Brugada syndrome (BrS) is a cardiac channelopathy characterized by specific findings, such as accentuated J wave and ST-segment elevation in the right precordial leads on ECG, in the absence of structural heart diseases.1–3 BrS patients sometimes suffer from syncpe, and have a risk of sudden cardiac death caused by rapid polymorphic ventricular tachycardia or ventricular fibrillation.1–3 Approximately 35% of BrS patients have a family history of the disease, which is consistent with the autosomal dominant inheritance, and mutations in 12 different genes have been reported as associated with BrS, of which the majority are mutations in SCN5A encoding a large subunit of the cardiac sodium channel Nav1.5.4–12 The prevalence of BrS in East Asia including Japan is much higher, reaching 1 in 1,000–2,000, than the worldwide prevalence of approximately 1 in 10,000.13–15

**Background:** Brugada syndrome (BrS) is characterized by specific alterations on ECG in the right precordial leads and associated with ventricular arrhythmia that may manifest as syncpe or sudden cardiac death. The major causes of BrS are mutations in SCN5A for a large subunit of the sodium channel, Nav1.5, but a mutation in SCN3B for a small subunit of sodium channel, Navβ3, has been recently reported in an American patient.

**Methods and Results:** A total of 181 unrelated BrS patients, 178 Japanese and 3 Koreans, who had no mutations in SCN5A, were examined for mutations in SCN3B by direct sequencing of all exons and adjacent introns. A mutation, Val110Ile, was identified in 3 of 178 (1.7%) Japanese patients, but was not found in 480 Japanese controls. The SCN3B mutation impaired the cytoplasmic trafficking of Nav1.5, the cell surface expression of which was decreased in transfected cells. Whole-cell patch clamp recordings of the transfected cells revealed that the sodium currents were significantly reduced by the SCN3B mutation.

**Conclusions:** The Val110Ile mutation of SCN3B is a relatively common cause of SCN5A-negative BrS in Japan, which has a reduced sodium current because of the loss of cell surface expression of Nav1.5. (Circ J 2013; 77: 959–967)

**Key Words:** Brugada syndrome; Electrophysiologic study; Genetics; Ion channels; Sodium
patient with BrS, although some other SCN3B mutations have been reported in other hereditary arrhythmias, including IVF, SIDS, and AF.

We report a SCN3B mutation, Val110Ile, found in 3 unrelated Japanese BrS patients. Functional studies in transfected cells demonstrated that the mutation decreased the cell surface expression of Nav1.5 and reduced the peak current of $I_{Na}$.

**Figure 1.** Mutational analysis of SCN3B in Brugada syndrome (BrS). (A) Direct sequencing analysis of exon 3 in a control (Left) and a patient, BrS-H-1 (Right), shows that the patient was heterozygous for GTC and ATC (Ile) at codon 110, where the control was homozygous for GTC (Val). The same pattern was obtained from the other proband patients, BrS-O-1 and BrS-S-1, as well as from BrS-O-2 who was a daughter of BrS-O-1 (Table 1). (B) Amino acid sequences of Navβ3 from various species aligned with the V110I mutation. (C) Representative ECG recording from BrS-O-1 shows typical coved-type ST-segment elevation in leads V1–2. Pedigrees of BrS families with the V110I mutation: BrS-O-1 (D) and BrS-S-1 (E). Squares and circles indicate male and female, respectively. Filled and open symbols represent affected and unaffected individuals, respectively. Shaded symbols indicate subjects with undefined arrhythmia (Ar). Arrows indicate the proband patients. Presence of the mutation is noted as +. Ages at blood sampling or death are indicated. HF, heart failure.
Table 1. Clinical Phenotypes of Individuals Carrying the SCN3B Val110Ile Mutation

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (years)/Sex</th>
<th>ST-elevation type</th>
<th>Symptoms</th>
<th>Family history of arrhythmia/SCD</th>
<th>ICD</th>
<th>EPS</th>
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<td>Yes</td>
<td>VF</td>
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<tr>
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<td>–</td>
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<td>NSVT</td>
</tr>
<tr>
<td>BrS-H-1</td>
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<td>Coved</td>
<td>Syncope</td>
<td>No</td>
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<td>–</td>
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</table>

BrS, Brugada syndrome; EPS, electrophysiologic study; ICD, implantable cardioverter defibrillator; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VF, ventricular fibrillation.

**Methods**

**Subjects**
We studied 178 genetically unrelated Japanese and 3 Korean patients with BrS. Age at the diagnosis of 145 male patients was 45.3±15.7 (range 7–76) years, and that of the 17 female patients was 46.8±17.3 (range 11–72) years. Episodes of syncope and/or arrhythmia had occurred in 93 patients, but the others were asymptomatic. There was a family history of sudden death and/or arrhythmias for 19 patients, but nothing certain for the others. Blood samples were obtained from each subject after informed consent for gene analysis was given. The patients had been analyzed for mutations in SCN5A by using specific primer pairs (Table S1), and no disease-related mutation was found. The control subjects were 480 genetically unrelated individuals who were selected at random without ECG records.

The research protocol was approved by the Ethics Review Committee of the Medical Research Institute, Tokyo Medical and Dental University and the Institutional Review Board of Samsung Medical Center.

**Mutational Analysis**
Genomic DNA extracted from the peripheral blood leukocytes of each individual was subjected to polymerase chain reaction (PCR) using primer pairs specific to SCN3B (Table S2). PCR products were analyzed for mutations by direct DNA sequencing using Big Dye Terminator version 3.1 and ABI3100 DNA sequencer. Genomic DNA extracted from the peripheral blood leukocytes of each individual was subjected to polymerase chain reaction (PCR) using primer pairs specific to SCN3B (Table S2). PCR products were analyzed for mutations by direct DNA sequencing using Big Dye Terminator version 3.1 and ABI3100 DNA sequencer.

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</tbody>
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BrS, Brugada syndrome; EPS, electrophysiologic study; ICD, implantable cardioverter defibrillator; NSVT, nonsustained ventricular tachycardia; SCD, sudden cardiac death; VF, ventricular fibrillation.

**Alignment of Amino Acid Sequences**
Amino acid sequences of human Navβ3 protein predicted from the nucleotide sequences (GenBank™ NM_018400) were aligned with those of chimpanzee (XM_522210), macaque (NM_00194283), mouse (NM_153522), rat (NM_139097), rabbit (ENSOCUP00000009050), bovine (NM_001046495), horse (ENSECAT000000025763), dog (XM_847682), elephant (ENSLAFFT00000000307), opossum (XM_001379934), platypus (ESQANT000000023925), chicken (XM_417884), Xenopus (NM_001011299), and zebrafish (NM_001080802).

**Constructs for Nav1.5 and Navβ3**
We obtained a cDNA fragment of human Navβ3 by reverse transcription-PCR from human adult heart cDNA. Mutant cDNA fragments of Navβ3 containing a T to C substitution in codon 10 (for Leu10Pro mutation) or a G to A substitution at codon 110 (for Val110Ile mutation) were created by the primer-mediated mutagenesis method using specific primers (Table S3). Wild-type (WT) or mutant cDNA fragments were cloned into pcDNA3.1-myc, His-B (myc-His-Navβ3) (Invitrogen, San Diego, CA, USA) and pIRES-CD8 (pIRES-CD8-Navβ3). The cDNA fragment of human SCN5A was a gift from Dr A.L. George (Vanderbilt University). A Flag-tagged Nav1.5 was constructed by inserting a Flag epitope (DYKDDDDK) into the extracellular linker 1 (L1) between S1 and S2 in the D1 domain after position aa154 in the Nav1.5 construct (L1-Flag-Nav1.5), as described previously. All constructs were sequenced to ensure that no errors were introduced.

**Immunofluorescence Microscopy**
We seeded 4.0×10⁴ tsA-201 cells, a derivative line of HEK cells, onto poly-D-Lysine 8-well culture slides (BD Biosciences, San Jose, CA, USA), and 24 h later, myc-His-Navβ3 (0.1 μg) alone, or L1-Flag-Nav1.5 (0.1 μg) plus myc-His-Navβ3 (0.1 μg) were added to the wells with Lipofectamine 2000 Reagent (Invitrogen) (0.2 or 0.4 μl, respectively). After 18 h, the cells were washed with phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde for 15 min at room temperature and permeabilized by 0.15% Triton X-100 in PBS with 3% bovine serum for 20 min at room temperature. The cells were then incubated with the primary rabbit anti-Flag polyclonal antibody (Ab) (1:250, Sigma, CA, USA) and mouse anti-myc monoclonal Ab (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and secondary Alexa Fluor 568 goat anti-rabbit IgG (1:500, Molecular Probes, Eugene, OR, USA) and Alexa Fluor 488 rabbit anti-mouse IgG (1:1,000, Molecular Probes), respectively, in PBS with 3% bovine serum. All cells were mounted on glass slides using Mowiol 4-88 Reagent (Calbiochem, Darmstadt, Germany), and images were collected and analyzed with an LSM510 laser-scanning microscope (Carl Zeiss Microscopy, Jena, Germany). To quantify the membrane expression of Nav1.5, fluorescence intensity of the total cell and the plasma membrane (peripheral, 2 μm) areas in the middle xy images of z series stack were measured, and the ratios of peripheral to total cell area fluorescence intensity (PTAFI) were calculated as described previously.

**Electrophysiological Studies**
The tsA-201 cell line was used in the electrophysiological study, as described previously. Cells transfected with pIRES-CD8 or pIRES-CD8-Navβ3 were briefly preincubated with Dynabeads M-450 CD8 (Dynal, Oslo, Norway) prior to the recordings. Sodium currents were recorded from the cells that were labeled with Dynabeads using the whole-cell patch clamp technique. Currents and cell capacitances were recorded using Axopatch 200B amplifier (Axon Instruments, CA, USA) and series resistance errors were reduced by 60–70% using electronic circuitry.
Figure 2. SCN3B mutations reported in arrhythmias and distribution of Nav1.5 and Navβ3 in transfect- ed tsA-201 cells. (A) Schematic representation of Navβ3 mutations found in arrhythmias. SCN3B mutations in BrS (red), IVF (purple), SIDS (light green), and AF (light blue) are mapped on the Navβ3. Extracellular domain and transmembrane domain are indicated. The V110I mutation found in this study is indicated in red. (B, C) tsA-201 cells transfected with myc-His-Navβ3-WT (a,b), -L10P (c,d) or -V110I (e,f), and the combination of L1-Flag-Nav1.5 and myc-His-Navβ3-WT (g–i), -L10P (j–l), or -V110I (m–o). The cells were permeabilized by 0.15% Triton X-100 and stained with anti-myc Ab (Navβ3, green; a,c,e,h,k,n) and anti-Flag Ab (Nav1.5, red; g,j,m). Nuclei were stained with DAPI and merged images are shown in b,d,f,l, and o. Scale bar=10μm. (D) The ratio of peripheral to total cell area fluorescence intensity (PTAFI) of expressed L1-Flag-Nav1.5 in the transfected cells. Numbers of analyzed cells are indicated at the bottom of each bar. Data are expressed as mean±SEM. *P<0.001. AF, atrial fibrillation; BrS, Brugada syndrome; IVF, idiopathic ventricular fibrillation; SIDS, sudden infant death syndrome.
tronic compensation. Holding potentials were −120 mV and pipette resistance was 1.0–1.5 MΩ. The bath solution contained 36 mmol/L N‐NaCl, 109 mmol/L N‐MgCl₂, 4 mmol/L KCl, 1.8 mmol/L CaCl₂, 1 mmol/L MgCl₂, and 10 mmol/L HEPES, pH 7.35, while the pipette solution contained 10 mmol/L NaCl, 110 mmol/L CsF, 20 mmol/L CsCl, 10 mmol/L EGTA, and 10 mmol/L HEPES, pH 7.35. All signals were acquired at 20–50 kHz (Digidata 1332, Axon Instruments) with a personal computer running Clampex 8 software (Axon Instruments) and filtered at 5 kHz with a 4‐pole Bessel low‐pass filter. Experiments were done at room temperature. Membrane currents were analyzed with Clampfit 8 software (Axon Instruments) and Sigmaplot (Systat Software, CA, USA). The current‐voltage relationships were fit to the Boltzmann equation,

\[ I = \frac{I_{\text{rev}}}{1 + \exp \left( \frac{V - V_{1/2}}{\kappa} \right)} \times G_{\text{max}} \times \left[ 1 + \exp \left( \frac{V - V_{1/2}}{\kappa} \right) \right]^{-1} \]

where \( I \) is the peak sodium current during the test pulse potential \( V \). The parameters estimated by the fitting are \( V_{\text{rev}} \) (reversal potential), \( G_{\text{max}} \) (maximum conductance), and \( \kappa \) (slope factor). Steady‐state availability was fit with the Boltzmann equation,

\[ I / I_{\text{max}} = \left[ 1 + \exp \left( \frac{V - V_{1/2}}{\kappa} \right) \right]^{-1} \]

where \( I_{\text{max}} \) is the maximum peak sodium current, to determine the membrane potential for \( V_{1/2} \) (half‐maximal inactivation) and \( \kappa \) (slope factor). The time course of inactivation was fit with a 2‐exponential function,

\[ I(t) / I_{\text{max}} = A_0 + A_1 \times \exp(-t / \tau_1) + A_2 \times \exp(-t / \tau_2) \]

where \( A \) and \( \tau \) are amplitudes and time constants, respectively, \( t \) and \( t \) refer to current and time, respectively.

### Co‐Immunoprecipitation (co‐IP) Assay

The tsA‐201 cells were transiently transfected with a combination of L1‐Flag‐Nav1.5 (2 μg) and myc‐His‐Navβ3 (2 μg). Cellular extracts were prepared from the transfected cells and equal amount of extracted proteins were used for the co‐IP assay using the Catch and Release version 2.0 reversible immunoprecipitation system, according to the manufacturer’s instructions (Millipore, Milford, MA, USA), with rabbit anti‐Flag polyclonal Ab (Sigma). Eluted samples were separated by SDS‐PAGE, transferred to a nitrocellulose membrane, pre‐incubated with 5% skimmed milk in PBS, and incubated with primary mouse anti‐c‐myc monoclonal Ab (1:100) followed by secondary rabbit anti‐mouse (for monoclonal Ab) IgG HRP‐conjugated Ab (1:1,000; Dako A/S, Glostrup, Denmark).

### Statistical Analysis

Numerical data are expressed as mean±SEM. Statistical differences were analyzed using 1‐way analysis of variance (ANOVA) followed by Dunnett’s test. P<0.05 was considered to be statistically significant.

### Results

#### Mutational Analysis of SCN3B

We analyzed 181 BrS patients, who were negative for SCN5A mutations, for sequence variations in SCN3B and found a synonymous and another non‐synonymous variant in 1 and 3 patients, respectively. The synonymous variant, p.Asn133Asn (c.399C>T), was rare and was not considered to be a disease‐causing mutation because no functional effect was deduced. On the other hand, the non‐synonymous variant, p.Val110Ile (V110I, c.328G>A) (Figure 1A), was found in 3 unrelated Japanese patients; a 42‐year‐old male (BrS‐O‐1), a 33‐year‐old male (BrS‐S‐1), and a 51‐year‐old male (BrS‐H‐1) (Table 1). The V110I variant was predicted to affect an evolutionary conserved residue of Navβ3 (Figure 1B) and was not found in 960 control chromosomes. As summarized in Table 1, BrS‐O‐1 had experienced repetitive syncpe, and showed spontaneous coved‐type ST‐elevation in ECG (Figure 1C). His daughter (BrS‐O‐2) was asymptomatic, but exhibited a BrS‐like ECG pattern and carried the same mutation (Figure 1D). His father and sister had arrhythmia, although the details could not be evaluated (Figure 1E). BrS‐H‐1 had experienced syncpe, and showed spontaneous coved‐type ST‐elevation in ECG, but the family history of arrhythmia was uncertain. These 3 proband patients were also analyzed for mutations in the other known disease genes for BrS (Table S1) and none had any mutation.

### Cell Surface Expression of Nav1.5 in the Presence of Mutant Navβ3

Navβ3 modulates the function of the Nav1.5 channel, and several SCN3B mutations have been reported in association with arrhythmias, including BrS. IVF, SIDS, and AF (Figure 2A). Because the Leu10Pro (L10P) mutation was the only mutation previously reported in only 1 BrS patient, which resulted in the reduction of Nav in transfected cells,25 we investigated the functional alterations caused by the V110I mutation as compared with the L10P mutation. Membrane surface expression of Nav1.5 was examined in tsA‐201 cells transfected with myc‐His‐Navβ3 alone or in combination with L1‐Flag‐Nav1.5. It was observed that Navβ3‐WT was expressed on the cell
Figure 3. Electrophysiological profiles of sodium currents recorded from tsA-201 cells transiently expressing Nav1.5 and Navβ3. (A) Representative sodium currents recorded from tsA-201 cells cotransfected with pCDNA3.1-Nav1.5 and pIRESCD8 or pIRES-CD8-Navβ3. The traces were recorded with a whole-cell configuration. (B) Current-voltage relationship for peak $I_{Na}$. (C) Sodium current density at –25 mV. Co-expression of Nav1.5 and Navβ3-WT increased the peak current densities by 83.9%, as compared with Nav1.5 alone, while co-expression of Nav1.5 with Navβ3-L10P or -V110I showed significantly smaller peak current densities than co-expression of Nav1.5 with Navβ3-WT by 37.5% and 42.5%, respectively ($n=11$ for WT, $n=13$ for L10P, $n=15$ for V110I). Co-expression of Nav1.5 and Navβ3-WT plus -L10P or -V110I also showed significantly smaller peak current densities than co-expression of Nav1.5 and Navβ3-WT by 25.2% or 29.4%, respectively ($n=13$ for WT/L10P, $n=13$ for WT/V110I). (D) Transfected cells of pCDNA3.1-Nav1.5 and pIRES-CD8-Navβ3-WT show a leftward shift in the voltage dependence of steady-state fast inactivation and activation, compared with transfected cells of pCDNA3.1-Nav1.5 and pIRES-CD8. Voltage dependences recorded from the transfected cells of pCDNA3.1-Nav1.5 in combination with pIRES-CD8-Navβ3-L10P, or -V110I were similar to that from the cells cotransfected with pCDNA3.1-Nav1.5 and pIRES-CD8-Navβ3-WT. (E) Transfected cells of pCDNA3.1-Nav1.5 and pIRES-CD8-Navβ3-WT show a leftward shift in the recovery from inactivation assessed by the double-pulse protocol, compared with transfected cells of pCDNA3.1-Nav1.5 and pIRES-CD8. Recovery from inactivation was nearly identical among Navβ3-WT, and -V110I, whereas L10P showed a significant rightward shift. The 2-pulse protocol is shown in the inset. WT, wild-type.
surface, whereas both Navβ3-L10P and Navβ3-V110I were retained in the cytoplasm (Figures 2B-a-i). In the cells co-transfected with Nav1.5 and myc-His-Nav3, Nav1.5 was clearly expressed on the cell surface in the presence of Navβ3-WT (Figures 2C-g-4), but its cytoplasmic trafficking was disturbed by both Navβ3-L10P and Navβ3-V110I (Figures 2C-j-o). To express the trafficking defects quantitatively, we measured the fluorescence intensity of Nav1.5 in both the plasma membrane region and the entire cell area to obtain the ratios of PTAFL. As shown in Figure 2D, both the L10P and V110I mutation of SCN3B significantly reduced the cell surface expression of Nav1.5 by approximately 70%.

**Altered Electrophysiological Characteristics of \( i_{Na} \) Caused by the SCN3B Mutations**

Because the V110I mutation impaired the intracellular trafficking of Nav1.5, we investigated the potential effect of V110I mutation on Nav1.5 kinetics. Whole-cell patch clamp recordings were obtained from tsA-201 cells transiently transfected with pcDNA3.1-Nav1.5 in combination with pRES-CD8, pRES-CD8-Navβ3-WT, -L10P or -V110I (Figure 3, Table 2). It was found that the peak current densities of \( i_{Na} \) from the cells cotransfected with pcDNA3.1-Nav1.5 and pRES-CD8-Navβ3-WT were significantly larger than that recorded from the cells cotransfected with pcDNA3.1-Nav1.5 and pRES-CD8 by 83.9% (Figures 3B-C, Table 2). However, the peak current densities of \( i_{Na} \) from the cells cotransfected with pcDNA3.1-Nav1.5 and pRES-CD8-Navβ3-L10P or -V110I were significantly smaller than that recorded from the cells cotransfected with pcDNA3.1-Nav1.5 and pRES-CD8-Navβ3-WT by 37.5% or 42.5%, respectively (Figures 3B-C, Table 2).

It also was observed that pRES-CD8-Navβ3-WT shifted the voltage dependence of activation and inactivation to more negative potentials compared with pRES-CD8, and neither pRES-CD8-Navβ3-L10P nor -V110I caused any significant changes in the activation and inactivation kinetics of \( i_{Na} \) compared with pRES-CD8-Navβ3-WT (Figure 3D, Table 2). In accordance with the previous report, 22 pRES-CD8-Navβ3-L10P caused a rightward shift in the time course of recovery from inactivation, whereas pRES-CD8-Navβ3-V110I did not show any significant changes (Figure 3E, Table 2). To analyze the functional impact of mutant Navβ3 in the heterogeneous state, the sodium current was recorded from cells expressing Nav1.5 in combination with WT and mutant Navβ3. It was demonstrated that the peak current densities of \( i_{Na} \) recorded from the cells cotransfected with pcDNA3.1-Nav1.5-transfected cells with pRES-CD8-Navβ3-WT+L10P or -WT+V110I were significantly smaller than that from the transfected cells with pRES-CD8-Navβ3-WT by 25.2% or 29.4%, respectively (Table 2). These data indicated that neither mutation exerted a dominant negative effect on the function of normal Navβ3.

**Binding Between Nav1.5 and Navβ3**

Because Navβ3 non-covalently interacts with Nav1.5, we investigated whether the V110I mutation would change the interaction, but there were no significant differences among Navβ3-WT, -L10P and -V110I in binding Nav1.5 (Figure 4), indicating that the altered sodium channel function was not caused by loss of binding between Nav1.5 and Navβ3.

**Discussion**

Arrhythmias can be caused by mutations in the genes for cardiac ion channels producing action potentials. In BrS, the inward sodium current (\( i_{Na} \)) is more frequently affected than the other currents such as calcium and potassium. 32 To date, more than 300 disease-causing SCN5A mutations have been reported, and have been detected in 11–28% of BrS patients. 11,32 On the other hand, the prevalence of BrS-causing mutations in the genes for modifier proteins of Nav1.5, including GPD1-L, Navβ1, Navβ3 and MOG1, is relatively low. 32,23,33 In the present study, we identified a novel SCN3B mutation, V110I, in 3 Japanese BrS patients. It affected the evolutionary conserved residue of Navβ3, not found in the control subjects, decreased the cell surface expression of Nav1.5, and impaired \( i_{Na} \) function. These observations strongly suggested that the SCN3B mutation was a BrS-causing mutation. It is noteworthy that, among these 3 patients, 2 had family histories of arrhythmia and/or sudden cardiac death, while the family history was uncertain in the other patient, indicating that the SCN3B mutation was rare but could be found in a considerable proportion of SCN5A-negative BrS, especially familial cases; 2 in 19 (10.5%) familial cases and 1 in 159 (0.6%) sporadic cases. Although there were no traceable genetic relations among the proband patients carrying the same mutation, the V110I mutation might be a founder mutation. Further investigation of the SCN3B mutation in a large cohort of familial BrS cases is required to assess the ancestral origin of mutation.

The cell surface expression of Nav1.5 was significantly reduced in the presence of Navβ3-L10P or -V110I in transfected cells. Although the cytoplasmic trafficking defect of Nav1.5 is well known to be an underlying mechanism for cardiac channelopathies, including BrS, 33 involvement of modifier proteins in the cell surface expression of Nav1.5 is poorly understood. 34 A pore-forming subunit of the voltage-gated sodium channel in the sensory nervous and atrial myocardium, Nav1.8, is highly homologous to Nav1.5. 35 It was reported that an en-doplasmic reticulum (ER) retention sequence, RRR, in the cytoplasmic loop I of Nav1.8 caused retention of Nav1.8 on the ER, and masking of the retaining signal by Navβ3 released Nav1.8 for trafficking to the cell surface. 36 Because the RRR sequence is conserved in the cytoplasmic loop I of Nav1.5, Navβ3 might alter Nav1.5 trafficking by masking its ER reten-
tion signal. However, immunofluorescence studies demonstrated cytoplasmic colocalization of Nav1.5 and Navβ3, even in the presence of SCN3B mutations. This, in turn, implied that the trafficking defect of Nav1.5 was not caused by impaired formation of the sodium channel complex but by retention of mutant Navβ3 in the ER.

To date, 5 different β-subunits of the sodium channel have been identified.23,24 In cardiomyocytes, Navβ1 and Navβ3 are preferentially expressed and modulate the function of Nav1.5 through non-covalent binding.25 The structure of the β-subunits is relatively simple; forming with an Ig loop at the extracellular N-terminal region, 1 transmembrane domain, and a small intracellular C-terminal domain. In this study, both the L10P and V110I mutations affected the peak current of I_Na in transfected cells via an affect on the trafficking of Nav1.5 to the cell surface. The binding of Nav1.5 and Navβ3, however, was not affected by the SCN3B mutations, suggesting that the Ig loop might not be involved in the binding to Nav1.5. On the other hand, these mutations showed different effects on the recovery from inactivation, indicating the possibility that the modulation of Nav1.5 function by Navβ3 might be controlled at multiple steps. It is interesting to note that the L10P mutation is also reported in AF.26 The underlying mechanism of AF is a reentrant circuit in atrial tissues, where electrical conduction is delayed.4,27 In patients with inherited AF who carried the SCN5A mutation, the delayed conduction is predicted to be induced by a slower upstroke of the action potential because of the loss-of-function mutation in SCN5A. As shown in Figure 3E, slower recovery from inactivation associated with the L10P mutation might partly contribute to the further delayed upstroke of the action potential by decreasing the fraction of channels enrolling in the subsequent depolarization. The difference in inactivation recovery might be related to the differences in arrhythmic phenotypes.

We revealed that the L10P mutation decreased peak sodium current density by 37.5%. On the other hand, Hu et al reported that the L10P mutation decreased the peak current density by 80%, and approximately 40% of the transfectants did not produce the current,28 and Olsen et al showed that the L10P decreased the peak currents by approximately 50%.29 The reasons for these functional differences might be related to the different experimental conditions, including cell lines, the ratio of vectors, the presence of Navβ1, and the chemical composition of the bath solution. These differences would complicate the understanding and comparing of functional alterations caused by the mutations.

In summary, we identified a SCN3B V110I mutation in 3 unrelated Japanese patients with BrS that impaired intracellular trafficking and affected the electrophysiological function of Nav1.5, a hallmark of BrS. This is the first replicating report demonstrating a SCN3B mutation as a disease gene for BrS.

Acknowledgments

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Disclosures

Conflict of Interest: None declared.

References

SCN3B Mutation in BrS


Supplementary Files

Supplementary File 1

Table S1. Nucleotide Sequences of Primers Used for Mutational Analysis of the Other Known Brugada Syndrome Genes

Table S2. Nucleotide Sequences of Primers Used in the Mutational Analysis of SCN3B

Table S3. Nucleotide Sequences of Primers Used in the Construction of Nav3 Constructs

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-12-0995