The gene for the β-subunit of the epithelial sodium channel (βENaC) is one of the most prominent candidate genes being analyzed for an association with human essential hypertension. It is known that a deletion or alteration of PY motif in exon 12 of βENaC is responsible for Liddle’s syndrome. Although the localization of genetic polymorphisms of βENaC is unique to each population, intensive analysis of individuals of white and African ancestry has demonstrated that genetic variants are localized in exons 8 and 12, with two frequent polymorphisms, G442V in exon 8 and T594M in exon 12. These two mutations are both found in individuals of African ancestry, and might be associated with elevated blood pressure (BP). Previously, we have screened the last two-thirds of exon 12 in the Japanese population, and demonstrated the absence of the T594M mutation and the presence of a novel P592S mutation. In the present study, we further examined the rest of exon 12 and exon 8 in a general population from Ohasama, Japan (the Ohasama Study), using single-strand conformational polymorphism (SSCP) analysis. We screened 803 subjects randomly selected from the representative participants, who measured their home and casual BP. The PCR products presenting a shift in SSCP gels, as well as controls, were directly sequenced by autoanalyzer to identify the mutation. A novel gel shift was noted in exon 12 (n = 8) and sequencing identified a polymorphism at codon Ser 520, leading to no change in amino acid sequence (G77576C TCG→TCC). In exon 8, all three SSCP variants were heterozygous for V434M (GTG→ATG), which is coincident with a rare polymorphism in whites. The G442V mutation, however, was absent from the Japanese population. A novel mutation of exon 12 was not associated with a significant difference in clinical features. These results indicate that Japanese people possess three polymorphisms in exon 12, all of which are unique, and one in exon 8. These genetic variants of βENaC may not influence the BP level of Japanese people.
previous study also confirms the requirement to screen individual populations to fully determine the linkage between genetic variants of ENaC and clinical manifestations.\textsuperscript{10}

To date, all genetic polymorphisms, missense or silent (leading to no amino acid sequence changes), in $\beta$ENaC have been found only in exons 8 and 12, indicating the importance of screening these two exons.\textsuperscript{5,7} Therefore, in the present study, we intensively screened the rest of exon 12 and exon 8 using the same DNA samples used in the previous study, as the subjects represent a general population of rural Japan (the Ohasama Study).\textsuperscript{11,12}

The subjects of the present study are included in the Ohasama Study, and performed self-measurement of blood pressure at home (home BP). All studies, except for our previous study concerning the linkage between hypertension and genetic polymorphisms, have been performed on the basis of measurement of conventional casual BP (casual BP).\textsuperscript{13} Home BP, however, has been reported to be more reliable than casual BP because it avoids clinical biases.\textsuperscript{14,15} We have also demonstrated by analysis of Ohasama Study data that home BP has a stronger predictive power for mortality than does casual BP.\textsuperscript{16,17} Therefore, the linkage analysis of novel variants found in the present study was performed using home BP in addition to casual BP.

**Methods**

**Design**

The present report is based on the subjects who participated in our home BP measurement project in a rural community of Ohasama, Iwate Prefecture, Japan. The characteristics of this area and the details of the study project have been described previously.\textsuperscript{11–13,16,17} The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government.

**Study Population**

DNA samples were obtained from 1301 of the 1789 participants 40 years or older who measured their home BP.\textsuperscript{16,17} The selection and the clinical characteristics of these study subjects have been described previously.\textsuperscript{10,17} All study subjects gave written informed consent. Screening of exons 8 and 12 was performed in 803 individuals randomly selected from these 1301 subjects. Genotyping was performed in duplicate for each individual, and to prevent observer bias, the investigator was blinded to the origin of the sample.

**Biochemical and Hormonal Measurements**

Plasma blood urea nitrogen, creatinine, and electrolytes were measured using an autoanalyzer. Plasma renin activity was determined by angiotensin I generation (angiotensin I per milliliter of plasma per hour of incubation).

**Detection of Mutations**

A single-strand conformational polymorphism (SSCP) analysis was performed in all subjects as previously described.\textsuperscript{10} Primer sets for exons 12 and 8 of $\beta$ENaC were prepared as described in the study by Persu et al.\textsuperscript{7} The 5’ ends of the sense primers were FITC labeled (Amersham Pharmacia Biotech, Tokyo, Japan). Human genomic DNA extracted from leukocytes was amplified by 35 cycles of polymerase chain reaction (PCR), separated on the SSCP gel, and then visualized by a Photo Analyzer (FMBIO II Multi-View; Hitachi, Japan). Finally, PCR products showing a shift in the SSCP gel, as well as controls, were directly sequenced by autoanalyzer (ABI PRISMMTM 310; Perkin Elmer, Norwalk, CT) to identify the mutation.

**BP Measurements**

**Home BP**

Physicians or public health nurses instructed subjects on how to perform home BP measurements. Subjects were asked to measure their BP every morning, within 1 h of waking, in the sitting position after more than 2 min of rest and to record the results for 4 weeks. The home BP of an individual was defined as the mean of all measurements obtained for that individual. The mean number of home BP measurements was 20.8 ± 8.3 (mean ± SD), ranging from 3 to 38.

**Casual BP**

Casual health check-ups, including BP measurements, are available to all Japanese citizens aged 40 years or older. Nurses or technicians measured BP twice consecutively with a semiautomatic device, with subjects in the sitting position after at least 2 min of rest. Casual BP was defined as the average of the two readings.

**BP Measuring Devices**

Home BP was measured with the HEM 401C (Omron Life Science Co. Ltd, Tokyo, Japan), a semiautomatic device based on the cuff oscillometric method that generates a digital display of both systolic BP (SBP) and diastolic BP (DBP).\textsuperscript{18} Casual BP was measured with an USM-700F (UEDA Electronic Works Co. Ltd., Tokyo, Japan), a fully automatic device based on the Korotkoff sound technique (microphone method). The circumference of the arm was less than 34 cm in most cases, therefore we used a standard arm cuff for both BP measurements. The devices used to measure home BP and casual BP have been previously validated\textsuperscript{18} and meet the criteria of the Association for the Advance-ment of Medical Instrumentation.\textsuperscript{19}

**Data Analysis**

The SAS software, version 6.12 (SAS Institute Inc., Cary, NC) was used for all statistical calculations. Fisher’s exact test, $t$ test, or analysis of covariance (ANCOVA) was used as appropriate. Data are shown as the mean ± SD. A $P$
value < .05 was accepted as indicating statistical significance.

Results
Detection of Genetic Variants
The SSCP analyses using the primer sets for exon 12 demonstrated novel gel shift in 8 subjects (Fig. 1B). Sequencing revealed polymorphism (G77576C) at codon Ser 520, leading to no change in amino acid sequence (TCG→TCC). In exon 8, we found gel shifts (Fig. 1A) in 3 subjects, all of whom showed a V434M (GTG→ATG) mutation by sequencing.

Clinical and Biological Characteristics
Because a relatively high frequency (∼1% of the Japanese population of Ohasama) of G→C polymorphism at codon Ser 520 was noted, the clinical characteristics of individuals with this variant of exon 12 were analyzed. The results showed no significant differences between those with and without mutation in home and casual BP values, age, body mass index, biochemical profile such as electrolytes or plasma renin activity, and the distributions of gender and use of antihypertensive medication.

In Table 1, we show the clinical features of the three V434M variants found in the present study.

Discussion
Intensive screening of βENaC in the Japanese population revealed a novel mutation, although silent, in exon 12 and the V434M mutation in exon 8 that has been reported in whites. Although Japanese individuals harbor neither of the two frequent mutations of βENaC, T594M or G442V, they possess rare unique polymorphisms in exon 12 and the V434M mutation in exon 8. Persu and co-workers have screened βENaC in white and black populations, and found five missense mutations and three polymorphisms leading to no change in amino acid sequence in exon 12. All of these polymorphisms are quite rare (frequency <1%), except for the T594M mutation. In exon 8 they also

Table 1. Clinical characteristics of each subject with V434M in Exon8

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>Case</th>
<th>Case</th>
<th>Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>54</td>
<td>49</td>
<td>69</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>Home SBP/DBP (mm Hg)</td>
<td>120/72</td>
<td>126/84</td>
<td>103/65</td>
</tr>
<tr>
<td>Casual SBP/DBP (mm Hg)</td>
<td>114/70</td>
<td>121/76</td>
<td>135/73</td>
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<td>Biochemical parameters</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mmol/L)</td>
<td>144</td>
<td>144</td>
<td>146</td>
</tr>
<tr>
<td>K⁺ (mmol/L)</td>
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<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
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<td>1.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Renin activity (ng/mL/h)</td>
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<td>2.0</td>
<td>0.3</td>
</tr>
</tbody>
</table>

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure.
found G442V and V434M mutations; G442V was observed mostly in individuals of African ancestry, but also in one white person. Combining these observations, each population appears to have unique mutations in exon 12, whereas mutations in exon 8 occur in different populations. This population-based difference in the locations of mutations also suggests that new mutations could be found in other exons in the Japanese population.

The present study demonstrated the absence of the G442V mutation in the Japanese population. This mutation is frequent in individuals of African ancestry and is thought to be closely associated with hypertension. Together with our previous report showing the absence of the T594M mutation, which is also frequent and associated with hypertension, the significance of genetic polymorphisms of βENaC appears to be less important in the Japanese population. However, plasma sodium concentration tended to be high in all individuals with the V434M variant (Table 1). One previously reported case of this mutation also showed slightly elevated plasma sodium and clinical manifestations of hypertension. Therefore, further extended study, such as a meta-analysis, is required to determine the clinical implications of this quite rare polymorphism of βENaC.

In conclusion, there are three polymorphisms of βENaC in exon 12 and one polymorphism in exon 8 in the Japanese population. Although the rare V434M mutation in exon 8 is considered to be a potential candidate for further examination, Japanese people do not harbor the relatively frequent polymorphisms of βENaC, such as the T594M or G442V mutations, which have close association with hypertension.

Acknowledgments
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References