Association of Sodium Channel \( \gamma \)-Subunit Promoter Variant With Blood Pressure

Naoharu Iwai, Shunroku Baba, Toshifumi Mannami, Tomohiro Katsuya, Jitsuo Higaki, Toshio Ogihara, Jun Ogata

Abstract—The \( \text{SCNN1G} \) gene, located on human chromosome 16p12, encodes the \( \gamma \)-subunit of the amiloride-sensitive epithelial sodium channel, and mutations in \( \text{SCNN1G} \) can result in Liddle’s syndrome or pseudohypoaldosteronism type I. We identified sequence variations in the promoter region of \( \text{SCNN1G} \) and examined the association between this polymorphism and blood pressure in a large cohort (\( n = 4075 \)) representing the general population in Japan. We found \( T(-1290)C \), \( T(-501)G \), \( G(-173)A \), and \( G(-104)T \) polymorphisms in the promoter region of \( \text{SCNN1G} \) and confirmed the existence of \( T387C \) and \( T474C \) polymorphisms in exon 3 and the \( C1947G \) polymorphism in exon 13. Because the genotypes of the \( T(-1290)C \), \( T(-501)G \), \( G(-104)T \), and \( T474C \) polymorphisms were in tight linkage disequilibrium, we selected the \( T474C \) and \( G(-173)A \) polymorphisms for an association study. The \( G(-173)A \) polymorphism of \( \text{SCNN1G} \) had a significant effect on systolic pressure (\( P = 0.0050 \)) and pulse pressure (\( P = 0.0050 \)). The \( AA \) genotype was associated with an 11 mm Hg drop in systolic pressure and an 8 mm Hg drop in pulse pressure and with a higher prevalence of hypotension (\( P = 0.0195 \)). A transient transfection assay using MDCK cells and human renal epithelial cells indicated that the promoter activity of the \( G(-173) \) allele was higher than that of the \( A(-173) \) allele. Although the effects of the \( A(-173) \) allele were recessive and although the \( AA \) genotype was found in just 0.7% of our study population, we observed that this variation of human \( \text{SCNN1G} \) had significant effects on blood pressure. (Hypertension. 2001;38:86-89.)

Key Words: genetics ■ hypertension, essential ■ polymorphism

The \( \text{SCNN1G} \) gene, located on human chromosome 16p12, encodes the \( \gamma \)-subunit of the amiloride-sensitive epithelial sodium channel, and mutations in \( \text{SCNN1G} \) can result in Liddle’s syndrome or pseudohypoaldosteronism type I.\(^1,\)\(^2\) Several researchers have studied the role of the human chromosome 16p12 locus in human essential hypertension and have reported conflicting results.\(^3\)-\(^9\) For example, on the basis of a case-control study, Persu and colleagues\(^5,\)\(^6\) concluded that \( \text{SCNN1B} \) and \( \text{SCNN1G} \) did not play important roles in essential hypertension. On the other hand, Wong et al\(^9\) found significant linkage between systolic blood pressure and markers at chromosome 16p12 on the basis of an identity-by-descent sibling-pair analysis. This discrepancy may be due to the study design, inasmuch as a small-scale association study has relatively weak statistical power.\(^10\)

In the present study, we screened for sequence variations in the promoter region of \( \text{SCNN1G} \) and evaluated the significance of polymorphisms in essential hypertension by using a large cohort (4075 subjects) that was representative of the general Japanese population.

Methods

Subjects
The selection criteria and design of the Suita study have been described previously.\(^11\) The sample consisted of 14,200 men and women age 30 to 79 years stratified by gender and 10-year age groups, selected randomly from the municipal population registry. They were all invited by letter to attend regular cycles (every 2 years) of follow-up examinations. DNA from leukocytes was collected from participants who visited the National Cardiovascular Center between May 1996 and February 1998. All of the participants were Japanese, and only those who gave their written informed consent for genetic analyses of the amiloride-sensitive sodium channel genes were included in the present study. In the present study, of the 4104 DNA samples available, the genotypes of 4075 samples could be determined.

The characteristics of the subjects analyzed in the present study are summarized in Table 1. Blood pressure was measured in the subjects after at least 10 minutes of rest in a sitting position. Systolic and diastolic blood pressure values were the means of 2 physician-obtained measurements (recorded \( > 3 \) minutes apart).

Hypertension was defined as systolic blood pressure \( \geq 140 \) mm Hg or diastolic blood pressure \( \geq 90 \) mm Hg or the current use of antihypertensive medication. Hypotension was defined as systolic blood pressure \( < 100 \) mm Hg.
TABLE 2. Primers and Probes for Genotype Determination

<table>
<thead>
<tr>
<th>Region Polymorphism</th>
<th>GenBank Frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter T(−1290)C G(−501)G G(−173)A G(−104)T</td>
<td></td>
</tr>
<tr>
<td>Exon 2 A164G U78937 0.75/0.25</td>
<td></td>
</tr>
<tr>
<td>Exon 3 T387C X87160 0.75/0.25</td>
<td></td>
</tr>
<tr>
<td>Intron 10 G216A X87160 0.85/0.15</td>
<td></td>
</tr>
<tr>
<td>Intron 12 G31A U53853 0.95/0.05</td>
<td></td>
</tr>
<tr>
<td>Intron 13 C1947G X87160 0.85/0.15</td>
<td></td>
</tr>
</tbody>
</table>

*Allele frequencies described are based on those in the 20 subjects used for variation screening.

The region covered by these primers was between −437 and +81. The polymorphisms in this region were G(−173)A and G(−104)T. The haplotypes determined were G/G, G/T, A/G, and A/T. The PCR products were purified, blunted, and ligated to the Smal-cut luciferase reporter vector pGL2-Basic (Promega), which does not contain any promoter sequence or enhancer. The sequences of the recombinant genes with different alleles were confirmed.

Transfection with the SCNN1G gene promoter/luciferase fusion gene was performed with LipofectAmine Plus Reagent ( Gibco BRL) according to the manufacturer’s recommendations. DNA-LipofectAmine complex was contacted with cells in Opti-MEM medium ( Gibco BRL) without serum, and the transfection medium was replaced with culture medium 3 hours later. MDCK cells were cultured in a MEM/10% FBS. Human renal epithelial (HRE) cells were cultured according to the manufacturer’s recommendation (BioWhittaker). The HRE cell was positive for pancytokeratin, and the expression of SCNN1G mRNA was confirmed with reverse transcriptase–PCR (data not shown). PRL-CMV vector ( Promega), in which Renilla luciferase is under the control of the cytomegalovirus (CMV) promoter, was included in the transfection mixture as an internal standard. Cells were harvested 30 hours after transfection.

Results

Detection of Genetic Variants

We found T(−1290)C, T(−501)G, G(−173)A, and G(−104)T polymorphisms in the promoter region of SCNN1G and confirmed the existence of T474C and T387C polymorphisms in exon 3 and the C1947G polymorphism in exon 13. Moreover, we found 4 polymorphisms in SCNN1G (Table 3). The allele of T(−1290) completely corresponded with the alleles of T(−501) and G(−104) in the 20 subjects sequenced. The allele T474 completely corresponded with the
allele T387 in the 20 subjects sequenced. The A164G polymorphism of SCNN1G was in tight linkage disequilibrium with exon 3 polymorphisms. The T allele of the T(−1290)C polymorphism was in tight linkage disequilibrium with that of the T474C polymorphism (P<0.0001). However, the T allele of the T474C polymorphism showed a tendency to be in linkage disequilibrium with the G(−173)A allele of the G(−173)A polymorphism of SCNN1G (P=0.066). The G216 allele in intron 10 completely corresponded to the C1947 allele in the 20 subjects sequenced. The genotype frequencies of G(−173)A and T474C polymorphisms were consistent with Hardy-Weinberg equilibrium.

### Association Study

Because the genotypes of the T(−1290)C, T(−501)G, G(−104)T, and T474C polymorphisms were in tight linkage disequilibrium, we selected the T474C and G(−173)A polymorphisms for an association study. The C1947G polymorphism in exon 13 was not studied because our preliminary study yielded no association of this polymorphism with blood pressure. Table 4 shows blood pressure levels according to each genotype of these 2 polymorphisms. Although the T474C polymorphism had no significant effects on blood pressure, the G(−173)A polymorphism significantly affected both systolic pressure (P=0.02) and pulse pressure (P=0.02).

The AA genotype was associated with a lower systolic pressure and a lower pulse pressure. These effects on systolic pressure (P=0.005) and pulse pressure (P=0.005) became much more significant after adjusting for age, sex, waist/hip ratio, alcohol consumption, and body mass index. Multiple logistic analyses in which age, gender, body mass index, waist/hip ratio, alcohol consumption, and body mass index were included as independent variables indicated that the AA genotype was associated with a lower prevalence of hypertension (P=0.050). On the other hand, multiple logistic analyses in which age, body mass index, and the genotype of the G(−173)A polymorphism of SCNN1G (GG+GA=0, AA=1) were included as independent variables indicated that the AA genotype was associated with a higher prevalence of hypertension (P=0.0195).

### Functional Significance of the G(−173)A Polymorphism

Because the G(−173)A polymorphism was associated with blood pressure status, we next examined the functional significance of the G(−173)A polymorphism in vitro by using 2 cell lines, MDCK and HRE cells (Figure). In both types of cells, 2-way ANOVA indicated that the G(−173)A but not the G(−104)T polymorphism affected promoter activity.

![Assessment of promoter activity.](image-url)
activity. No significant interaction between the genotypes was observed. The allele \(G(-173)\) had \(\approx 2.5\)-fold and \(\approx 1.6\)-fold higher promoter activity than the allele \(A(-173)\) in MDCK and HRE cells, respectively.

**Discussion**

In the present study, we found previously unidentiﬁed sequence variations in the promoter region of \(SCNN1G\) on chromosome 16p12. We then evaluated the signiﬁcance of these polymorphisms in blood pressure regulation by using a large cohort consisting of \(\approx 4000\) subjects. The AA genotype of \(SCNN1G\) was found to be associated with lower systolic and pulse pressures and a higher prevalence of hypotension.

Reporter analyses on promoter activity suggest that the AA genotype of \(SCNN1G\) is associated with lower promoter activity in vivo. A lower expression of \(SCNN1G\) subunit might lead to lower sodium reabsorption in the kidney. Several mutations in the \(\gamma\) subunit have been reported to lead to autosomal recessive pseudohypoaldosteronism type 1.3,4 It is likely that the clinical characteristics of subjects with the AA genotype may resemble those of subjects with pseudohypoaldosteronism type I. This awaits further investigation. The recessive effects of the \(A(-173)\) allele may indicate an existence of a threshold expression level of the \(\gamma\)-subunit for normal channel activity.

The promoter activity assessed in vitro may not necessarily indicate the activity in vivo, inasmuch as our promoter-luciferase construct covered only up to -421 from the initiation site, although this region has been reported to have almost full activity.12 The promoter region of this gene has been studied in promoter region polymorphisms, our present results may not be worthwhile to assess the signiﬁcance of the \(G(-173)A\) polymorphism in white populations. Moreover, the present study suggests that dense single-nucleotide polymorphism mapping will be necessary to accurately assess the signiﬁcance of the gene with association studies.15

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**References**


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The authors of the article “Association of Sodium Channel γ-Subunit Promoter Variant With Blood Pressure” by Iwai et al (Hypertension. 2001;38:86–89) wish to make the following correction. In Table 3, GenBank accession of the promoter sequence corresponds to U48937, not U78937.