Dopamine D1 Receptor Gene Polymorphism Is Associated With Essential Hypertension

Mikano Sato, Masayoshi Soma, Tomohiro Nakayama, Katsuo Kanmatsuse

Abstract—Dopamine has been shown to influence renal sodium excretion through a direct interaction with the dopamine receptor (DR). The dopamine D1 receptor (DRD1) has been localized to the proximal tubules and is known to increase sodium excretion by inhibiting Na-H exchanger and Na,K-ATPase activity. Defective renal dopamine production and/or DR function have been reported in essential hypertension (EH) as well as in genetic models of animal hypertension. With a restriction fragment length polymorphism of the DRD1 gene, we performed an association study in patients with EH. One hundred thirty-one subjects with EH and 136 age-matched normotensive (NT) controls were studied. Polymerase chain reaction was used to amplify the A-48G polymorphic site in the DRD1 gene, and restriction analysis of the polymerase chain reaction product was used to score the A and G alleles. The allele frequencies in the EH group and NT group were then compared. The G allele was observed more frequently in the EH group than in the NT group, and the allele frequencies in the 2 groups differed significantly (χ²=6.5, P=0.01). Multiple logistic linear regression analysis revealed that the genotype frequencies of A/A, A/G, and G/G differed significantly (odds ratio=2.1; 95% CI=1.19 to 3.66) between the EH and NT groups. EH patients who possess the G allele had a higher diastolic blood pressure than those lacking the G allele (P<0.01). Thus, the alleles detected by this restriction fragment length polymorphism in the DRD1 gene are associated with EH, and they appear to influence the diastolic blood pressure of Japanese EH patients. (Hypertension. 2000;36:183-186.)

Key Words: hypertension, essential receptors, dopamine genes polymorphism

Dopamine plays an important role in the control of renal sodium excretion through a specific dopamine receptor (DR). The DRs are classified into 2 families: D1-like (includes D1 and D5) and D2-like (includes D2, D3, and D4) dopamine receptors grounded on the stimulation and inhibition of adenylyl cyclase, respectively.1–4 D1-like receptors exist on the smooth muscle of renal arteries, the juxtaglomerular apparatus, and the renal tubules.1–3,5 Because the blood pressure (BP) of some individuals with essential hypertensive (EH) is influenced by alterations in salt intake, it has been assumed that an aberrant renal dopaminergic system may play a role in the pathogenesis of some forms of hypertension.1,2,6

Renal dopamine D1A receptor (DRD1A) number and expression have been studied in both animal models of hypertension and EH patients. Watanabe et al7 reported that renal cortical DRD1A density was diminished in spontaneously hypertensive rats (SHR) compared with normotensive Wistar-Kyoto rats (WKY) at 18 weeks of age, but it was similar in both rats at 3 and 7 weeks of age. DRD1A density and expression in renal proximal tubules were similar in SHR compared with WKY.8,9 While DRD1A expression in inner medulla was decreased in SHR compared with controls.9 There is defective transduction of the DRD1A signal in renal proximal tubules of SHR, resulting in decreased inhibition of sodium transport by dopamine.10 In obese Zucker rats, a model of hypertension and obesity, DRD1A binding sites in proximal tubules were reduced compared with those in lean Zucker rats.11 Albrecht et al12 reported that mice lacking the DRD1A gene have impaired regulation of renal sodium transport and represented the impaired regulation of renal sodium transport and elevated systolic (SBP) and diastolic blood pressure (DBP). DRD1 protein abundance in renal proximal tubules is similar in normotensive (NT) and EH humans.13 Nevertheless, it is possible that decreased renal DRD1 may be present in some patients with EH.

EH is a complex, polygenetic disease. Association studies using the candidate gene approach may provide important clues regarding the etiology of hypertension and define a basis for further genetic investigation.14,15 Recently, the human DRD1 gene has been cloned16 and localized to chromosome 5 at q35.1,17,18 and its gene structure has been described.19 A polymorphism, A-48G, has been identified at −48 bp of the 5′ untranslated region.20,21 Although the DRD1 gene polymorphism has been studied extensively in psychiatric diseases,21–24 there is no report linking it to EH. Therefore, we examined the association between the A-48G polymorphism in the DRD1 gene and EH in Japanese individuals.

Hypertension is available at http://www.hypertensionaha.org

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Methods

Subjects
The study group consisted of 131 patients, aged 49±9 years (mean±SD), with EH diagnosed according to the World Health Organization/International Society of Hypertension criteria, including a sitting SBP of >160 mm Hg and/or DBP of >95 mm Hg. Patients with secondary forms of hypertension were excluded after appropriate clinical and laboratory examinations. One hundred thirty-six healthy subjects, aged 49±10 years, were also studied as a NT control group. The NT subjects had no family history of hypertension, and, in all cases, their SBP was <140 mm Hg and their DBP was <85 mm Hg. A positive family history was defined as hypertension diagnosed in grandparents, parents, or siblings.

The association between BP and genotype was assessed in 90 patients with EH who had not been treated by antihypertensive drugs. Fasting plasma renin activity (PRA) and aldosterone concentrations were measured in the untreated patients. Informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee at Nihs University.

Biochemical Analysis
The plasma concentration of total cholesterol, the serum concentrations of creatinine and uric acid, and PRA and aldosterone concentration were measured by standard methods in the clinical laboratory department of our university hospital.

Analysis of the DRD1 Gene Restriction Fragment Length Polymorphism
Genomic DNA was extracted from peripheral blood leukocytes by standard methods. A restriction fragment length polymorphism assay was developed to detect a G allele at nucleotide 48 upstream in the 5’ untranslated region of the human DRD1 gene according to the methods of Cichon et al. Amplification with the forward primer F1 (5-GGC TTT CTG GTG CCC AAC ACA GTG-3) and the reverse primer R1 (5-AGC ATG GAT CAC ACA GTG-3) resulted in a 405-bp product in a 50-µL total reaction volume containing 200 ng of genomic DNA according to the manufacturer’s specifications (TaKaRa Ex Taq, TaKaRa Shuzo Co, Ltd). After an initial 5 minutes of denaturation at 94°C, 35 cycles were performed, consisting of 30 seconds at 94°C, 30 seconds at 63°C, and 1 minute at 72°C, followed by a final extension step of 5 minutes at 72°C. Each PCR product was recovered by ethanol precipitation, and a 20-µL aliquot was incubated overnight with 1 U of DdeI according to the manufacturer’s recommendations (Bio Labs Inc). Fragments were separated in 1.5% agarose gel and visualized by ethidium bromide staining.

Statistical Analysis
Data are presented as mean±SD. Allele frequencies were calculated from the genotypes of all subjects. Hardy-Weinberg equilibrium was assessed by χ² analysis. Significant differences between the total number of alleles on all chromosomes for the EH and NT groups were assessed by χ² analysis with 1 df. Associations between genotype and hypertension were evaluated by multiple logistic linear regression analysis. EH was regarded as the dependent variable, and genotype, gender, and age were entered as independent variables. Differences in the clinical data between the EH and NT groups and between genotypes were assessed by ANOVA followed by Bonferroni’s test. A P value of <0.05 was considered significant.

Results
The clinical characteristics of the EH and NT subjects are shown in Table 1. SBP, DBP, pulse rate, and body mass index (BMI) were higher in the EH group than in the NT group. There were no significant differences between the 2 groups in regard to age, gender, serum concentrations of creatinine and uric acid, or plasma concentrations of total and HDL cholesterol.

The frequencies of the A and G alleles were 0.92 (249/272) and 0.08 (23/272) for the EH group and 0.84 (221/262) and 0.16 (41/262) for the EH group, respectively (χ²=6.5, P=0.01). Genotypes A/A, A/G, and G/G were detected with frequencies of 0.83 (113/136), 0.17 (23/136), and 0 (0/136) in the NT group and 0.71 (93/131), 0.27 (35/131), and 0.02 (3/131) in the EH group, respectively. The genotype distribution in the NT group was in Hardy-Weinberg equilibrium (P>0.10). Multiple logistic linear regression analysis revealed the DRD1 genotype to be significantly associated with EH (odds ratio=2.1; 95% CI=1.19 to 3.66). The allele or genotype frequency was not associated with BMI or pulse rate (Table 2).

The clinical characteristics of the untreated EH patients with different genotypes are shown in Table 3. DBP was significantly higher in the A/G subjects than in the A/A subjects among the untreated EH patients (P=0.0067). We combined the A/G and G/G subjects for statistical analysis because of the small number of G/G individuals (n=2). DBP was significantly higher in subjects who possessed the G allele in comparison to those not possessing the G allele among the untreated EH patients (P=0.0098). SBP of untreated EH patients and DBP and SBP of NT subjects were not significantly different between the genotypes. The DRD1 genotype did not influence PRA or aldosterone concentration in untreated EH patients (Table 3).

Discussion
In this study an A-48G polymorphism in the DRD1 gene was associated with EH. In association analysis using genetic markers, subject selection is a critical and difficult problem. Given that BP is influenced by age, we selected closely age-matched subjects for the NT and EH groups. Additionally, we strictly defined NT subjects and omitted anyone whose family history was EH-positive, since such individuals might become hypertensive later in life. We also omitted borderline hypertensive subjects. Allele and genotype frequencies of the control group were almost identical to those of controls in an association study of Japanese schizophrenia patients and were consistent with
Hardy-Weinberg equilibrium, confirming that our selection of controls was appropriate.

The diverse physiological actions of dopamine are mediated by at least 5 distinct G protein–coupled receptor subtypes.1,2 D1-like receptors have been localized to the proximal tubules and are known to increase sodium excretion by inhibiting Na-H exchanger and Na,K-ATPase activity.1,2,6 In humans, EH is associated with a reduced proximal tubular response to D1-like receptor stimulation.25 The human DRD1 gene was cloned,16–18 and it consists of 2 exons, an upstream noncoding exon 1 of ≈450 bp and a longer exon 2 that codes for the entire receptor protein. These 2 exons are separated by a small intron of 116 bp.19

The A-48G variant is 1 of 4 common coding frame polymorphisms in the DRD1 gene.20 We have demonstrated a significant association between the A-48G polymorphism in the 5′ untranslated region of exon 2 of the DRD1 gene and EH and with DBP in untreated EH patients. Recently, Krushkal et al26 reported the importance of the distal end of the long arm of chromosome 5 containing the DRD1 gene in influencing human SBP variations in young Caucasians. Accordingly, the DRD1 gene might be involved in regulation of arterial pressure.

The intermediate phenotype is thought to be important to binding genetic background to final phenotype. The DRD1A subtype, known as DRD1 in humans, is expressed in juxtaglomerular cells and regulates renin secretion in rats.27 Therefore, we measured PRA and aldosterone concentration, but there appeared to be no association with the DRD1 genotype. However, a sodium balance study and the response to selective DRD1 agonist in each genotype may be required to elucidate the receptor function.

We found that the G allele was much less frequent than was reported by Cichon et al20,21 and Liu et al,23 as summarized in Table 4. This discrepancy may be attributable to ethnic differences as observed in angiotensinogen28 and in the

### Table 2. Clinical Characteristics of Subjects With Different Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>206</td>
<td>58</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>147/58</td>
<td>39/19</td>
<td>2/1</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>50±10</td>
<td>49±10</td>
<td>50±7</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>142±35</td>
<td>151±38</td>
<td>171±8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>87±20</td>
<td>95±25*</td>
<td>102±13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse, bpm</td>
<td>74±12</td>
<td>73±13</td>
<td>67±12</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>71±18</td>
<td>80±18</td>
<td>71±0</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.2±1.0</td>
<td>5.2±1.0</td>
<td>4.5±0</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid, mmol/L</td>
<td>0.32±0.08</td>
<td>0.33±0.1</td>
<td>0.35±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6±3.5</td>
<td>23.7±3.4</td>
<td>27.7±0.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean±SD. Distribution of gender was compared between groups by χ² analysis. Data of others were compared between groups by ANOVA followed by Bonferoni’s test.

*Compared with A/A group.

### Table 3. Clinical Characteristics of Untreated EH Patients With Different Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>A/A</th>
<th>A/G</th>
<th>G/G</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>66</td>
<td>22</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>42/24</td>
<td>14/8</td>
<td>1/1</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±10</td>
<td>49±11</td>
<td>47±4</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>176±26</td>
<td>182±21</td>
<td>167±4</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>106±11</td>
<td>115±15*</td>
<td>103±18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pulse, bpm</td>
<td>76±15</td>
<td>77±18</td>
<td>70±14</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>71±18</td>
<td>80±27</td>
<td>71±0.0</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1±1.1</td>
<td>5.3±0.9</td>
<td>4.5±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Uric acid, mmol/L</td>
<td>0.34±0.08</td>
<td>0.34±0.09</td>
<td>0.33±0.0</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.7±4.0</td>
<td>24.5±4.6</td>
<td>27.7±0.9</td>
<td>NS</td>
</tr>
<tr>
<td>PRA, ng (mL·h)</td>
<td>1.4±1.0</td>
<td>1.4±0.9</td>
<td>1.2±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma aldosterone, pmol/L</td>
<td>0.32±0.13</td>
<td>0.31±0.14</td>
<td>0.33±0.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean±SD. Distribution of gender was compared between groups by χ² analysis. Data of others were compared between groups by ANOVA followed by Bonferoni’s test.

*Compared with A/A group.
endothelial nitric oxide synthase gene.\textsuperscript{29,30} Detection of functional allele polymorphism may be needed because this allele alone does not seem to influence the gene function,\textsuperscript{18} and this region might be spliced out in the kidney.\textsuperscript{31}

In conclusion, the A-48G polymorphism in the 5' untranslated region of the DRD1 gene was associated with EH in Japanese subjects. This allele may be in linkage disequilibrium with the actual defect, suggesting that one cause of EH is a polymorphism in the DRD1 gene itself or in another gene close to this one.

**Acknowledgments**

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**References**


**TABLE 4. Ethnic Differences in the A-48G Polymorphism of the DRD1 Gene**

<table>
<thead>
<tr>
<th>Population</th>
<th>Allele</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian (n=338)</td>
<td>0.61</td>
<td>0.39</td>
<td>ND ND ND ND 23</td>
</tr>
<tr>
<td>German (n=45)</td>
<td>0.66</td>
<td>0.34</td>
<td>0.39 ND ND ND 20, 21</td>
</tr>
<tr>
<td>Japanese (n=148)</td>
<td>0.93</td>
<td>0.07</td>
<td>0.85 0.15 0 24</td>
</tr>
<tr>
<td>Japanese* (n=136)</td>
<td>0.92</td>
<td>0.08</td>
<td>0.83 0.17 0 *This study</td>
</tr>
</tbody>
</table>

ND indicates not described. *NT subjects.
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