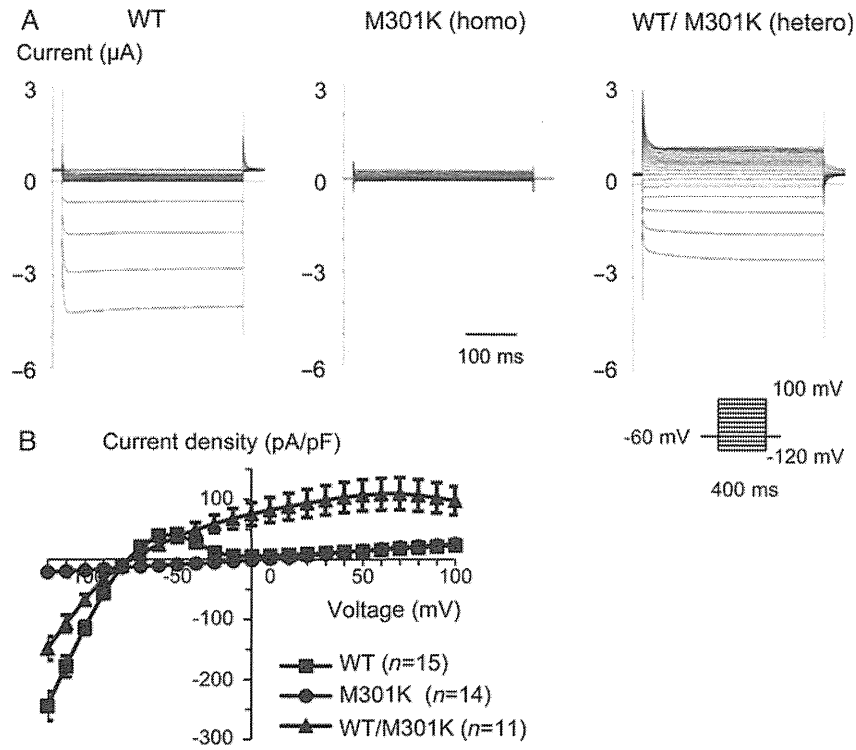

Figure 3 Voltage-clamp recordings from transfected HEK 293 cells. (*A*) Representative current traces of WT, M301K, and WT/M301K. Currents were elicited by 400 ms depolarizing voltage steps from −120 to +100 mV and from a holding potential of −60 mV. (*B*) Current–voltage relationships are plotted as the current. Current density was calculated by dividing the whole-cell current amplitude by cell capacitance. No functional currents were recorded in the homozygous M301K channels. On the other hand, the mean current densities of the WT/M301K channels are significantly larger than the WT ($P < 0.05$) at each voltage from −30 to +100 mV, and smaller at each voltage from −120 to −90 mV ($P < 0.05$).

3.6 Action potentials recording in *KCNJ2*-M301K-transfected NRVMs

We investigated the impacts of M301K mutant Kir2.1 channels on NRVMs' action potentials using a transient transfection method. *Figure 5A* shows typical action potentials recorded for non-transfected (control) NRVMs (*Figure 5A*, left panel), and NRVMs transfected with *KCNJ2* WT or WT/M301K (*Figure 5A* middle and right panels, respectively). Phase 3 repolarization was accelerated in the *KCNJ2* WT- and WT/M301K-overexpressed groups (*Figure 5A* middle and right panels, respectively) and we could further note that the dome is nearly lost in the WT/M301K group. APD_{90} was significantly abbreviated in the *KCNJ2* WT-overexpressed group (28.2 ± 3.4 ms, $n = 10$, $P < 0.001$, *Figure 5A*, middle panel) in comparison with the control group (123.3 ± 12.2 ms, $n = 11$, *Figure 5A*, left panel; bar graphs in *Figure 5B*). Additionally, APD90 was significantly shorter in the WT/M301K mutant-overexpressed group (9.4 ± 2.1 ms, $n = 16$, $P < 0.001$, *Figure 5A*, right panel; bar graph in *Figure 5B*) than in the WT-overexpressed group.

4. Discussion

4.1 Major findings

In the present study, we identified a novel heterozygous *KCNJ2* mutation, M301K, in a patient with a markedly shortened QT interval. The QT interval, 172 ms, of this patient is the shortest among previous SQTS reports,[2–7,16] to our knowledge. The methionine at position 301 is located in the C-terminus of Kir2.1 channel, and is considered to form a pore-facing loop region.[13] Functional assays using a heterologous expression system revealed that homozygous M301K Kir2.1 channels carried no currents with preserved plasma membrane expression; however, heterozygous WT/M301K Kir2.1 channels attenuated inward rectifying properties, which resulted in increased outward currents for positive voltages and negative voltages down to −30 mV. Significant increases in outward currents within the voltage range of the action potentials shortened APD by accelerating membrane repolarization as shown in *Figure 5*, which is implicated in increased cardiac vulnerability.

4.2 Impaired inward rectification of Kir2.1 currents: a novel mechanism predisposing SQTS

In 2005, Priori *et al.*[4] first reported a heterozygous gain-of-function *KCNJ2* mutation, D172N, in a patient with SQTS. In the report, homozygous D172N Kir2.1 channels displayed larger outward currents compared with WT Kir2.1 alone, and heterozygous channels yielded intermediate results. In both homozygous and heterozygous D172N mutant channels, the inward rectification properties of Kir2.1 currents were preserved. In heterozygous M301K mutant channels identified in our patient, however, the inward rectification was significantly reduced, allowing ample outward potassium currents at positive potentials. In addition, it should be emphasized that the homozygous M301K mutant channels were non-functional. These functional changes, such as the impaired inward rectification of the

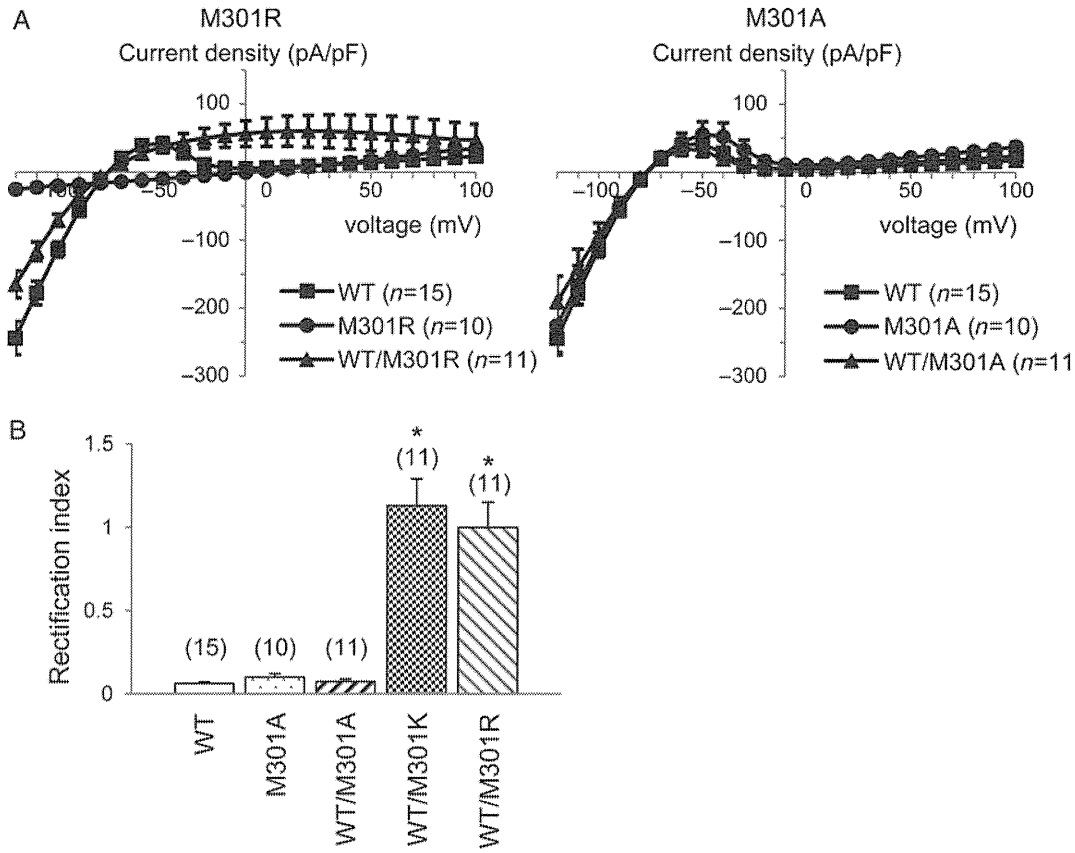

Figure 4 Comparison of macroscopic currents through WT Kir2.1 and mutants. (*A*) Current–voltage relationships for WT, M301R, and M301A are shown. M301R mutant channels displayed no functional currents and WT/M301R mutant channels displayed decreased inward rectification. On the other hand, the currents recorded in the homozygous M301A and heterozygous WT/M301A mutant channels showed no significant difference from WT. (*B*) Rectification index for WT ($n = 15$), M301A ($n = 10$), WT/M301A ($n = 11$), WT/M301K ($n = 11$), and WT/M301R ($n = 11$) channels. The rectification index was calculated by dividing the value of the outward currents measured at 0 mV by the absolute value of the inward currents measured at −100 mV. $*P < 0.001$.

Kir 2.1 currents resulting in increased outward currents, are a novel *KCNJ2* gain-of-function mechanism predisposing SQTS.

The phenotypic characteristics of our index patient somewhat differ from those of the *KCNJ2*-D172N mutation carriers.[4] No apparent arrhythmias were recorded with D172N mutation carriers. On the other hand, our M301K patient showed paroxysmal AF and multiple disorders. Additionally, mechanical stimulation by a Swan–Ganz catheter induced paroxysmal supraventricular tachycardia and VF. Moreover, the QTc interval in our patient was much shorter (QTc = 194 ms, *Figure 1*) than that of the D172N carriers (QTc = 315 and 320 ms).[4] Another gain-of-function *KCNJ2* mutation, V93I, was reported in a familial AF case.[17] Their functional analysis showed a similar result with D172N, but the affected members had normal QT intervals. These diverse clinical manifestations may be related to the extent and the different gain-of-function mechanisms of the Kir2.1 currents.

4.3 Relationship between impaired inward rectification and charged amino acid residues at 301

Kir currents exhibit strong inward rectification, which is thought to be due to pore blocking induced by multivalent ions from intracellular Mg^{2+}.[18–20] Channel blockade by physiological concentrations of Mg^{2+} is influenced by the electrostatic negativity within the cytoplasmic pore.[15] Negative charges on the inner wall of the cytoplasmic pore are therefore key determinants of the strength of the inward rectification. Many amino acid residues inside the pore demonstrate interactions with the ion over long distances, suggesting that mutations potentially affect ion or blocker energetics over the entire pore profile.[14,21] The M301K mutation causes the change of the amino acid residue at 301 from a non-charged amino acid residue, methionine, to a positively charged residue, lysine. In order to evaluate the importance of the charge at 301, additional whole-cell patch-clamp recordings were carried out on M301A (remained neutral) and M301R (neutral to positive) (*Figure 4*). Inward rectification of Kir2.1 currents was well preserved in both homozygous and heterozygous M301A channels. Heterozygous M301R channels, however, attenuated inward rectification, and homozygous M301R channels were non-functional similar to that of the M301K channels. These electrophysiological results indicate that the neutral amino acid residue at 301 plays an important role in generating Kir2.1 inward rectification. The decrease in the net negative charge within the cytoplasmic pore may facilitate the reduction in both the susceptibility of the channel to Mg^{2+} block and the voltage dependence of the blockade. It

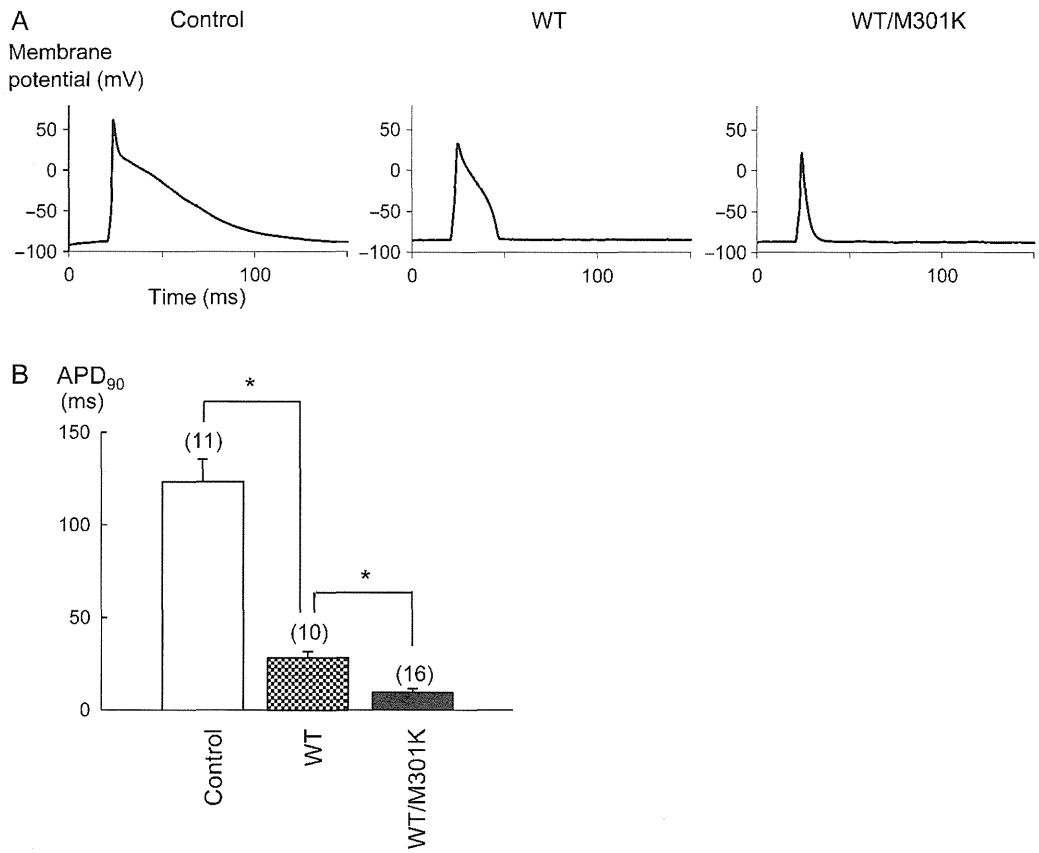

Figure 5 Effects of the M301K mutation on NRVM action potentials. Typical action potentials were demonstrated in a non-transfected cell (*A*), in a WT-overexpressed cell (*B*), and in a heterozygous overexpressed cell (*C*). Graphs show APD at 90% repolarization from the overshoot (*D*). In WT-overexpressed NRVMs, the plateau phase of the cardiac AP was markedly abbreviated, resulting in short repolarization. Under the heterozygous overexpressed condition, the results exhibited virtually no plateau phase, and the mean APD_{90} was significantly shorter in comparison with WT overexpressed alone. $^*P < 0.001$.

remains unknown why only tentative hetero-multimers of WT and M301K are active and lose their inward rectification properties. In homozygous M301K channels, all of the tetrameric subunits must have a positively charged lysine at 301, which may impair potassium ion permeation due to a conformational change in the near-pore region.

4.4 Heterozygous *KCNJ2*-WT/M301K overexpression shortened APD in NRVMs

In cardiomyocytes, Kir2.1, Kir2.2, and Kir2.3 channels are supposed to be able to co-assemble in order to modulate their channel properties.[22] Thus, there can be a multitude of Kir2.x heteromultimers, and to date a wide range of single-channel conductances of inward rectifier channels have been reported in studies conducted on various mammalian myocytes, including human.[23–25] This variety at the individual channel level may contribute to the different stoichiometry of the tetrameric channels.[26] Because Kir2.1 is a major component of IK1 in the myocardium, we overexpressed the *KCNJ2* M301K mutant channels in NRVMs to examine the effects of the mutation on APD. Overexpression with WT alone resulted in shorter APD in comparison with non-transfected myocytes (*Figure 5B*). These results are consistent with a previously published report.[27] Notably, heterozygous overexpression with WT and M301K further amplified the shortened APD (*Figure 5C*). These results were compatible with the electrophysiological changes assessed in HEK 293 cells, because the heterozygous WT/M301K channels showed a larger outward current than WT Kir2.1 channels under the physiological range of membrane potentials (*Figure 3*). Weak inward rectification observed in the heterozygous WT/M301K channels suggests that potassium ion can get through Kir2.1 channel at depolarized potential, probably resulting in loss of the action potential dome recorded in the *KCNJ2* WT/M301K-overexpressed group. The experiments were performed using a transient overexpression system that was different from the patient's heart, and the amount of overexpressed channels was difficult to be estimated accurately. But, these results are beneficial in understanding that the heterozygous *KCNJ2* M301K mutation could abbreviate APD and cause an extremely short-QT interval in the patient's ECG.

4.5 Clinical features of the index patient with *KCNJ2*-M301K

Regarding the clinical criteria for the diagnosis of SQTS, they have yet to be defined. However, we should consider SQTS in a patient presenting with a QTc <340 ms and other factors suggestive of arrhythmia (such as syncope or family history of sudden death).[28] A prominent clinical manifestation of SQTS is arrhythmias, such as AF

and VF.[1–5,7] In this patient, however, additional medical histories not limited to arrhythmias, such as severe mental retardation, abnormal proliferation of the oesophageal blood vessels, epilepsy, and Kawasaki' disease, were also documented. Because *KCNJ2* is known to be expressed in a variety of tissues, such as cardiac and skeletal muscle, the brain, arterial smooth muscle cells and developing bony structures of the craniofacial region, extremities, and vertebrae,[29–31] some of her compound disorders may be attributed to the *KCNJ2* mutation. In fact, loss-of-function mutations in *KCNJ2* cause Andersen–Tawil syndrome, which is characterized by prolonged repolarization, dysmorphic features, and periodic paralysis.[10,32] In the family of our female patient, we could not perform extensive genetic testing. We cannot exclude the possibility of the presence of other affected genes. Further analyses using knock-in mice or induced pluripotent stem cells would culminate monumental insight into the relationship between the *KCNJ2* M301K mutation and the patient's extra-cardiac phenotypes.

4.6 Conclusions

We described a novel *KCNJ2* gain-of-function mutation, M301K, in a patient with SQTS. Functional assays revealed no functional currents in the homozygous channels, whereas impaired inward rectification in the heterozygous channels manifested in larger outward currents, which is a novel mechanism predisposing SQTS.

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Conflict of interest: none declared.

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Electrocardiographic Characteristics and *SCN5A* Mutations in Idiopathic Ventricular Fibrillation Associated With Early Repolarization

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Background—Recently, we and others reported that early repolarization (J wave) is associated with idiopathic ventricular fibrillation. However, its clinical and genetic characteristics are unclear.

Methods and Results—This study included 50 patients (44 men; age, 45±17 years) with idiopathic ventricular fibrillation associated with early repolarization, and 250 age- and sex-matched healthy controls. All of the patients had experienced arrhythmia events, and 8 (16%) had a family history of sudden death. Ventricular fibrillation was inducible by programmed electric stimulation in 15 of 29 patients (52%). The heart rate was slower and the PR interval and QRS duration were longer in patients with idiopathic ventricular fibrillation than in controls. We identified nonsynonymous variants in *SCN5A* (resulting in A226D, L846R, and R367H) in 3 unrelated patients. These variants occur at residues that are highly conserved across mammals. His-ventricular interval was prolonged in all of the patients carrying an *SCN5A* mutation. Sodium channel blocker challenge resulted in an augmentation of early repolarization or development of ventricular fibrillation in all of 3 patients, but none was diagnosed with Brugada syndrome. In heterologous expression studies, all of the mutant channels failed to generate any currents. Immunostaining revealed a trafficking defect in A226D channels and normal trafficking in R367H and L846R channels.

Conclusions—We found reductions in heart rate and cardiac conduction and loss-of-function mutations in *SCN5A* in patients with idiopathic ventricular fibrillation associated with early repolarization. These findings support the hypothesis that decreased sodium current enhances ventricular fibrillation susceptibility. **(*Circ Arrhythm Electrophysiol.* 2011;4:874-881.)**

Key Words: arrhythmia ■ sodium channel ■ electrophysiology ■ genetics ■ mutations

Early repolarization or J-wave is characterized by an elevation at the junction between the end of the QRS

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complex and the beginning of the ST-segment (J-point) in a 12-lead ECG and generally has been considered benign for decades.[1] However, early repolarization can be observed under various negative biological conditions, such as low body temperature and ischemia,[2–4] and there is increasing evidence that early repolarization is associated with an increased risk of ventricular fibrillation and sudden cardiac death.[5–7]

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In previous studies, including our own, early repolarization in the inferior or lateral leads was associated with pathogenesis in idiopathic ventricular fibrillation.[5,6] Moreover, early repolarization in the right precordial leads also has been associated with idiopathic ventricular fibrillation.[8] Heritability of early repolarization has been shown in a recent population-based study,[9] and as in other arrhythmia syndromes such as long QT syndrome and Brugada syndrome,[10] ion channel genes are responsible for idiopathic ventricular fibrillation associated with early repolarization.[11–13] A mutation in *KCNJ8*, which encodes a pore-forming subunit of the ATP-sensitive potassium channel, has been identified in idiopathic ventricular fibrillation with early repolarization.[11,14] Mutations in L-type calcium channel genes, including *CACNA1C*, *CACNB2B*, and *CACNA2D1*, also have been associated with idiopathic ventricular fibrillation with early repolarization.[12]

In this study, we compared electrocardiographic parameters between patients with idiopathic ventricular fibrillation and healthy controls and found that heart rate and cardiac conduction were slow in patients with idiopathic ventricular fibrillation. Furthermore, we screened patients with idiopathic ventricular fibrillation for mutations in *SCN5A*, which encodes the predominant cardiac sodium channel α subunit and is critical for cardiac conduction. Here, we present the clinical and in vitro electrophysiological characteristics in idiopathic ventricular fibrillation associated with early repolarization.

Methods

Study Populations

This study included patients with idiopathic ventricular fibrillation and early repolarization who were referred to our institutions. Patients were diagnosed with idiopathic ventricular fibrillation if they had no structural heart disease as identified using echocardiography, coronary angiography, and left ventriculography. Baseline electrophysiological studies without antiarrhythmic drugs were performed based on the indication of each institution. Early repolarization was defined as an elevation of the J-point, either as QRS slurring or notching $\geq$0.1 mV $\geq$2 consecutive leads in the 12-lead ECG.[5] Patients were excluded if they had a short QT interval (corrected QT interval using Bazett formula <340 ms) or a long QT interval (corrected QT interval >440 ms) in the 12-lead ECG.[15,16] All patients received sodium channel blocker challenge, and patients with Brugada type ST-segment elevations at baseline or after sodium channel blocker challenge were excluded.[17] Twelve-lead electrocardiograms recorded in the absence of antiarrhythmic drugs were compared between patients with idiopathic ventricular fibrillation and control subjects who were matched to patients with idiopathic ventricular fibrillation based on gender and age (patient: control ratio, 1:5). Control subjects were selected from 86 068 consecutive electrocardiograms stored in the ECG database in Niigata University Medical and Dental Hospital from May 7, 2003 to July 2, 2009.[18] Control subjects who had a normal QT interval (corrected QT interval, 360 to 440 ms) and no cardiovascular disease or medication use were included. Control subjects with Brugada type ST-segment elevations or early repolarization were excluded.

Genetic Analysis

All probands and family members who participated in the study gave written informed consent before genetic and clinical investigations in accordance with the standards of the Declaration of Helsinki and local ethics committees. Genetic analysis was performed on genomic DNA extracted from peripheral white blood cells using standard methods. The coding regions of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ8* were amplified by PCR using exon-flanking intronic primers,[19–21] and direct DNA sequencing was performed using ABI 310, 3130, and 3730 genetic analyzers (Applied Biosystems, Foster City, CA).[22]

Generation of Expression Vectors and Transfection in Mammalian Cell Lines

Full-length human *SCN5A* cDNA was subcloned into the mammalian expression plasmid pcDNA3.1+ (Invitrogen, Carlsbad, CA).[22] Mutant constructs were prepared using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions. The human cell line tsA201 was transiently transfected with wild-type or mutant *SCN5A* plasmid using Lipofectamine LTX (Invitrogen), in combination with a bicistronic plasmid (pCD8-IRES-hβ1) encoding CD8 and the human sodium channel β1 subunit (hβ1) to visually identify cells expressing heterologous hβ1 using Dynabeads M-450 CD8 (Invitrogen).[22] Electrophysiological measurements were performed 24 to 72 hours after transfection.

Electrophysiology

Sodium currents were recorded using the whole-cell patch clamp technique as previously described.[22] Electrode resistance ranged from 0.8 to 1.5 mol/LΩ. Data were acquired using an Axopatch 200B patch clamp amplifier and pCLAMP8 software (Axon Instruments). Sodium currents were filtered at 5 kHz (−3 dB, 4-pole Bessel filter) and were digitally sampled at 50 kHz using an analog-to-digital interface (Digidata 1322A; Molecular Devices, Sunnyvale, CA). Experiments were performed at room temperature (20 to 22°C). Voltage errors were minimized using series resistance compensation (generally 80%). Cancellation of the capacitance transients and leak subtraction were performed using an online P/4 protocol. The time from establishing the whole-cell configuration to the onset of recording was consistent (5 minutes) between cells to exclude possible time-dependent shifts of steady-state inactivation. The pulse protocol cycle time was 10 s. The data were analyzed using Clampfit 10 (Molecular Devices) and SigmaPlot 9 software (Aspire Software International, Ashburn, VA). The holding potential was −120 mV. The bath solution contained the following (in mmol/L): 145 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, pH 7.35 (adjusted with NaOH). The pipette solution (intracellular solution) contained the following (in mmol/L): 10 NaF, 110 CsF, 20 CsCl, 10 EGTA, and 10 HEPES, pH 7.35 (adjusted with CsOH).

Immunocytochemistry

For immunocytochemistry, the FLAG epitope was inserted between residues 153 and 154 of the extracellular linker S1-S2 in domain I. The FLAG insertion into the S1-S2 linker previously has been shown to have no effect on channel gating or cell surface expression.[22,23] Immunocytochemistry was performed in HEK293 cells transfected with wild-type or mutant *SCN5A* plasmid as described previously.[22,24] After 48 hours of transfection, the cells were washed with phosphate-buffered saline, fixed in 4% paraformaldehyde, and permeabilized with 0.15% Triton X-100 in phosphate-buffered saline with 3% bovine serum albumin. Then the cells were stained with anti-FLAG polyclonal antibody (F7425; Sigma-Aldrich, St Louis, MO; 1:100) for 1 hour at room temperature. Protein reacting with antibody was visualized with Alexa Fluor 568–labeled secondary antibody (A-11011, Invitrogen, 1:1000). Images were collected using a Zeiss LSM 510 laser confocal microscope and analyzed using LSM 4.0 software.

Data Analysis

Differences in parameters between patients with idiopathic ventricular fibrillation and control subjects were analyzed using conditional logistic regression models. To exclude the effects of multicollinearity among electrocardiographic parameters, each electrocar-

Table 1. Electrocardiographic Parameters

	IVF Patients N=50	Controls N=250	OR (95% CI)/ 10 Unit Increase	*P* Value
Male sex, N (%)	44 (88)	220 (88)	...	...
Age, y	45±17	45±16	...	...
Heart rate, beats/min	62±9	70±14	0.62 (0.47–0.81)	<0.001
PR interval, ms	175±34	147±20	1.32 (1.22–1.43)	<0.001
QRS interval, ms	96±14	89±8	1.63 (1.31–2.02)	<0.001
QTc, ms	388±25	397±22	0.85 (0.75–0.98)	0.02

IVF indicates idiopathic ventricular fibrillation; OR, odds ratio; QTc, corrected QT interval.

diographic parameter was separately tested in the logistic models. All statistical analyses were performed with SPSS, version 12.0 (SPSS Inc, Chicago, IL). A 2-sided $P<0.05$ was considered statistically significant. Values are expressed as mean±SD. The study protocol was approved by the ethics committee of each institution.

Results

We identified 50 patients with idiopathic ventricular fibrillation and early repolarization (44 men [88%]; mean age, 45±17 years). All of the patients had experienced arrhythmia events, and 8 (16%) had a family history of sudden death.

Electrocardiographic parameters were compared between 50 patients with idiopathic ventricular fibrillation and 250 healthy control subjects without cardiovascular disease and not taking medication who were matched with gender and age (Table 1). The heart rate was slower, and the PR interval and QRS duration were longer in patients with idiopathic ventricular fibrillation compared with control subjects. The corrected QT interval was shorter in patients with idiopathic ventricular fibrillation than control subjects. No patient with idiopathic ventricular fibrillation showed type I Brugada electrocardiograms in repeated recordings.[25] Sodium channel blockers were administered in all patients, and Brugada type electrocardiograms were not provoked in any of these patients.[25] Electrophysiological study was performed in 29 patients. His-ventricular interval was 48±9 ms, and 4 patients had prolonged His-ventricular time ≥55 ms.[26] Ventricular fibrillation was inducible by programmed electric stimulation in 15 patients (52%).

We screened for mutations in *SCN5A* in 26 unrelated patients with idiopathic ventricular fibrillation and identified 3 mutations (A226D, R367H, and L846R) in 3 patients (Figure 1, Table 2). R367H and L846R are predicted to be located in the pore region. These mutations were not found in the genomes of 200 healthy control individuals. Two of the patients exhibited prolongation of the PR interval, and sodium channel blocker challenge was negative for Brugada syndrome in all of them. Alignment of the amino acid sequences from multiple species demonstrated that the amino acids substituted by mutations are highly conserved, supporting the importance of these amino acids. A226D and L846R, but not R367H, are predicted to change the electric charge of substituted amino acids.

A missense mutation, A226D (Figure 1A), was identified in a 36-year-old man (patient 1) resuscitated from ventricular fibrillation. He had experienced multiple episodes of syncope. The physical examination and echocardiography were normal. His ECG showed prolongation of the PR interval and early repolarization in leads II, III, and aVF, and J-point/ST-segment elevation in lead V1 (Figure 2A). Administration of pilsicainide augmented early repolarization in the inferior leads and induced ventricular fibrillation, but did not produce a type I Brugada ECG in the right precordial leads (Figure 2B). Electrophysiological study revealed prolongation of His-ventricular interval (68 ms), and ventricular fibrillation was induced by programmed electric stimulation. The patient's family history was negative for syncope, sudden cardiac death, and epilepsy.

A missense mutation L846R (Figure 1B) was identified in a 27-year-old man (patient 2). He was admitted after multiple episodes of syncope, and polymorphic ventricular tachycardia was documented when he lost consciousness. The physical examination and echocardiography were normal. His ECG

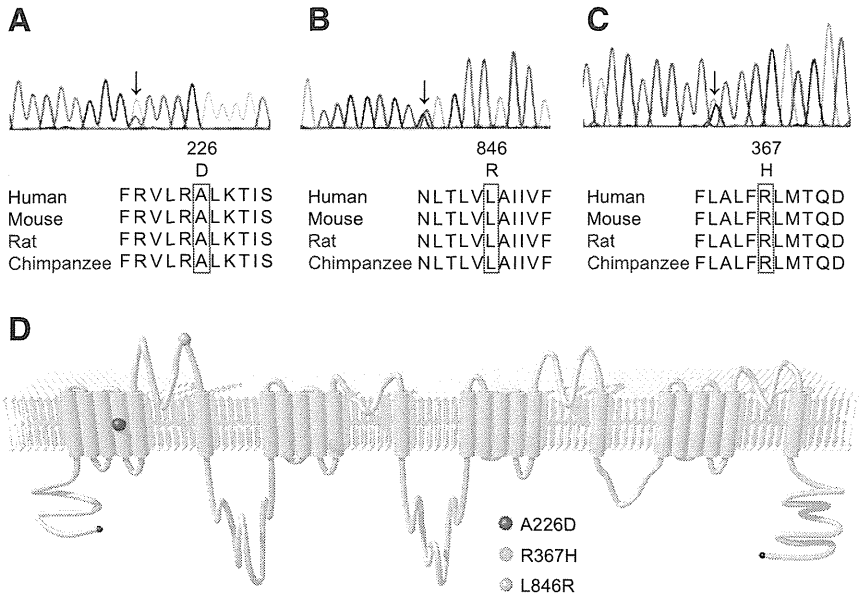

Figure 1. Mutations in *SCN5A* identified in patients with idiopathic ventricular fibrillation associated with early repolarization. **A**, The c.677C→A mutation in *SCN5A* resulting in p.A226D found in patient 1. **B**, The c.2537T→G mutation in *SCN5A*, resulting in p.L846R found in patient 2. **C**, The c.1100G→A mutation in *SCN5A*, resulting in p.R367H found in patient 3. We previously reported the R367H mutation (modified from Takehara et al[27]). **D**, Predictive topology of the SCN5A channel. Circles indicate the locations of the mutations.

Table 2. Characteristics of Idiopathic Ventricular Fibrillation Patients With *SCN5A* Mutations

Patient No.	Sex	Age at Onset (y)	Family History of SCD	Presenting Symptom	Location of J Wave	Other ECG Abnormalities	Response to Sodium Channel Blocker	Amino Acid Substitution
1	M	36	N	Aborted SCD	II, III, aVF, V1	PR prolongation	Augmentation of J-point amplitude and VF	A226D
2	M	27	Y	Aborted SCD	I, II, III, aVF	PR prolongation	Marked QRS prolongation and VF	L846R
3	F	37	N	Aborted SCD	II, III, aVF, V2	N	Augmentation of J-point amplitude and marked QRS prolongation	R367H

ECG indicates electrocardiogram; SCD, sudden cardiac death.

showed prolongation of the PR interval and early repolarization in lead III (Figure 2C). During the recovery phase of exercise testing, the amplitude of the J-point/ST-segment was augmented in leads I, II, III, and aVF, and ventricular fibrillation was induced. Pilsicainide caused marked prolongation of QRS duration and augmented the J-point/ST-segment amplitude in leads V1 and V2, followed by the development of ventricular fibrillation (Figure 2C and 2D). Pilsicainide did not produce a type I Brugada ECG. During electrophysiological study, His-ventricular interval was 55 ms. His uncle died suddenly.

We previously reported a missense mutation R367H in patient 3 as a case with Brugada syndrome (Figure 1C).[27] However, idiopathic ventricular fibrillation associated with early repolarization was diagnosed at a later time because a type 1 Brugada ECG has never been seen spontaneously or after the administration of sodium channel blocker in more than 1 right precordial lead, and thus the diagnostic criteria for Brugada syndrome were not fulfilled.[25] When the patient admitted to the hospital after recurrent episodes of syncope, early repolarization was present in the inferior and right precordial leads (Figure 2E). After sinus pause, early repolarization was augmented in leads II, III, and aVF, followed by the development of ventricular fibrillation after a few hours of the admission (Figure 2F). Procainamide further exaggerated early repolarization but did not produce a type I

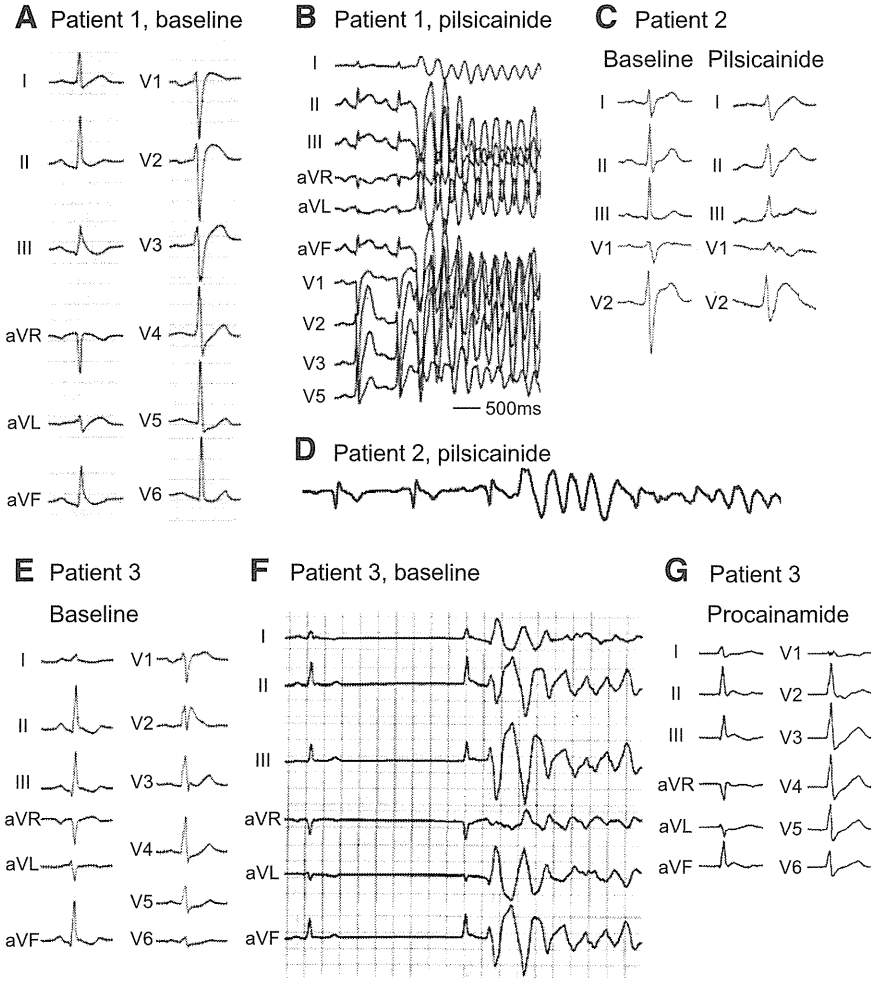

Figure 2. Electrocardiograms of patients with idiopathic ventricular fibrillation and a mutation in *SCN5A*. **A**, Early repolarization was present in the inferior and right precordial leads in patient 1. **B**, After administration of pilsicainide, early repolarization was augmented and ventricular fibrillation developed. **C** and **D**, Pilsicainide caused marked prolongation of QRS duration and J-point elevation in the right precordial leads, followed by the development of ventricular fibrillation in patient 2. **E**, Early repolarization was present in the inferior leads and right precordial leads in patient 3. **F**, The augmentation of early repolarization after sinus pause, followed by ventricular fibrillation. **G**, After the administration of procainamide, early repolarization was augmented in the inferior. In all patients, sodium channel blockers did not provoke a type I Brugada ECG. **E**, **F**, and **G** were modified from Takehara et al.[27]

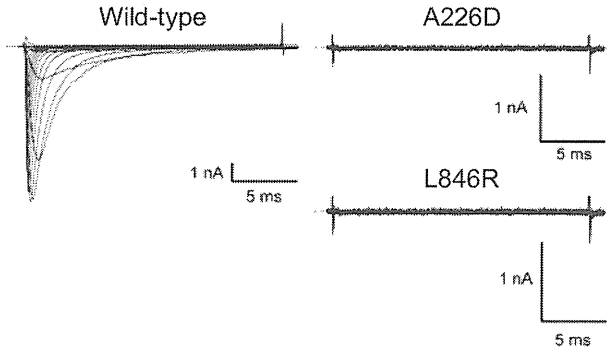

Figure 3. Electrophysiological characteristics of the *SCN5A* mutants. Representative traces of sodium current demonstrating that all of the mutant channels failed to generate any currents. We previously reported that R367H mutant fails to generate any currents.[27]

Brugada ECG (Figure 2G). During electrophysiological study, His-ventricular time was prolonged (65 ms) and ventricular fibrillation was not induced. The patient's family history was negative for syncope, sudden cardiac death, and epilepsy.

The electrophysiological characteristics of the mutant sodium channels were assessed in transfected mammalian cells using the whole-cell patch-clamp technique. Figure 3 shows representative current traces in cells expressing wild-type or mutant SCN5A channels. There was no detectable current in A226D, R367H,[27] and L846R mutant channels. Immunostaining revealed that cells expressing A226D channels showed cytoplasmic fluorescence, while cells expressing wild-type channels showed marked peripheral fluorescence, suggesting that the mutation results in trafficking defect (Figure 4). Cells expressing R367H channels and those expressing L846R channels showed a similar fluorescence pattern to wild-type channels, suggesting that these mutations do not affect trafficking.

Discussion

In this study, patients with idiopathic ventricular fibrillation associated with early repolarization exhibited slower heart rate and slower cardiac conduction properties than did controls. We found rare, nonsynonymous variants in *SCN5A* in patients who had idiopathic ventricular fibrillation associated with early repolarization. These variants affect highly conserved residues, and all of the mutant SCN5A channels failed to generate any currents when expressed in heterologous expression systems. Immunostaining experiments suggested 2 possible mechanisms for the sodium channel dysfunction by the *SCN5A* mutations, a defect of channel trafficking to cell surface in A226D and critical alterations of the structures required for the sodium ion permeation or gating in R367H and L846R that are predicted to be located at the pore region.

Loss-of-function mutations in *SCN5A* are associated with a wide range of inherited arrhythmia syndromes, including Brugada syndrome, progressive cardiac conduction disease, and sick sinus syndrome.[28–30] Furthermore, our results suggest that *SCN5A* is a causative gene of idiopathic ventricular fibrillation associated with early repolarization. Evidence supporting disease causality of the mutations includes the

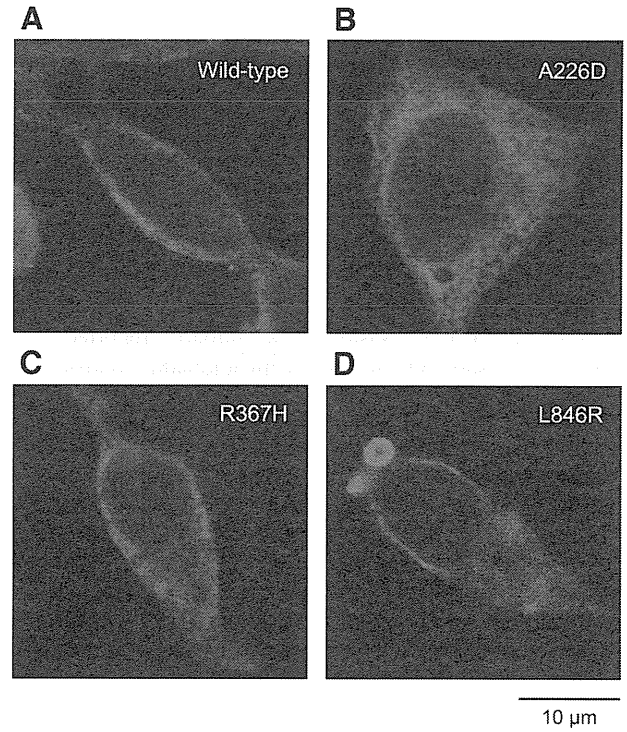

Figure 4. Representative confocal microscopy images. **A**, Cells expressing wild-type SCN5A channels showed marked peripheral fluorescence. **B**, Cells expressing A226D channels showed cytoplasmic fluorescence. **C** and **D**, Cells expressing R367H channels and those expressing L846R channels showed a similar fluorescence pattern to wild-type channels.

identification of 3 mutations in 3 unrelated probands who shared similar clinical phenotypes and the loss of sodium channel function effects in heterologous expression systems in all of the mutant channels.

Although our findings suggest that loss of sodium channel function plays a role in idiopathic ventricular fibrillation associated with early repolarization, the mechanisms of early repolarization are not understood well. In wedge preparations of canine ventricles, early repolarization results from increased action potential notches at the ventricular epicardium by either a decrease in inward currents or an increase in outward currents.[31] A mutation in *KCNJ8*, which encodes the ATP-sensitive potassium channel, recently has been identified in idiopathic ventricular fibrillation associated with early repolarization.[11] The *KCNJ8* mutation has shown gain-of-function effects in ATP-sensitive potassium channels in heterologous expression studies,[14] and augmentation of ATP-sensitive potassium currents results in the development of ventricular fibrillation in wedge preparations.[32] Decreased calcium currents also have been proposed as a mechanism for idiopathic ventricular fibrillation associated with early repolarization.[33] Mutations in L-type calcium channel genes, including *CACNA1C*, *CACNB2B*, and *CACNA2D1*, recently have been identified; however, functional studies are not yet available.[12] Our findings that mutant *SCN5A* channels displayed loss of sodium channel function, resulting in a decrease of inward currents, are consistent with findings in prior studies and with the proposed mechanism.[11,12,14,33]

In this study, heart rate and cardiac conduction were slower in patients with idiopathic ventricular fibrillation than in healthy controls. Furthermore, His-ventricular interval was prolonged in all of the patients carrying an *SCN5A* mutation. Reductions in heart rate and conduction may result from underlying electrophysiological abnormalities in idiopathic ventricular fibrillation. In addition to the maintenance of the action potential dome, normal impulse generation and propagation are dependent critically on normal sodium channel function,[34] and reductions in heart rate and conduction we observed here can be partially explained by loss-of-function mutations in *SCN5A*. Viskin et al initially reported the association of short QT interval with idiopathic ventricular fibrillation,[35] and the recent study also showed that corrected QT interval is shorter in idiopathic ventricular fibrillation patients with early repolarization than those without early repolarization.[5] In this study, corrected QT interval was shorter in patients with idiopathic ventricular fibrillation than in healthy controls, in line with the previous findings.[5,35] Furthermore, we have previously reported that early repolarization is frequently found in patients with short QT syndrome.[18] There may be the association between short QT interval and early repolarization, although the mechanism is unknown.

Idiopathic ventricular fibrillation associated with early repolarization and Brugada syndrome characterized by J-point/ST-segment elevation in the right precordial leads share genetic, clinical, and pharmacological characteristics.[5,8,12,17,25,33,36–41] Rare variants in genes encoding L-type calcium channel and ATP-sensitive potassium channel have been associated with both diseases.[12,14,36] Defects in *SCN5A* are responsible for Brugada syndrome, and we found that mutations in *SCN5A* were possible causative genetic factors in idiopathic ventricular fibrillation associated with early repolarization. Furthermore, an R367H *SCN5A* mutation identified in this study also has been reported in a family affected by Brugada syndrome.[37] However, the mechanism by which loss of sodium channel function results in either Brugada syndrome or idiopathic ventricular fibrillation associated with early repolarization is unknown, similar to that in other arrhythmia phenotypes caused by loss of function mutations in *SCN5A*, the so called cardiac sodium channelopathies.[42] There may be other genetic or environmental factors that modify the clinical phenotype. Although the association of inferolateral early repolarization with idiopathic ventricular fibrillation has been initially reported,[5] early repolarization in the right precordial leads, where Brugada type electrocardiograms can be seen, also has been associated with idiopathic ventricular fibrillation.[8,25] In this study, 2 of the 3 patients carrying an *SCN5A* mutation showed J-point elevation in the right precordial leads, but did not show diagnostic Brugada type ST-segment elevations in multiple ECG recordings even after sodium channel blocker challenge. Sinus node dysfunction and conduction disorders often are seen in Brugada syndrome, and we observed similar electrocardiographic characteristics in idiopathic ventricular fibrillation.[17,25] Bradycardia-dependent augmentation of J-point amplitude has been reported in both diseases and we observed similar changes of J-wave in a patient carrying *SCN5A* mutation.[43,44] The recent studies have shown that early repolarization is found in 14 to 24% of patients with Brugada syndrome, and that early repolarization is associated with the increased risk of arrhythmia events,[12,45] although the role of early repolarization in Brugada syndrome is not clear. The electrocardiographic manifestations of Brugada syndrome may be unmasked or augmented by sodium channel blockers.[17,25] In our present and prior studies, the administration of sodium channel blockers resulted in the augmentation of J-point amplitude or development of ventricular fibrillation in patients with idiopathic ventricular fibrillation.[46] The efficacy of isoproterenol and quinidine also is common in both diseases.[8,17,25,38–41]

In conclusion, we have shown reductions in heart rate and cardiac conduction in patients with idiopathic ventricular fibrillation associated with early repolarization. We identified *SCN5A* mutations in patients with idiopathic ventricular fibrillation and showed that mutant channels did not generate any currents. These findings implicate that *SCN5A* is a disease gene for idiopathic ventricular fibrillation associated with early repolarization, and that it plays a role in the electrocardiographic characteristics of idiopathic ventricular fibrillation, at least in part.

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CLINICAL PERSPECTIVE

Idiopathic ventricular fibrillation associated with early repolarization is a new arrhythmia syndrome entity, although early repolarization has been considered benign for decades. Early repolarization is a heritable electrocardiographic phenotype and there is a positive family history in 10 to 20% of patients with idiopathic ventricular fibrillation associated with early repolarization. Recent studies have identified the causative genes of the arrhythmia, all of which are associated also with Brugada syndrome. In this study, SCN5A, which encodes the predominant cardiac sodium channel α subunit and is critical for cardiac conduction, was screened in patients with idiopathic ventricular fibrillation associated with early repolarization. The screening identified 3 patients carrying an SCN5A mutation, and His-ventricular interval was prolonged in all patients. All of the mutations are predicted to substitute amino acids highly conserved across species and failed to produce any detectable sodium current. To identify electrophysiological characteristics in idiopathic ventricular fibrillation associated with early repolarization, we compared electrocardiograms between patients with the arrhythmia and healthy controls. We found that patients with the arrhythmia exhibited slower heart rate and slower cardiac conduction properties than controls. Our findings suggest that there are underlying electrophysiological abnormalities resulting in slow heart rate, slow cardiac conduction, early repolarization, and ventricular fibrillation, partially explained by sodium channel dysfunction. Idiopathic ventricular fibrillation associated with early repolarization and Brugada syndrome share genetic, clinical, and pharmacological characteristics, but other factors that modify the clinical phenotypes are unknown. Further studies to identify the modifiers are warranted.

SCN5A promoter haplotype affects the therapeutic range for serum flecainide concentration in Asian patients

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Objective **An increased slowing of cardiac conduction induced by sodium channel blockers is remarkably observed in carriers of an Asian-specific promoter haplotype [haplotype B (HapB)] of the cardiac sodium channel gene (*SCN5A*). We investigated the effect of HapB on the therapeutic range for serum flecainide concentration in Asian patients.**

Patients and methods **We examined the serum concentration and antiarrhythmic efficacy of flecainide, together with the *SCN5A* promoter haplotype, in 146 patients with supraventricular tachyarrhythmias. Trough serum flecainide concentrations were determined by HPLC. The antiarrhythmic efficacy of flecainide was assessed for at least 2 months through examination of symptomatology, ECG, and Holter monitoring.**

Results **The serum flecainide concentration did not differ between the wild-type HapA homozygotes and HapB carriers under treatment with the usual dose. A genetic difference in the antiarrhythmic efficacy of flecainide was observed between the HapA homozygotes and HapB carriers at serum flecainide concentrations less than 300 ng/ml (42.9 vs. 68.8%; $P=0.022$). PR prolongation and QRS widening were observed more commonly among the HapB carriers with serum flecainide concentrations of at least 300 ng/ml than in the HapA homozygotes (PR, 210±25 vs. 195±25 ms; $P=0.036$; and QRS, 112±10 vs. 105±9 ms; $P=0.030$).**

Conclusion **These findings suggest that the therapeutic range for serum flecainide concentration is lower in HapB carriers than in HapA homozygotes. *Pharmacogenetics and Genomics* 23:349–354 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: arrhythmia, flecainide, promoter haplotype, SCN5A serum concentration, therapeutic range

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Introduction

The voltage-gated cardiac sodium channel type V alpha (SCN5A) is responsible for the fast depolarization upstroke of the cardiac action potential. It acts as a molecular target for antiarrhythmic drugs [1]. Mutations in the *SCN5A* gene result in long QT syndrome, Brugada syndrome, atrial fibrillation, dilated cardiomyopathy, and sick sinus syndrome [2–10]. A haplotype B (HapB) consisting of six individual *SCN5A* promoter polymorphisms has been shown to produce lower *SCN5A* transcription activity in Asians (allele frequency 24%) [11]. Increased slowing of cardiac conduction induced by a sodium channel blocker challenge test was remarkably observed in HapB carriers compared with that in wild-type HapA homozygotes [11]. Thus, a *SCN5A* promoter haplotype status may modify a patient's response to sodium channel blockers by modulating the activity of SCN5A [12]. The influence of HapB on the antiarrhythmic efficacy of the daily dosage of sodium channel blockers is unclear.

Flecainide acetate, a potent sodium channel blocker, is a class Ic antiarrhythmic agent commonly used for the treatment of a variety of supraventricular tachyarrhythmias [13–15]. Maintaining the serum flecainide concentration within the recommended therapeutic range (200–1000 ng/ml) ensures proven efficacy without the development of severe adverse events such as proarrhythmia [16–18]. Severe adverse events have occasionally occurred in patients whose serum flecainide concentration exceeded 1000 ng/ml [19]. The lower limit of the recommended therapeutic range for serum flecainide concentration, which remains somewhat controversial, varies from 200 to 400 ng/ml for suppression of ventricular arrhythmia [16,18,20]. We previously recommended to maintain serum flecainide concentration at least 300 ng/ml in patients with supraventricular tachyarrhythmias [21–23]. However, according to a previous report, some patients showed sufficient effectiveness of flecainide at concentrations less than 300 ng/ml [21]. We hypothesized that interindividual differences in the flecainide therapeutic concentration may result from differences in the activity of the drug target, such as reduction in the sodium current density due to the presence of *SCN5A* promoter HapB. To confirm this hypothesis, we examined the effects of the *SCN5A*

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promoter haplotype status on the therapeutic range for serum flecainide concentrations in Asian patients with supraventricular tachyarrhythmias.

Methods

Patients

Patients treated with oral flecainide for supraventricular tachyarrhythmias were enrolled into this study during an outpatient visit to our hospital (Table 1). The exclusion criteria were as follows: history of unstable angina or myocardial infarction, recent cardiac surgery, a higher-degree atrioventricular nodal block, pacemaker-dependent rhythms, permanent atrial fibrillation, Brugada syndrome, concomitant therapy including other class I antiarrhythmic drugs or amiodarone, and left ventricular dysfunction (i.e. ejection fraction < 50% or history of heart failure or both). The patients had received oral flecainide (1.2–5.0 mg/kg/day as flecainide acetate) for 2–100 months (mean, 18±24 months). They received other drugs as per requirement: digoxin, β-blockers (carvedilol, atenolol, nadolol, bisoprolol, metoprolol, and propranolol), Ca^{2+} antagonists (verapamil, diltiazem, nifedipine, amlodipine, nisoldipine, and nicardipine), angiotensin-converting enzyme inhibitors (enalapril, lisinopril, and temocapril), angiotensin II receptor blockers (valsartan, telmisartan, olmesartan, losartan, and candesartan), anticoagulants (warfarin, aspirin, and ticlopidine), H_2-blockers (famotidine and ranitidine), or other drugs (lipid-decreasing drugs and HMG–CoA reductase inhibitors). This study was approved by the ethical committee of the University of Tsukuba (approval number, 63-1). Written informed consent was obtained from all patients.

Sample collection and determination of serum flecainide

The patients had received flecainide for at least 2 months before the study. Blood draws for determining flecainide trough levels were performed between 8:30 and 11:00 during an outpatient visit. On sample collection days, the patients postponed taking their morning flecainide until after the collection. The last dose of flecainide before sample collection was taken between 19:00 and 21:00. Serum samples separated from whole blood were stored at −20°C until analysis.

Flecainide acetate and an internal standard [*N*-(2-piperidinylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide acetate] were kindly supplied by Eisai Co. Ltd. (Tokyo, Japan). Serum flecainide was determined by HPLC on a conventional octadecylsilyl silica column with a fluorescence detector, as described previously [24]. Assay precision was evaluated by intraday and interday validation at 200 and 1000 ng/ml flecainide. The coefficients of variation for the intraday and interday assays were 2.7–5.3% and 7.0–8.4%, respectively.

Clinical evaluation of antiarrhythmic efficacy

The antiarrhythmic efficacy of flecainide was assessed for at least 2 months through examination of symptomatology, 12-lead ECG, and Holter monitoring. At discharge, the patients were given a form and asked to record the incidence of subjective symptoms (e.g. palpitation, chest oppression, chest pain, shortness of breath, and presyncope). Reported subjective symptoms and an ECG were recorded at each patient visit. For evaluation of arrhythmias ($n = 49$), Holter monitoring was performed

Table 1 Patient characteristics

	HapA homozygotes	HapB carriers	*P*-value
Number of patients	91	54	
Female sex	20 (22.0)	11 (20.4)	0.82
Age (years)	61.2±11.3	60.4±14.0	0.91
Weight (kg)	67.2±10.7	64.1±10.8	0.096
Diagnosis			
Paroxysmal atrial fibrillation	67 (73.6)	46 (85.2)	0.42
Persistent atrial fibrillation	7 (7.7)	4 (7.4)	
Paroxysmal atrial fibrillation and atrial flutter	10 (11.0)	2 (3.7)	
Paroxysmal supraventricular tachycardia	6 (6.6)	2 (3.7)	
Wolff–Parkinson–White syndrome	1 (1.1)	0 (0)	
Comorbidities			
Hypertension	49 (53.8)	20 (37.0)	0.050
Hyperlipidemia	24 (26.4)	14 (25.9)	0.95
Diabetes mellitus	17 (18.7)	8 (14.8)	0.55
Chronic kidney disease	18 (19.8)	10 (18.5)	0.85
Concomitant antiarrhythmic drugs			
Digoxin	7 (7.7)	8 (14.8)	0.17
β-Blockers	51 (56.0)	26 (48.1)	0.36
Ca^{2+} antagonists	17 (18.7)	8 (14.8)	0.55
CYP2D6 allele frequency			
EM allele	60.4	57.4	0.88
IM allele	36.2	38.9	
PM allele	3.3	3.7	

Data are presented as number (%), mean±SD, or %. *CYP2D6*1* and **2* alleles, extensive metabolizer (EM) alleles; **10*, an intermediate metabolizer (IM) allele; **4*, **5*, **14*, **21*, and **36*, poor metabolizer (PM) alleles.
HapA, haplotype A.

for 24 h. Presence of arrhythmias was defined as an occurrence of symptoms suggestive of tachycardia and an episode of arrhythmias documented on an ECG or by Holter monitoring during the assessment period.

Genotyping of *SCN5A* promoter polymorphism and *CYP2D6* polymorphism

Genomic DNA was isolated from peripheral blood using an extraction kit (Takara Bio, Shiga, Japan).

Genotyping for *SCN5A* T-1418C and T-1062C was performed by restriction fragment length polymorphism analysis, as described previously [11]. The corresponding *SCN5A* gene fragments were amplified using the primers 5′-ACC TAA GGC GTC CAA CGA AGC-3′ (forward) and 5′-CCA GGG TCT CAG AGG GCA CAG-3′ (reverse) for T-1418C and 5′-CCC TGA TGG CCT GTT TTG TTT-3′ (forward) and 5′-ACT CAG AGA CAT GGT CAC AGG CA-3′ (reverse) for T-1062C. The PCR amplification conditions were as follows: 94°C for 4 min (initial denaturation); followed by 30 cycles of 94°C for 30 s (denaturation), 59°C for 30 s (annealing), and 72°C for 30 s (extension); and 72°C for 2 min (final extension). DNA fragments generated after restriction enzyme (*Ear*I for T-1418C and *Hae*III for T-1062C) digestion were separated on a 4% agarose gel. These SNPs were used to assign *SCN5A* promoter haplotypes: A (−1418T and −1062T), B (−1418C and −1062C), and C (−1418C and −1062T).

Genotyping for the *SCN5A* H558R polymorphism (1673A > G) was performed using restriction fragment length polymorphism analysis, as described previously, with minor modifications [6]. The corresponding *SCN5A* gene fragment was amplified using the primers 5′-GAG ACC TGG GTT CTG AAG CA-3′ (forward) and 5′-TCA GTT TGG GAG ACC AGA CC-3′ (reverse). The PCR amplification conditions were as follows: 94°C for 4 min (initial denaturation); followed by 30 cycles of 94°C for 30 s (denaturation), 59°C for 30 s (annealing), and 72°C for 30 s (extension); and 72°C for 2 min (final extension). DNA fragments generated after restriction enzyme (*Aci*I) digestion were separated on 4% agarose gel.

*CYP2D6*1*, **2* (C2850T), **4* (G1846A), **5* (*CYP2D6* gene deletion), **10* (C100T), **14* (G1758A), **21* (2573 C insertion), **36* (*CYP2D6* gene conversion to *CYP2D7P* in exon 9), and *CYP2D6xN* (*CYP2D6* gene duplication) were determined using allele-specific PCR with mismatch primers (ASPCR-MP) and step-down PCR [25]. SNP typing kits for cytochrome P450 (STK-121, 122, 124, 125, 126 for ASPCR-MP; STK-123, 127, 128 for step-down PCR) were obtained from Toyobo (Tokyo, Japan). We designated *CYP2D6*1* and **2* alleles as extensive metabolizer (EM) alleles, **10* as an intermediate metabolizer (IM) allele, and **4*, **5*, **14*, **21*, and **36* as poor metabolizer (PM) alleles.

Statistical analyses

Patient characteristics and pharmacokinetic and ECG data were compared between the HapA homozygotes and HapB carriers using Student's *t*-test, Welch's *t*-test, or the Mann–Whitney *U*-test. Comparison of proportions was performed using the χ^2-test, Fisher's exact probability test, or the Mann–Whitney *U*-test. Observed haplotype pair frequencies were compared with those expected under the Hardy–Weinberg equilibrium. *P* values less than 0.05 were considered statistically significant.

Results

SCN5A promoter polymorphisms and patient characteristics

SCN5A promoter polymorphisms were examined in 146 patients treated with oral flecainide for supraventricular tachyarrhythmias. The frequencies of *SCN5A* promoter haplotypes A, B, and C were 0.788, 0.205, and 0.007, respectively. The overall frequency of haplotypes genotyped in this study was comparable to that reported for the Japanese population [11]. The number of patients with each haplotype pair were as follows: 91 with AA, 47 with AB, six with BB, one with BC, and one with AC. The frequencies of the haplotype pair were not different from those obtained from Hardy–Weinberg equilibrium ($P = 0.78$).

The 91 *SCN5A* promoter HapA homozygotes and 54 HapB carriers were included in the comparative analysis. The lone patient with haplotype pair AC was excluded from the analysis. Patient characteristics for each haplotype group are summarized in Table 1. Sex, age, body weight, diagnosis of supraventricular tachyarrhythmias, and comorbidities did not significantly differ between the HapA homozygotes and HapB carriers (Table 1). The frequency of the R558 polymorphism, a major *SCN5A* coding region polymorphism, did not differ significantly between the HapA homozygotes and HapB carriers (7.7 vs. 12.0%; $P = 0.21$). The frequencies of *CYP2D6* polymorphisms did not differ between the HapA homozygotes and HapB carriers (Table 1). The flecainide daily dose did not differ between the *CYP2D6* EM allele homozygotes and the IM/PM allele carriers in both HapA homozygotes (2.3±0.8 vs. 2.4±0.8 mg/kg; $P = 0.49$) and HapB carriers (2.4±0.8 vs. 2.4±0.8 mg/kg; $P = 0.77$).

SCN5A promoter haplotype and antiarrhythmic efficacy of flecainide

The flecainide daily dose and serum flecainide concentration did not differ significantly between the HapA homozygotes and HapB carriers (Table 2). Tachyarrhythmias were well controlled in 63.0% of HapB carriers and 51.6% of HapA homozygotes; this difference was not significant ($P = 0.18$; Table 2). The influence of the *SCN5A* promoter haplotype on the antiarrhythmic efficacy of flecainide was compared between the patients with serum flecainide concentrations less than 300 ng/ml

Table 2 Effect of the *SCN5A* promoter haplotype on the antiarrhythmic efficacy of flecainide

	HapA homozygotes	HapB carriers	*P*-value
Number of patients	91	54	
Flecainide daily dose (mg/kg)	2.34±0.78	2.39±0.83	0.81
Serum flecainide concentration (ng/ml)	300±154	309±161	0.74
Efficacy of flecainide (effective/ineffective)			
Overall	47/44 (51.6%)	34/20 (63.0%)	0.18
Serum flecainide concentration of <300 ng/ml	21/48 (42.9%)	22/10 (68.8%)	0.022
Serum flecainide concentration of ≥ 300 ng/ml	26/16 (61.9%)	12/10 (54.5%)	0.57

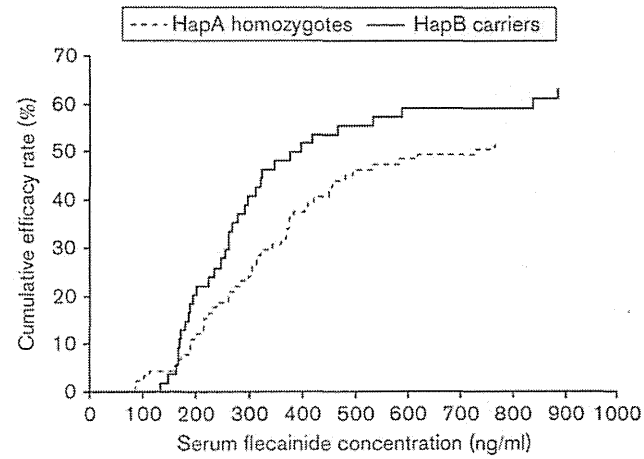
Data are presented as number, mean±SD, or number (efficacy rate).
HapA, haplotype A.

and those who had concentrations of at least 300 ng/ml, according to previous reports [21–23]. The HapB carriers achieved clinically relevant flecainide efficacy more frequently (68.8%) when the serum flecainide concentrations were less than 300 ng/ml, compared with the HapA homozygotes (42.9%; $P = 0.022$; Table 2). The efficacy of flecainide at serum concentrations of at least 300 ng/ml did not differ significantly between the two groups (54.5 vs. 61.9%; $P = 0.57$; Table 2).

The serum concentration–cumulative efficacy rate curves for prevention of supraventricular arrhythmias in the HapA homozygotes and HapB carriers are shown in Fig. 1. The concentration–efficacy curve for HapB carriers had a sharper increase at serum flecainide concentrations less than 300 ng/ml than that for HapA homozygotes. The concentration–efficacy curve was shifted to the left in the case of HapB carriers compared with HapA homozygotes; the cumulative efficacy rate (24%) was achieved at 300 ng/ml for HapA homozygotes and at around 200 ng/ml for HapB carriers (Fig. 1).

ECG data for the sinus rhythm and the absence of bundle branch block under treatment with flecainide were compared between the HapA homozygotes ($n = 59$) and HapB carriers ($n = 39$) (Table 3). No significant difference was observed in the heart rate, PR interval, QRS duration, and QTc interval between the HapA homozygotes and HapB carriers at serum flecainide concentrations less than 300 ng/ml (Table 3). In contrast, the PR interval and QRS duration were significantly prolonged in the HapB carriers compared with that in the HapA homozygotes at serum flecainide concentrations of at least 300 ng/ml ($P = 0.036$ and 0.030, respectively, Table 3); however, the heart rate and QTc interval did not differ between the HapA homozygotes and HapB carriers.

Fig. 1



Serum concentration–cumulative efficacy rate curves for the prevention of supraventricular arrhythmias in HapA homozygotes and HapB carriers. The cumulative efficacy rate was calculated as the percentage of patients with a positive effect accumulated up to each serum flecainide concentration in each patient group. HapA, haplotype A.

Discussion

The present study revealed that an Asian-specific *SCN5A* promoter haplotype influences the antiarrhythmic efficacy of daily doses of flecainide. HapB carriers achieved clinically relevant flecainide efficacy even at drug concentrations less than 300 ng/ml (Table 2 and Fig. 1). This finding suggests that the therapeutic range for serum flecainide concentration was lower for the HapB carriers because of their low expression of SCN5A. Thus, a *SCN5A* promoter haplotype is an important factor for explaining the difference in flecainide efficacy at lower serum drug concentrations in Asian patients.

We previously reported that the serum flecainide concentration should be maintained at at least 300 ng/ml to control the paroxysms of supraventricular tachyarrhythmias [21–23]. The recommended serum flecainide concentration in HapB carriers, however, is likely to be at least 200 ng/ml, because the cumulative efficacy rate at around 200 ng/ml was similar to that at 300 ng/ml in the HapA homozygotes (Fig. 1). In contrast, ECG data associated with the risk of cardiovascular side effects [20] showed an excess slow down of cardiac conduction in the HapB carriers with serum flecainide concentrations of at least 300 ng/ml; the PR interval (210±25 ms) and QRS duration (112±10 ms) were significantly longer than those in the HapA homozygotes (Table 3). These values were also remarkable when compared with the data from postmarketing surveillance of flecainide acetate among Japanese patients with atrial fibrillation/flutter (PR interval: 183±34 ms and QRS

Table 3 Effects of the *SCN5A* promoter haplotype on the ECG data for sinus rhythm while under treatment with flecainide

	HapA homozygotes	HapB carriers	*P*-value
Number of patients with ECG data for sinus rhythm	59	39	
Serum flecainide concentration of <300 ng/ml	27	23	
Heart rate (beats/min)	61±8	63±10	0.47
PR interval (ms)	178±20	181±25	0.66
QRS duration (ms)	101±8	102±8	0.63
QTc	422±29	419±27	0.90
Serum flecainide concentration of ≥ 300 ng/ml	32	16	
Heart rate (beats/min)	60±10	62±10	0.57
PR interval (ms)	195±25	210±25	0.036
QRS duration (ms)	105±9	112±10	0.030
QTc	437±23	434±24	0.69

Data are presented as number or mean±SD.
HapA, haplotype A; HapB, haplotype B.

duration: 102±20 ms) [26]. The upper limit of the therapeutic range for serum flecainide concentration may also be lower in HapB carriers because of the excess slow down in the cardiac conduction.

Many factors can influence the antiarrhythmic effects of sodium channel blockers. The variability in SCN5A function due to polymorphisms in the *SCN5A* coding region may be a genetic factor explaining the inter-individual difference in antiarrhythmic efficacy. A common polymorphism in the *SCN5A* coding region such as H558R, a risk factor for lone atrial fibrillation, may contribute to the drug response observed with sodium channel blockers [6,7]. However, in the present study, the frequency of the R558 polymorphism is similar between the HapA homozygotes and HapB carriers. The other genetic factor may be the variant in MOG1, a cofactor of the cardiac sodium channel, which regulates the channel expression on the surface of the cardiomyocytes and reduces the sodium current [27,28]. Although other factors such as concomitant antiarrhythmic drugs can influence the prevention of supraventricular tachyarrhythmias, coadministration of digoxin, β-blockers, and Ca^{2+} antagonists did not differ between the two groups in the present study (Table 1).

Our study has several limitations as regards the assessment of clinical efficacy, as well as a gene–dose effect in ECG parameters. First, asymptomatic atrial fibrillation could not be detected in every patient because Holter monitoring was available for only one-third of the present patients. Second, a clear gene–dose effect in the PR interval and QRS duration was not found in the present study because the number of HapB homozygotes was insufficient.

In Japan, the flecainide acetate package insert recommends an initial dose of 100 mg/day. This recommendation may be reasonable because one-third of Japanese patients are HapB carriers in whom clinically relevant flecainide efficacy is obtained at lower serum concentrations. Another Asian-specific genetic factor affecting flecainide efficacy is *CYP2D6*10*, a polymorphism in the gene encoding the main metabolic enzyme for flecainide. As about 60% of Japanese patients were reported to be *CYP2D6*10* carriers [29], this variant and the *SCN5A* promoter HapB may result in a lower flecainide dose requirement in this population [30,31].

Conclusion

HapB carriers more frequently achieve clinically relevant flecainide efficacy even at lower concentrations. The present study suggests that the therapeutic range for serum flecainide concentrations in HapB carriers is likely to be lower than that for HapA homozygotes.

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Conflicts of interest

There are no conflicts of interest.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Novel *SCN3B* Mutation Associated With Brugada Syndrome Affects Intracellular Trafficking and Function of Nav1.5

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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 syncope 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

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 syncope, 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.[3–12] 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]

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In cardiomyocytes, 5 distinct sodium channel β-subunits, Navβ1, Navβ1b, β2, β3 and β4, are known to be expressed. In particular, Navβ1 and Navβ3, encoded by *SCN1B* and *SCN3B*, are abundantly expressed, and these auxiliary β-subunits and a pore-forming subunit, Nav1.5, comprise the cardiac sodium channel complex.[4,16] Inward sodium current (I_{Na}) generated by the sodium channel complex is crucial for the cardiac action potential,[16,17] and functional alterations of I_{Na} caused by gene mutations have been reported in a wide range of arrhythmias, including long QT syndrome,[18] idiopathic ventricular fibrillation (IVF),[19] sudden infant death syndrome (SIDS),[20] and atrial fibrillation (AF).[21] In BrS patients, disease-causing mutations were found not only in *SCN5A* but also in the genes

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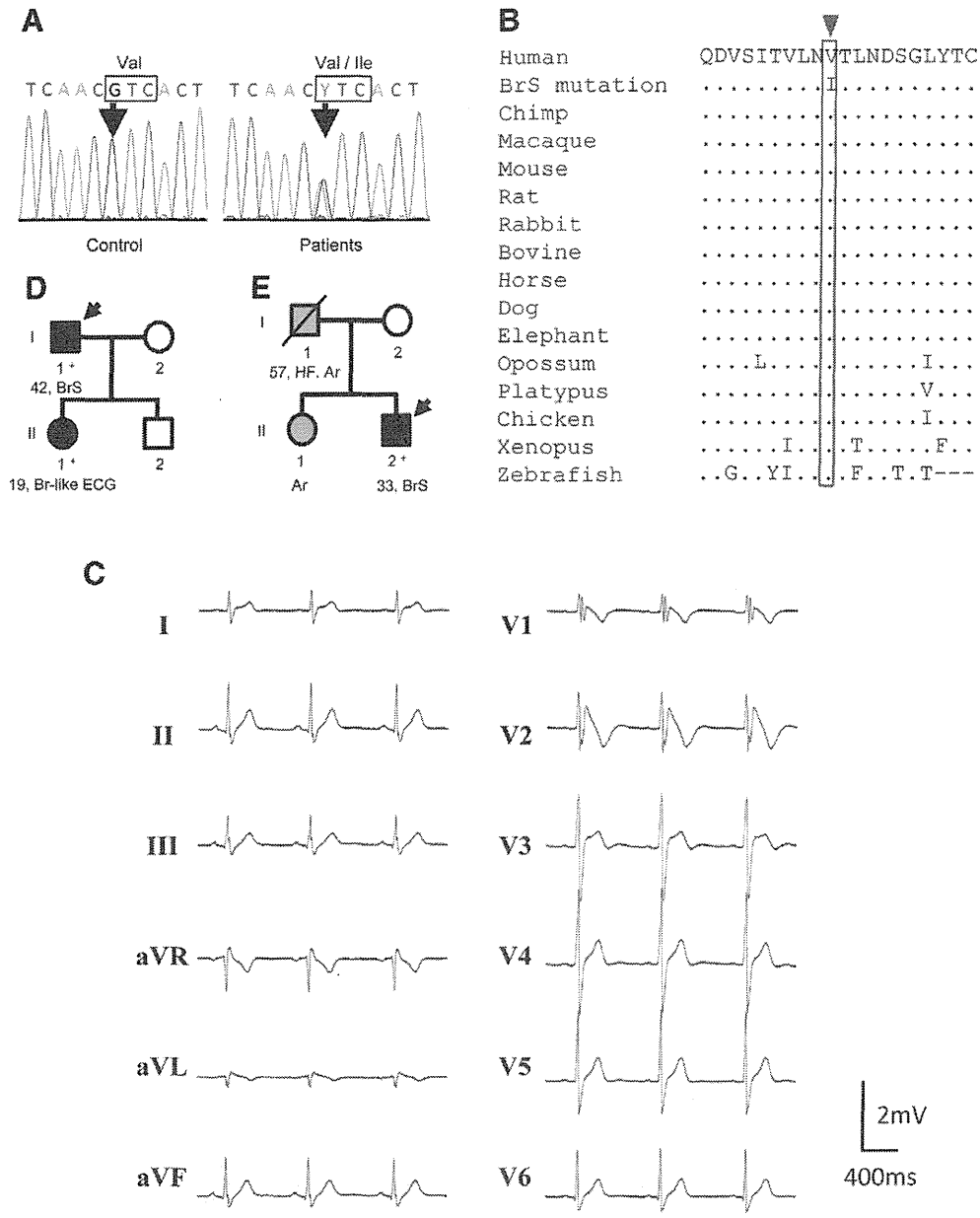

Figure 1. Mutational analysis of *SCN3B* in Brugada syndrome (BrS). (**A**) Direct sequencing analysis of exon 3 in a control (**Left**) and a patient, BrS-H-1 (**Right**), shows that the patient was heterozygous for GTC and ATC (Ile) at codon 110, where the control was homozygous for GTC (Val). The 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.

encoding modifier proteins of Nav1.5, which causes functional loss of I_{Na}.[8,12,22–25] Among the modifier proteins, Navβ3, which does not form the ion-conducting pore, modifies the function of Nav1.5 by modulating channel gating and increasing the cell surface expression of Nav1.5,[26] and hence *SCN3B* mutations could be responsible for BrS. Nevertheless, there is only 1 report of a *SCN3B* mutation, Leu10Pro, in an American patient with BrS,[23] although some other *SCN3B* mutations have been reported in other hereditary arrhythmias, including IVF,[27] SIDS,[28] and AF.[29,30]

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 the I_{Na}.