Genetic Polymorphism of CYP11B2 Gene and Hypertension in Japanese

Shinji Tamaki, Naoharu Iwai, Yasuyuki Tsujita, Masahiko Kinoshita

Abstract—Low-renin hypertension is characterized by a high ratio of aldosterone to plasma renin activity (ALD/PRA), which may suggest inappropriately increased aldosterone biosynthesis. The genes for the enzymes involved in aldosterone synthesis may contribute to low-renin hypertension. We investigated the associations between genetic variations of CYP11B2 (aldosterone synthase) T(−344)C and hypertension in 482 Japanese subjects. Subjects older than 50 years with a blood pressure <140/85 mm Hg were considered normotensive (n=227 subjects), and subjects younger than 65 years old with a BP >160/95 mm Hg were considered hypertensive (n=255 subjects). The frequency of the TC+CC genotypes in the normotensive group was significantly lower than in the hypertensive group. Logistic analysis on 482 subjects revealed that body mass index, gender, and the genotype of CYP11B2 T(−344)C were significantly associated with hypertension. ALD and PRA were assessed in 97 subjects with hypertension, and the TC+CC genotypes were significantly associated with higher ALD/PRA. Sixty-five subjects with hypertension were assessed by 24-hour ambulatory blood pressure monitoring, and the frequency of nondippers (a difference in mean blood pressure of <10% between the daytime [6 AM to 9 PM] and nighttime [9 PM to 6 AM] hours) was significantly higher in subjects with the TC+CC (hetero+homo mutation) genotype than in subjects with the TT (wild-type) genotype. Echocardiographic assessment (n=136) revealed that the ratio of left ventricular end-diastolic dimension to height tended to be higher in subjects with the TC+CC genotype than in subjects with the TT genotype. The present study suggests that the (−344)C allele of the CYP11B2 gene may be a genetic marker for low-renin hypertension in Japanese. (Hypertension. 1999;33[part II]:266-270.)

Key Words: genetics ■ CYP11B2 ■ aldosterone ■ renin ■ blood pressure monitoring, ambulatory ■ hypertension, low-renin

Low-renin hypertension is characterized by high ratio of aldosterone to plasma renin activity (ALD/PRA), which may suggest inappropriately increased aldosterone biosynthesis. Approximately 10% to 20% of patients with essential hypertension exhibit high ALD/PRA. In a study by Komiy et al,1 which characterized the renin profile in 421 Japanese patients with essential hypertension, 39 patients exhibited a high (>30) ALD/PRA. The genes for the enzymes involved in aldosterone biosynthesis may contribute to low-renin hypertension. Indeed, the genetic variant (Lys173Arg) P450c11AS (aldosterone synthase) has been suggested to be involved in low-renin hypertension in Chilean patients.2 Several frequent polymorphisms have recently been described in the transcriptional regulatory region of the CYP11B2 gene, and the T(−344)C polymorphism has recently been reported to be associated with left ventricular mass in young Finnish adults free of clinical heart diseases.3 Pohga et al4 reported that the T(−344)C polymorphism in the aldosterone synthase gene was associated with significant differences in plasma aldosterone levels. However, they reported that hypertension was not associated with this T(−344)C polymorphism.

In the present study, we investigated possible associations between genetic variations of CYP11B2 [Lys173Arg and T(−344)C] and hypertension in a Japanese population.

Methods

Subjects
The study population was selected at our outpatient clinic, as previously reported.5 From among the 850 subjects at our outpatient clinic for whom DNA samples were available, we selected 482 for the present study. The blood pressures (BPs) of these 482 subjects were measured on more than 2 separate occasions with subjects under no drug treatment. A normotensive control group (NT) was composed of 227 subjects. The criteria for normotension were age older than 50 years, BP <140/85 mm Hg, and no cardiovascular or other diseases. The remaining 255 subjects had systolic BPs >160 mm Hg or diastolic BPs >95 mm Hg; no history of myocardial infarction, cerebrovascular accident, life-threatening arrhythmia, or other serious diseases; and were younger than 65 years old. These 255 subjects comprised a hypertensive group (HTN).

Sequence Analysis and Genotype Determination
DNA was isolated from peripheral leukocytes, and the genotype of polymorphisms in the CYP11B2 gene was determined by a polymerase chain reaction (PCR)–based method. Since CYP11B1 is highly

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homologous to CYP11B2. We focused on the promoter regions of the two genes, where the sequence homology is not high, and each gene could be amplified separately by PCR. The promoter region of CYP11B2 (from −677 to −42) was amplified by PCR using the following two primers: 5′-CAGGGGATGACAGGACAGC-ACAG-3′ (sense; from −677 to −651) and 5′-CTCACCGAGGAC-ACCTGCTCTGAAATACA-3′ (antisense; 2 from −71 to −42). The PCR profile included 34 cycles of denaturing at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and polymerization at 72°C for 45 seconds. The PCR-amplified fragments were electrophoresed on a 1.5% agarose gel (Nippon Gene Co Ltd) and purified using glass beads (Qiagen II Gel Extraction Kit, Qiagen). Purified PCR products were directly sequenced using a cycle sequencing kit (TaKaRa Taq Cycle Sequencing Kit, Takara Shuzou). For the sequence analysis, we selected 11 hypertensive subjects—5 with low PRA (<0.2 ng angiotensin I [Ang I]·h⁻¹·mL⁻¹), 3 with normal PRA, and 3 with high PRA (>3 ng Ang I·h⁻¹·mL⁻¹)—who had a positive family history of hypertension. In our direct sequence analyses, we found the T(−344)C and C(−470)T polymorphisms in CYP11B2. No other sequence variation was found in promoter regions of the CYP11B2 gene.

The genotype of CYP11B2 was determined by PCR amplification using the primers described above and digestion by the HaeIII restriction enzyme (Takara Shuzou). The amplified fragments were digested with the HaeIII restriction enzyme and then subjected to electrophoresis on a 2.0% agarose gel and visualized under UV light. Fragments of 231 bp (T allele) and 140–91 bp (C allele) were detected.

Genetic variations of the CYP11B2 (Lys173Arg) gene were detected as described in the literature. Briefly, the 5′ and 3′ primers were 5′-AGGCCAGTTCTACACGGCCAGTACT-3′ (sense) and 5′-CACCCTCCTCCGAAATCTCATCCTT-A-3′ (antisense), respectively. Genomic DNA was amplified as previously reported with a program that consisted of denaturation at 94°C for 1 minute, followed by 33 cycles of 94°C for 45 seconds, 61°C for 45 seconds, and 72°C for 150 seconds. The amplified fragments were digested with the Bsa36I restriction enzyme (New England Biolabs) and then subjected to electrophoresis on a 2.0% agarose gel and visualized under UV light. Fragments of 1286 bp (Lys allele) and 1037+249 bp (Arg allele) were detected.

Statistical Analysis
Statistical analyses were performed using StatView 4. Frequency was compared by a contingency table analysis. Numerical data were analyzed by an unpaired t test or one-way ANOVA. Multiple regression analysis was performed to investigate the possible influence of the genotype of CYP11B2 on ALD/PRA; gender, age, body mass index (BMI), hypertension (negative=0, positive=1), and the genotype of the CYP11B2 gene (TT, CC, CT, homozygous=0, heterozygous=1) were included as independent variables. Moreover, multiple regression analysis was performed to investigate the possible influence of the genotype of CYP11B2 on M-mode echocardiographic measurements. Gender, age, BMI, systolic BP, diastolic BP and the genotype of the CYP11B2 gene (TT=0, TC+CC=1) were included as independent variables. Backward selection was used and a P value of 0.10 or greater was required for a variable to be removed. To examine the independent contribution of CYP11B2 gene polymorphisms to hypertension, while adjusting for the effects of other clinical characteristics, we used a logistic analysis. For hypertension, a baseline logistic model was developed by applying backward stepwise selection with the following factors as covariates: gender (female=0, male=1), BMI, and polymorphisms of the CYP11B2 gene (TT=0, TC+CC=1). Backward selection was used, and a P value of 0.10 or greater was required for a variable to be removed from the model.

M-Mode Echocardiographic Measurements
M-mode echocardiographic measurements were taken in 136 subjects who were not receiving antihypertensive therapy. Left ventricular end-diastolic dimension (LVDd) was calculated from M-mode echocardiographic measurements of the left ventricle using an SS160A system with 3.75-MHz transducers (Toshiba). M-mode measurements (guided in two dimensions) of LVDd, left ventricular end-systolic dimension (LVDes), end-diastolic interventricular septal thickness, and end-diastolic posterior wall thickness were performed at the left ventricular minor axis at the level of the chordae tendinae just beyond the mitral leaflet tips, as recommended by the American Society of Echocardiography. Each measurement was taken three times, and the average value was used.

Plasma Aldosterone and Renin Activities
Plasma aldosterone (ALD) and plasma renin activity (PRA) were determined at our outpatient clinic in 97 hypertensive subjects who were under no drug treatment after subjects had rested 30 minutes. ALD was determined by radioimmunoassay (SPAC-S Aldosterone Kit). PRA was determined by measuring Ang I by radioimmunoassay (SRL, Japan).

Twenty-Four–Hour BP Measurement
Twenty-four–hour ambulatory BP monitoring (ABPM) was performed in 65 subjects with hypertension in the absence of drug treatment. We usually recommended that all first-visit patients with possible hypertension have their BP levels evaluated by ABPM. Of all the subjects, only 65 approved to undergo ABPM. ABPM was performed noninvasively every hour with an automatic oscillometric device (model BP8800NC, Nippon Colin) that was attached to the upper left arm. Subjects were allowed to perform their usual daily activities between the measurements, except that they were asked to get up at 6 AM and go to sleep at 9 PM. BP and heart rate measurements were obtained at 30-minute intervals. A nondipper pattern was defined as a difference in mean BP of <10% between the daytime (6 AM to 9 PM) and nighttime (9 PM to 6 AM) hours. Mean BP was calculated as diastolic BP plus one third of the difference between systolic and diastolic BPs.

Results
Sequence Analyses
We determined the sequences corresponding to the promoter region of CYP11B2 in 11 subjects who had a positive family history of hypertension. Table 1 shows clinical and laboratory findings in these 11 subjects. Five subjects had low PRA (<0.2 ng Ang I·h⁻¹·mL⁻¹), 3 had normal PRA, and 3 had high PRA (>3 ng Ang I·h⁻¹·mL⁻¹). The T(−344)C and C(−470)T polymorphisms were found in the sequenced promoter region of the CYP11B2 gene. Sequence analyses in 14 CC(−344) and 12TT(−344) subjects revealed that T(−344)C and C(−470)T polymorphisms were in complete linkage disequilibrium.

Since the Arg173 allele in CYP11B2 completely corresponded to the C(−344) allele in our study population (n=100), only the T(−344)C genotype was analyzed further. In addition, since the CC genotype occurred at a low frequency, the TC and CC genotypes were combined into a single group.

Functional Significance of the Polymorphisms
To explore the possible functional significance of the C(−344)T polymorphism of CYP11B2, we investigated associations between the genotype of CYP11B2 and renin profile. Table 2 shows ALD/PRA according to the genotype of the CYP11B2 gene. Gender, age, BMI, and BP did not differ significantly in the two groups. The TT genotype was associated with a lower ALD/PRA than the TC+CC genotype (P<0.0017). Multiple regression analysis indicated that the genotype of the CYP11B2 gene (TT=0, TC+CC=1)
Table 4 shows the correlation between CYP11B2 and hypertension. The frequency of the TC genotype in the HTN group had a higher percentage of men and a higher age than in the NT group. The correlation between CYP11B2 and ABPM group was 43.2% (76/227), which was significantly higher than in the TT genotype (52.2%, 99/190). Logistic analysis of 482 subjects revealed that BMI (P=0.0012; odds ratio 1.093; 95% CI 1.003 to 2.104) were associated with hypertension (P<0.0001).

### Association of Polymorphisms With Hypertension

Table 3 shows the characteristics of the NT and HTN groups. Age did not differ significantly between the two groups. The frequency of the TC genotype in the NT group was 43.2% (76/227), which was significantly lower (P=0.0487) than that in the HTN group (52.2%, 102/311). Logistic analysis of 482 subjects revealed that BMI (P=0.0012; odds ratio 1.093; 95% CI 1.003 to 2.104) were predictors of ALD/PRA. The correlation between CYP11B2 polymorphism and M-Mode Measurements

Table 5 shows the correlation between CYP11B2 polymorphism and echocardiographic parameters. Echocardiographic assessment (n=136) revealed that LVDd/height tended to be greater (P=0.0987) in subjects with the TC+CC genotype than in subjects with the TT genotype. Other parameters, such as LVDs/height, end-diastolic interventricular septal thickness, and end-diastolic posterior wall thickness, were not significantly associated with CYP11B2 polymorphism (P=0.136, P=0.6128, and P=0.8791, respectively). Multiple regression analysis indicated that BMI (P=0.0012, β-coefficient=0.002), age (P=0.0487, β-coefficient=3.992), and the genotype of the CYP11B2 gene (TT=0, TC+CC=1) (P=0.0982, β-coefficient=0.008) were predictors of LVDd/height.

### Discussion

We have confirmed the existence of the previously reported T(−344)C, C(−470)T, and Lys173Arg polymorphisms of the CYP11B2 gene in a Japanese population. In the present study population, the T(−344) allele was completely linked with the Lys173 allele and the C(−470) allele. The C(−344) allele was associated with higher ALD/PRA and was also significantly associated with hypertension. Moreover, echocardiographic assessment revealed that LVDd/height tended to be higher in subjects with the TC+CC genotype than in subjects with the TT genotype.

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**Table 1. Clinical and Laboratory Findings in Study Subjects for the Sequence Analyses**

<table>
<thead>
<tr>
<th>Initial</th>
<th>Gender</th>
<th>Age, y</th>
<th>SBP, mm Hg</th>
<th>DBP, mm Hg</th>
<th>PRA, ng Ang I · h⁻¹ · mL⁻¹</th>
<th>ALD, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.M.</td>
<td>M</td>
<td>51</td>
<td>144</td>
<td>96</td>
<td>&lt;0.10</td>
<td>52.2</td>
</tr>
<tr>
<td>S.I.</td>
<td>M</td>
<td>58</td>
<td>200</td>
<td>140</td>
<td>&lt;0.10</td>
<td>58.8</td>
</tr>
<tr>
<td>S.A.</td>
<td>M</td>
<td>29</td>
<td>168</td>
<td>98</td>
<td>&lt;0.10</td>
<td>70.4</td>
</tr>
<tr>
<td>K.S.</td>
<td>M</td>
<td>41</td>
<td>140</td>
<td>98</td>
<td>0.10</td>
<td>58.6</td>
</tr>
<tr>
<td>M.Y.</td>
<td>F</td>
<td>40</td>
<td>210</td>
<td>125</td>
<td>0.15</td>
<td>218.0</td>
</tr>
<tr>
<td>T.K.</td>
<td>M</td>
<td>53</td>
<td>200</td>
<td>110</td>
<td>0.37</td>
<td>173.4</td>
</tr>
<tr>
<td>Y.K.</td>
<td>M</td>
<td>56</td>
<td>185</td>
<td>110</td>
<td>0.58</td>
<td>36.4</td>
</tr>
<tr>
<td>K.T.</td>
<td>M</td>
<td>61</td>
<td>186</td>
<td>104</td>
<td>1.05</td>
<td>86.0</td>
</tr>
<tr>
<td>N.K.</td>
<td>F</td>
<td>34</td>
<td>180</td>
<td>110</td>
<td>3.38</td>
<td>170.0</td>
</tr>
<tr>
<td>T.N.</td>
<td>M</td>
<td>56</td>
<td>185</td>
<td>115</td>
<td>5.88</td>
<td>54.3</td>
</tr>
<tr>
<td>S.K.</td>
<td>M</td>
<td>31</td>
<td>150</td>
<td>90</td>
<td>11.0</td>
<td>207.0</td>
</tr>
</tbody>
</table>

Correlation Between CYP11B2 Polymorphism and ABPM

Table 4 shows the correlation between CYP11B2 polymorphism and the nocturnal decline in BP. There were significantly more nondippers among subjects with the TC+CC genotype (45.7%, 11+5/23) than among subjects with the TT genotype (20.0%, 6/30) (P=0.029).
TABLE 3. Characteristics of NT and HTN Groups

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>HTN</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>227</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>Female/male</td>
<td>136/91</td>
<td>115/140</td>
<td>0.0012</td>
</tr>
<tr>
<td>Age, y</td>
<td>53.9 ± 8.8</td>
<td>55.4 ± 11.7</td>
<td>0.1263</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.7 ± 2.8</td>
<td>23.8 ± 3.7</td>
<td>0.0006</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>120.3 ± 15.2</td>
<td>168.8 ± 18.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>72.5 ± 9.0</td>
<td>96.9 ± 10.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

TABLE 4. Correlation Between CYP11B2 Polymorphism and ABPM

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT (80.0%)</th>
<th>TC+CC (54.3%)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipper</td>
<td>24</td>
<td>17 + 2</td>
<td>43</td>
</tr>
<tr>
<td>Nondipper</td>
<td>6</td>
<td>11 + 5</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>28 + 7</td>
<td>65</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

In a study by Fardella et al., the Arg173 allele was observed more often in hypertensive subjects (37.5%) than in normotensive subjects (22.5%); however, this change did not appear to have any functional significance. On the basis of our finding that the C(-344) allele was completely linked with the Arg173 allele in a Japanese population, the C(-344) allele may be associated with higher transcriptional activity. Indeed, Clyne et al. suggested that the promoter region between -413 and -221 might influence the responsiveness to angiotensin II. The T(-344)C variation of the CYP11B2 gene or some other unidentified polymorphism associated with T(-344)C may be associated with transcriptional levels. Because the region sequenced in the present study was just in 11 patients, other unidentified sequence variations might exist. Further studies will be necessary to confirm the functional significance of the C(-344)T polymorphism.

Kupari et al. reported that homozygotes for the C(-344) allele average, by cubic approximation, 28% greater end-diastolic volumes and 21% greater mass than homozygotes. Similarly, in our study, echocardiographic assessment revealed that LVd/height tended to be higher in subjects with the TC+CC genotype (P = 0.0987) than in subjects with the TT genotype.

The mechanism of the association of left ventricular size and mass with polymorphism of the CYP11B2 promoter is still unclear. Previous echocardiographic studies in humans have demonstrated that increases or decreases in dietary sodium result in corresponding changes in left ventricular volumes and mass. Kupari et al. also reported that in a regression model for left ventricular mass, a statistically significant interaction was observed between CYP11B2 promoter polymorphism and salt intake: The association of left ventricular mass with salt intake was strong and highly significant (P < 0.0001) in the -344CC group, intermediate (P = 0.0110) in the -344CT group, and nonexistent (P = 0.610) in the -344TT group. Thus, differences in the body’s sodium balance and intravascular volume related to the CYP11B2 genotype are plausible mechanisms for our observations of a dilated left ventricular dimension.

Another explanation may involve the indirect cardiovascular effects of aldosterone, ie, the induction of myocardial hypertrophy and fibrosis.11,12

We studied 24-hour ABPM in 65 subjects with hypertension who were under no drug therapy. The nondipper type was significantly more frequent among subjects with the TC+CC genotype (45.7%,11+5/35) than among subjects with the TT genotype (20.0%, 6/30) (P = 0.029). It has been reported that NaCl loading reduces the nocturnal decline in BP in salt-sensitive individuals but not in salt-resistant individuals. Oshima et al. also reported that in patients with essential hypertension, intracellular sodium accumulation and inadequate suppression of the renin-angiotensin system may be independently associated with NaCl sensitivity. The higher frequency of nondippers among subjects with the TC+CC genotype suggests that this genotype may be associated with salt sensitivity.

Recently, Brand et al. reported significant association of the T(-344) allele of the CYP11B2 gene and hypertension. However, our results indicated that the C(-344) allele of the CYP11B2 gene was associated with higher ALD/PRA and that the frequency of the C(-344) allele was higher in the HTN group in a Japanese population. The reasons for the differences between our data and those of Brand et al. are not evident. One possibility is that the C(-344) allele of the CYP11B2 gene may not have any functional significance itself but may be linked to an as yet unidentified mutation in the promoter region of the gene. Another possibility is that the predisposition to hypertension may be clarified only during high salt intake. Japanese salt intake is reportedly higher than that in whites. Environmental factors such as salt intake might influence a genetic predisposition to hypertension.

In conclusion, the C(-344) allele of the CYP11B2 gene was associated with higher ALD/PRA and that the frequency of the C(-344) allele was higher in the HTN group in a Japanese population. The reasons for the differences between our data and those of Brand et al. are not evident. One possibility is that the C(-344) allele of the CYP11B2 gene may not have any functional significance itself but may be linked to an as yet unidentified mutation in the promoter region of the gene. Another possibility is that the predisposition to hypertension may be clarified only during high salt intake. Japanese salt intake is reportedly higher than that in whites. Environmental factors such as salt intake might influence a genetic predisposition to hypertension.

In conclusion, the C(-344) allele of the CYP11B2 gene was associated with higher ALD/PRA in Japanese subjects. The present results suggest that the TC+CC genotype may be associated with salt sensitivity. Further investigation in a larger population might be necessary to confirm our results.

**References**


**TABLE 5. Correlation Between CYP11B2 Polymorphism and M-Mode Measurements**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT (n=75)</th>
<th>TC+CC (n=47+14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVd/dh, mm/cm</td>
<td>0.302±0.03</td>
<td>0.310±0.03</td>
<td>0.0987</td>
</tr>
<tr>
<td>LVds/h, mm/cm</td>
<td>0.198±0.03</td>
<td>0.204±0.03</td>
<td>0.2889</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>9.68±1.80</td>
<td>9.83±1.80</td>
<td>0.6128</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>9.09±1.48</td>
<td>9.13±1.38</td>
<td>0.8791</td>
</tr>
</tbody>
</table>

h indicates height; IVS, interventricular septal thickness; LVPWd, left ventricular end-diastolic posterior wall thickness.


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