center between May 1996 and February 1998. All participants were Japanese, and only those who gave informed consent for genetic analysis were enrolled in the present study. After excluding individuals without an adequate genotype, ACE I/D polymorphism data were obtained from 5014 participants who were eligible for genetic analysis.

**Measurements**

Basic clinical variables included age, BMI, smoking and drinking habits, alcohol consumption (mL/d), TC, TG, HDL-C, FPG, creatinine, antihypertensive therapy, and the presence of diabetes mellitus and IHD. After >10 minutes of rest, SBP and DBP were measured twice by a single physician. Hypertension was defined as a mean SBP of ≥160 mm Hg or a mean DBP of ≥95 mm Hg or currently under antihypertensive medication. Height and weight were also measured, and BMI was calculated by dividing weight in kilograms by height in meters squared.

**Determination of Genotype**

DNA was extracted from 200 μL of buffy coat separated from fresh blood with the use of a QIAamp Kit (QIAGEN). Template genomic DNA (100 ng) was amplified by polymerase chain reaction with a thermal cycler (Omnigene; Hybaid). I/D polymorphism was determined by agarose gel electrophoresis with ethidium bromide staining, and DD genotype was reconfirmed by insertion allele–specific amplification according to the Lindpaintner’s protocol18 with a minor modification.

**Statistical Analysis**

All statistical analyses were conducted with the use of StatView 4.5J (Abacus Concepts) and JMP 3.0 (SAS). The difference in genotype or allele distribution between hypertensive subjects and other subjects was examined by χ² analysis. The association between ACE I/D polymorphism and clinical variables was examined by the 1-way ANOVA. We assessed the quantitative effects of covariates by multiple logistic regression analysis using JMP. Since the inheritance manner of the D allele has not been clarified, we examined its effect separately as follows: recessive (DD vs ID+II), additive (DD vs ID vs II), or dominant (DD+ID vs II).

**Results**

**Study Population**

From a total of 5014 randomly selected residents of Suita, 1200 hypertensive subjects were chosen according to the criteria described above. A comparison of clinical characteristics between hypertensive subjects and other individuals revealed that age, percentage of men, body mass index (BMI), total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C), fasting plasma glucose (FGP), creatinine, and alcohol consumption were significantly higher in hypertensive subjects (Table 1). In contrast, smoking was less frequent among hypertensive subjects. Although 70.5% of hypertensive subjects were under antihypertensive medication, the mean systolic blood pressure (SBP) or diastolic blood pressure (DBP) of hypertensive subjects was apparently higher than those of other subjects. Parameters that revealed significant differences between hypertensive subjects and others (except SBP, DBP, and heart rate) were used for multiple logistic regression analysis as confounding factors.

**TABLE 1. Clinical Features of Study Participants**

<table>
<thead>
<tr>
<th>Hypertensive Subjects</th>
<th>Others</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>1200</td>
<td>3814</td>
</tr>
<tr>
<td>Age, y</td>
<td>65.9±0.34</td>
<td>57.7±0.19</td>
</tr>
<tr>
<td>Sex, % men</td>
<td>50.3</td>
<td>45.5</td>
</tr>
<tr>
<td>Family history of hypertension, %</td>
<td>42.8</td>
<td>32.0</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>151.6±0.42</td>
<td>121.8±0.27</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>89.4±0.26</td>
<td>77.1±0.15</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68.7±0.23</td>
<td>66.9±0.13</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.7±0.09</td>
<td>22.3±0.05</td>
</tr>
<tr>
<td>TC, mg/dl</td>
<td>212.4±0.95</td>
<td>207.9±0.54</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>140.5±2.54</td>
<td>116.0±1.48</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>57.7±0.45</td>
<td>60.1±0.25</td>
</tr>
<tr>
<td>FPG, mg/dl</td>
<td>100.8±0.53</td>
<td>96.8±0.30</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.79±0.008</td>
<td>0.70±0.005</td>
</tr>
<tr>
<td>Smoking habits, %</td>
<td>19.3</td>
<td>24.4</td>
</tr>
<tr>
<td>Drinking habits, %</td>
<td>47.3</td>
<td>47.4</td>
</tr>
<tr>
<td>Alcohol consumption, mL/d</td>
<td>16.0±0.67</td>
<td>14.4±0.38</td>
</tr>
</tbody>
</table>

Variables are mean±SEM.

Genotype Distribution of ACE I/D Polymorphism

ACE genotype distribution was significantly deviated from Hardy-Weinberg’s expectation in women (χ²=8.4, P=0.004) but not in total or in men (χ²=0.22, P=0.58). Although the ACE DD genotype frequency was similar between Japanese men and women (P=0.96), the frequency of the I allele and that of the II genotype were significantly higher in women than in men (P<0.04). The significant difference of ACE genotype distribution (χ²=9.9, P<0.008) between men and women was observed in Japanese.

**Sex Specificity in Association With Hypertension**

Both genotype and allele distribution of the ACE I/D polymorphism were significantly different between hypertensive subjects and other subjects (Table 2). The estimated odds ratio for hypertension in individuals with the D allele was 1.15 (95% CI 1.05 to 1.27). To examine the sex-specific association with hypertension, we compared genotype and allele distribution separately among men and women. The results showed that in men but not in women, ACE DD was significantly associated with hypertension (Table 2). The mean SBP in all of the tested men was significantly associated with the ACE genotype, whereas DBP was not. The association between major classic risk factors and the ACE genotype was not significant among the total number of tested women (Table 3). Multiple logistic regression analysis revealed that the ACE DD genotype is an independent risk for essential hypertension. The test of the effect of confounding factors for hypertension revealed that the effect of ACE DD is mild but significant in men (Table 4). After full adjustment for confounding factors (age, BMI, alcohol consumption, smoking habit, TC, TG, HDL-C, creatinine, FPG, presence of ischemic heart disease [IHD], and presence of diabetes mellitus), the estimated odds ratio of DD (vs II) was 1.75 (95% CI 1.21 to 2.53). When hypertension was defined...
through the use of only DBP > 95 mm Hg, the fully adjusted odds ratio of DD (vs II) was 1.71 (95% CI 1.17 to 2.47). In the estimation of the risk for systolic hypertension defined as DBP > 95 mm Hg and SBP > 160 mm Hg, the fully adjusted odds ratio was increased to 2.77 (1.09 to 6.54). On the other hand, the effect of the ID genotype did not reach a significant level in the increase of hypertension risk, suggesting that the effect of the D allele is recessive. No association was identified between the ACE genotype and hypertension in women (Tables 2 and 3 and Figure 1).

### Age Dependence in Association With Hypertension
To examine the age dependence in the association between ACE DD and hypertension, we compared the effect of ACE DD for hypertension in young and elderly subjects (Figure 2). Subjects ≤ 60 years old were categorized as “young”; subjects > 60 years old were categorized as “elderly.” The highest percentage of ACE DD was observed in elderly men, but the significance in the difference between hypertensive subjects and others was similar between young and elderly subjects (Figure 2a). Although the prevalence of hypertension increases along with age, the difference between young and elderly subjects in men is bigger than that in women. In the subjects with DD genotype, the incidence of hypertension in young men (25%) was significantly higher ($\chi^2 = 7.2, P = 0.008$) than that in young women (13%) (Figure 2b). In elderly subjects, however, the incidence of hypertension in men is similar to that in women ($\chi^2 = 0.2, P = 0.63$). The

### Table 2. Genotype and Allele Distribution of ACE ID Polymorphism in Hypertensive Subjects and Others

<table>
<thead>
<tr>
<th>Male</th>
<th>Hypertensive Subjects</th>
<th>Others</th>
<th>Female</th>
<th>Hypertensive Subjects</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>DD</td>
<td>103</td>
<td>17.1</td>
<td>204</td>
<td>11.8</td>
<td>88</td>
</tr>
<tr>
<td>ID</td>
<td>285</td>
<td>47.2</td>
<td>811</td>
<td>46.7</td>
<td>244</td>
</tr>
<tr>
<td>II</td>
<td>216</td>
<td>35.8</td>
<td>721</td>
<td>41.5</td>
<td>264</td>
</tr>
<tr>
<td>$\chi^2 = 13.3, P = 0.0013$</td>
<td>$\chi^2 = 2.0, P = 0.36$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Allele | D | 491 | 40.6 | 1,219 | 35.1 | 420 | 35.2 | 1,425 | 34.3 |
|        | I | 717 | 59.4 | 2,253 | 64.9 | 772 | 64.8 | 2,731 | 65.7 |
| $\chi^2 = 11.8, P = 0.0006$ | $\chi^2 = 0.37, P = 0.54$ |

OR = 1.27 (95% CI 1.11–1.45) OR = 1.04 (95% CI 0.91–1.19)

Genotype distribution significantly deviated from Hardy-Weinberg expectation; *$\chi^2 = 4.9, P = 0.027$; †$\chi^2 = 6.3, P = 0.012$.

### Table 3. Features of Men and Women by ACE Genotype

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACE Genotype</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n = 2340)</td>
<td>DD</td>
<td>ID</td>
</tr>
<tr>
<td>Age, y</td>
<td>61.4 ± 0.72</td>
<td>60.9 ± 0.38</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.2 ± 0.16</td>
<td>23.0 ± 0.09</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>40.1</td>
<td>40.3</td>
</tr>
<tr>
<td>Alcohol, mL/d</td>
<td>26.9 ± 0.16</td>
<td>26.2 ± 0.85</td>
</tr>
<tr>
<td>IHD, %</td>
<td>6.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>18.2</td>
<td>21.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>132.2 ± 1.2*</td>
<td>129.9 ± 0.63</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>82.5 ± 0.64</td>
<td>81.5 ± 0.34</td>
</tr>
<tr>
<td>Female (n = 2674)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58.7 ± 0.65</td>
<td>58.6 ± 0.36</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.6 ± 0.17</td>
<td>22.3 ± 0.09</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>8.0</td>
<td>8.9</td>
</tr>
<tr>
<td>Alcohol, mL/d</td>
<td>6.3 ± 0.61</td>
<td>4.8 ± 0.34</td>
</tr>
<tr>
<td>IHD, %</td>
<td>4.3</td>
<td>2.6</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>15.6</td>
<td>14.3</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>129.3 ± 1.1</td>
<td>127.9 ± 0.63</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.1 ± 0.58</td>
<td>78.7 ± 0.32</td>
</tr>
</tbody>
</table>

Variables are mean ± SEM.

*Significant difference ($P < 0.04$) was observed between DD and II by Schefé test.
estimation of fully adjusted odds ratios for hypertension in 4
groups indicates that the effect of ACE DD is strong in young
men but weak in elderly women (Figure 2c).

Association Between Presence of IHD and
ACE DD
On the other hand, the prevalence (%) of IHD tended to
increase among individuals with the DD genotype (Table 3).
The fully adjusted odds ratio (DD vs ID+II) for IHD was
1.56 (0.96 to 2.58) in men and 1.80 (0.96 to 3.19) in women.
In the whole population, the significant association was
observed between the presence of IHD and DD, and the fully
adjusted odds ratio (DD vs ID+II) was 1.62 (1.08 to 2.36).

Discussion
The effect of the D allele of ACE has been discussed within
the context of IHD. A recent review16 that applied meta-analysis
in examining the cause-and-effect relation between ACE
I/D polymorphism and cardiovascular-renal risk among
49 959 subjects could not identify a significant association
with hypertension but suggested its role as a marker of
atherosclerotic cardiovascular complications and diabetic
nephropathy. On the other hand, a large case-referent study that
used the Copenhagen City Heart Study of 10 150 individuals
did not detect any significant association in the development
of myocardial infarction or any other manifestations of
IHD.17 The previous study20 of a New Zealand population
showed that increased risk for IHD is associated with the AGT
T235 but not the ACE DD. In contrast, 2 recent reports6,9 that
found an association between the ACE locus and essential
hypertension suggest a unique sex-specific effect of ACE on
hypertension.

We determined the ACE genotype of >5000 individuals.
One advantage of this investigation is that the participants
were randomly selected urban residents. The selection bias
of cases and control subjects has been avoided because our
population was simply divided into 2 groups according to the
criteria for hypertension. Since the proportion of ACE genotype
in the mildly hypertensive subjects (140 mm Hg ≤ SBP
<160 mm Hg or 90 mm Hg ≤ DBP <95 mm Hg) is similar
to that of the normotensive subjects (SBP <140 mm Hg and
DBP <90 mm Hg), we examined the relation between hypertensive
subjects and others. Another advantage is that
the participants are all Japanese. The D allele frequency
among Japanese (35.5%) was significantly lower than that in
whites (51.1%) estimated by the Copenhagen City Heart
Study16 (χ² =625.1, P<0.0001). Despite the smaller preva-
ence of the DD genotype among Japanese (13.1%) compared
with whites (26.2%), the significance of the male-specific
association between hypertension and ACE DD genotype
is higher in the Suita Study than in the Framingham Study.8
Though many confounding factors were different between
hypertensive subjects and other subjects, the significant
independent association of ACE DD with hypertension re-
mained after these factors were adjusted. The DD but not the
ID genotype was associated with a significant increase in
relative risk for hypertension compared with the II genotype.
This finding suggested that the hypertensive effect of the D
allele appeared with recessive inheritance. Similar results
were obtained from two different races, suggesting that the
ACE DD polymorphism or an unknown linked gene has a
mild but certain effect in the pathogenesis of essential
hypertension.

The female ACE genotype distribution was not confirmed
to Hardy-Weinberg’s law in this study. One possible reason


![Figure 1](image)

**Figure 1.** Fully adjusted odds ratio for hypertension according to ACE genotype. II genotype is reference group. Odds ratio for hypertension was estimated with adjustment for other covariates (age, BMI, current smoking, alcohol consumption, TC, HDL-C, TG, FPG, creatinine, presence of IHD, and presence of diabetes mellitus). *χ² = 9.07, P = 0.0026.

<table>
<thead>
<tr>
<th>Confounding Factor</th>
<th>Wald χ²</th>
<th>P</th>
<th>Wald χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>116.0</td>
<td>&lt;0.0001</td>
<td>202.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>61.2</td>
<td>&lt;0.0001</td>
<td>77.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td>16.0</td>
<td>&lt;0.0001</td>
<td>1.6</td>
<td>0.21</td>
</tr>
<tr>
<td>Creatinine</td>
<td>13.2</td>
<td>0.0003</td>
<td>14.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>DD (vs ID+II)</td>
<td>9.7</td>
<td>0.0018</td>
<td>0.67</td>
<td>0.41</td>
</tr>
<tr>
<td>TG</td>
<td>5.1</td>
<td>0.024</td>
<td>3.2</td>
<td>0.073</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>2.5</td>
<td>0.12</td>
<td>0.86</td>
<td>0.35</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.68</td>
<td>0.41</td>
<td>2.9</td>
<td>0.087</td>
</tr>
<tr>
<td>TC</td>
<td>0.67</td>
<td>0.41</td>
<td>0.04</td>
<td>0.84</td>
</tr>
<tr>
<td>FPG</td>
<td>0.11</td>
<td>0.74</td>
<td>12.8</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

The effect of confounding factor was estimated with regard to presence of hypertension (0 with hypertension, 1 without hypertension).

TABLE 4. Test of Effects of Confounding Factors for Hypertension
for the excess of DD is that ACE DD associates with longevity, but ACE genotype proportion in senior subjects (>70 years of age) was not different from that in young or middle-aged subjects (data not shown). In our preliminary result, the basic genotype distributions of other genes were not different between men and women, suggesting that this observation is specific for the ACE gene.

On the other hand, it is notable that sex specificity in the association between ACE DD and hypertension was confirmed in this Japanese population. The mechanism of the sex specificity of association with hypertension remains unclear. One notion is that estrogen protects against hypertension, although O’Donnell et al could not detect a relation with estrogen replacement therapy or menopause status. Our results suggested that the sex specificity decreased in elderly subjects and increased in young subjects (Figure 2b). The fact that estrogen replacement therapy is uncommon in Japanese (<1% of Japanese women undergo this therapy) also supports our hypothesis.

A minor difference from the Framingham Study is that ACE DD was associated with SBP but not DBP. Since SBP reflects the increased cardiac output or resistance of large arteries, the hypertensive effect of ACE DD might be through an increased left ventricular mass or atherosclerosis of large arteries. Actually, the maximum odds ratio was observed in the association with systolic hypertension. We did not examine whether or not ACE and plasma concentration correlate, and the difference of SBP in men between DD and II was only 3.4 mm Hg in the present study (Table 3). Other limitations underlie this study. We did not make any adjustments for medication, dietary factors, habits, and exercise, which seem to affect blood pressure variance. However, it is also true that these interventions also produce bias and alter the results. We would rather conclude that ACE DD is associated with predisposition to hypertension in men in a general Japanese population.

Acknowledgments

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