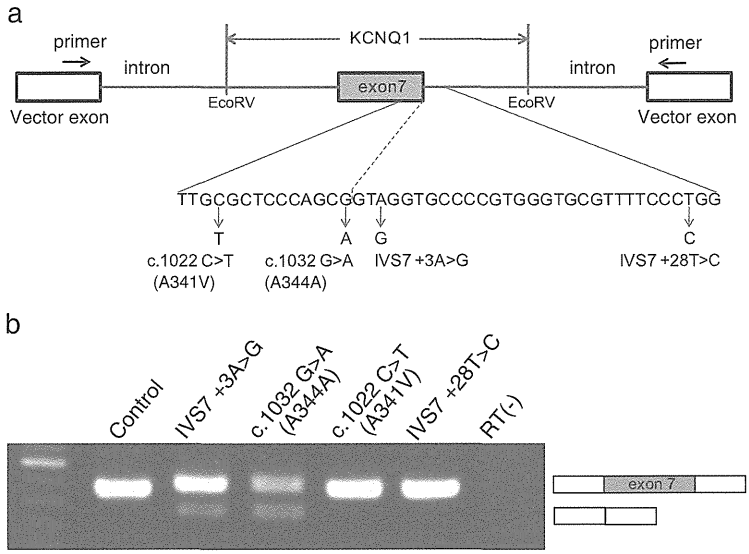

Fig. 3. Minigene analysis. (a) The structure of the minigene harboring KCNQ1 exon 7 (dark shaded box) and its flanking introns (lines between EcoRV sites) inserted into the pSPL3 vector. Open boxes and its flanking lines indicate pSLP3 vector exons and introns, respectively. Arrows upon the open boxes indicate the forward and reverse primers for RT-PCR. The locations of mutations studied in the assay are indicated. (b) RT-PCR from COS7 cells transfected with the minigene constructs. Two major bands were identified; one with KCNQ1 exon joined to vector exons, the other with vector exons only.

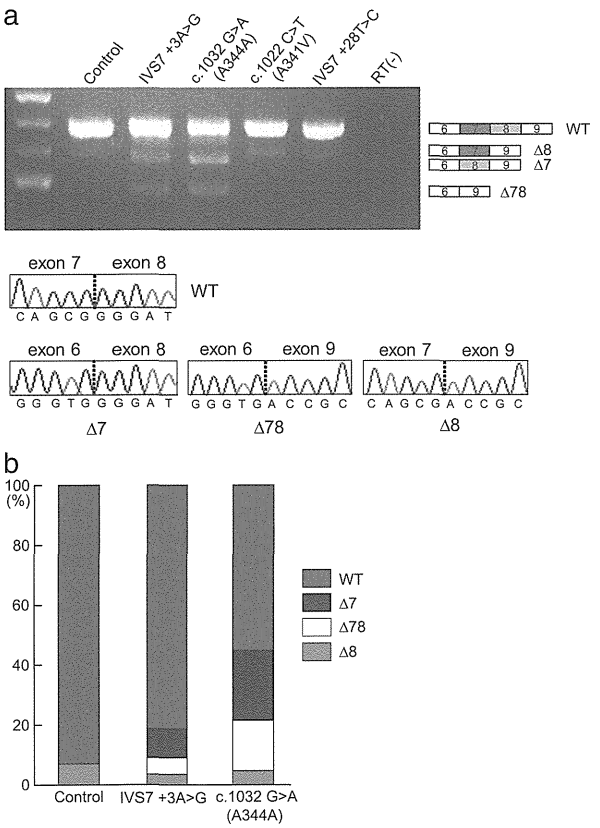

Fig. 4. RT-PCR analysis of patients' blood samples. (a) The result of RT-PCR with total RNA samples extracted from patients' lymphocytes. PCR was performed using primers spanning exons 5 though 10. Nucleotide sequence of each of the exon-skipping mRNAs is also shown. (b) The amounts of WT and mutant mRNAs expressed as a percentage of the total KCNQ1 mRNA. Controls: 4 normal healthy individuals, IVS7 +3A>G: the proband, c.1032G>A: 3 mutation carriers.

Fig. 6b presents regional action potentials of endocardial, mid-myocardial, and epicardial tissues of the 1D model during pacing at 1 Hz in the cases of control (black line), I_{Ks} 60% as a model of IVS7 +3A>G carrier (dark gray line), and I_{Ks} 60% with β-adrenergic stimulation as a model of IVS7 +3A>G carrier under exercise stress (light gray line). Simulated ECGs in the above 3 cases are also shown in the bottom. In the model of IVS7 +3A>G carrier, the β-adrenergic stimulation markedly prolonged the QTc (668 ms) while the I_{Ks} 60% alone did not (388 ms vs. 366 ms in control case).

Similar to Fig. 6b, c presents regional action potentials and simulated ECGs in the cases of control (black line), I_{Ks} 40% as a model of c.1032G>A carrier (dark gray line), and I_{Ks} 40% with β-adrenergic stimulation as a model of c.1032G>A carrier under exercise stress (light gray line). In the model of c.1032G>A carrier, the β-adrenergic stimulation markedly prolonged the QTc (741 ms) while the I_{Ks} 40% alone did not (405 ms).

Fig. 6d and e show regional action potentials and simulated ECGs for a longer period (21–40 s after the first pacing stimulus) in the cases of I_{Ks} 60% (IVS7 +3A>G carrier) with β-adrenergic stimulation and I_{Ks} 40% (c.1032G>A carrier) with β-adrenergic stimulation, respectively. Intriguingly, no arrhythmia was induced for IVS7 +3A>G carrier model (Fig. 6d) whereas tachyarrhythmia was induced for c.1032G>A carrier model (Fig. 6e). In the latter, the monomorphic ventricular tachycardia (VT) was initially derived from triggered activities due to delayed afterdepolarization (asterisks) in the epicardial region. The VT soon degenerated into the fibrillation-like activities (VF) because of marked long APD in the mid-myocardial region, causing decremental conduction (†, endocardial to mid-myocardial regions) followed by propagated graded response (mid-myocardial to endocardial regions) and phase-2 reentry (‡, epicardial activation originating from action potential plateau in the mid-myocardial region).

4. Discussion

4.1. Identification of the novel splicing mutation using minigene assay

A significant fraction of disease-causing mutations affect pre-mRNA splicing. In the present study, three potential splice mutations as well as one definite splice mutation (c.1032G>A) in the intron 7 5′ splice-site

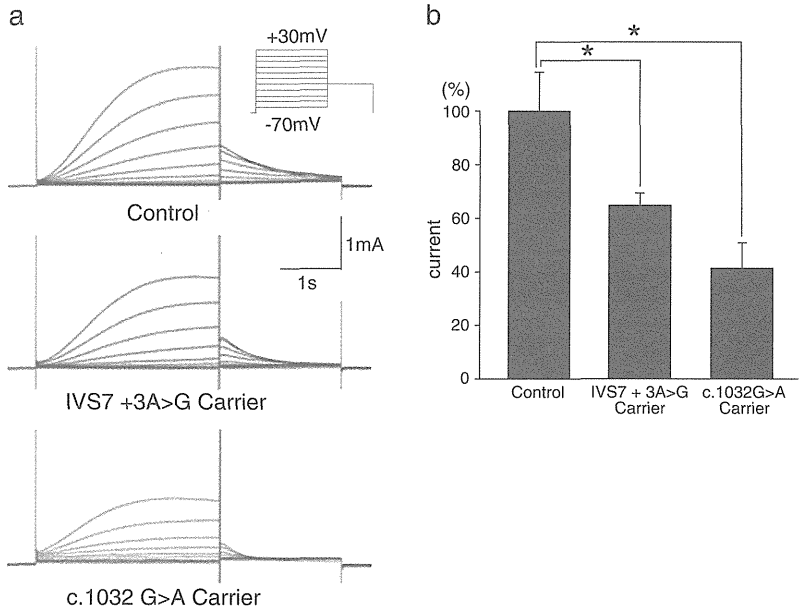

Fig. 5. Electrophysiological analysis. (a) Representative current traces recorded from two-electrode voltage-clamp of *Xenopus* oocytes simulating the proportions of mRNA of control individuals and mutation carriers. Currents were recorded at various membrane potentials from −70 to +30 mV for 3 s in 10 mV increments from a holding potential of −80 mV. A total of 10 ng of cRNA was injected with the relative ratios of WT and mutant KCNQ1 inferred from the data obtained in the real-time RT-PCR experiment. All the current recordings in the present study were performed in the presence of KCNE1 β-subunits (1 ng). (b) Pooled data of currents. Current amplitudes were measured at 1.8 s after the initiation of 3-s pulse applied to a +30 mV test potential. Background I_{Ks} current (22.9 nA) was subtracted. n = 7 for control, 8 for IVS7 +3A>G and 7 for c.1032G>A. *p<0.01 vs. control.

of KCNQ1 identified in clinically-diagnosed LQTS patients were studied. First, we assessed the effects exerted by these mutations on splicing of the KCNQ1 transcript using a hybrid minigene in transient transfection experiments, and found that c.1032G>A and IVS7 +3A>G resulted in the skipping of KCNQ1 exon 7 (Fig. 3). In good agreement with the minigene assay results, we confirmed the presence of exon-skipping transcripts in the blood samples of the mutation carriers (Fig. 4a). The minigene assay is a useful tool to screen for potential splicing mutations in clinically-diagnosed LQTS patients with no mutation in the KCNQ1 coding sequence.

4.2. Mechanistic basis of splicing abnormality

Splice sites are conserved sequences at both ends of an intron that are recognized during the initial steps of splicing [24]. Mutations at 5′ splice-site are frequent among mutations that cause human diseases [25,26]. The human 5′ splice-site consensus sequence is MAG/GTRAGT (M is A or C; R is A or G), spanning from position −3 to position +6 relative to the exon–intron junction. A (59%) and G (35%) are conserved at position +3 [27], but it has been shown that 5′ splice-site with disease-causing +3A>G mutations are frequently associated with non-consensus nucleotides at positions +4 and +5 [28]. Indeed, the KCNQ1 intron7 5′ splice-site sequence, GCG/GTAGGT, has non-consensus G at position +4, which presumably facilitates the skipping of exon under +3A>G mutation. These dependencies between +3 (A/G) and +4/+5 were demonstrated by other investigators'in in vitro experiment; the splicing defect in the +3A>G mutant was successfully fixed by converting either +4 or +5 independently to the consensus [28]. In contrast, G at position-1 is more strictly conserved, and mutations at this position, as c.1032G>A, cause robust splicing defects [28]. Hence, these differences in the strength of 5′ splice-site sequence dictate the extent to which splicing is disrupted, as indicated by the greater amount of exon-skipped transcripts in c.1032G>A compared with IVS7 +3A>G (Fig. 4b).

4.3. Significance of quantitative assessment of splicing abnormality in risk stratification

The family with IVS7 +3A>G splicing mutation showed mild LQTS phenotype; asymptomatic and had no history of sudden cardiac death, despite an exercise-induced QT prolongation (Fig. 1). Meanwhile, c.1032G>A mutation, a similar KCNQ1 splicing mutation, was more malignant; 8 families out of 9 were symptomatic with episodes of syncope (mostly during exercise or swimming) or sudden death, as we previously reported [10]. These observations strongly suggest the possible correlation between genotypes and clinical phenotypes in LQTS caused by aberrant splicing of KCNQ1. This profound suppression in I_{Ks} currents may underlie the pathophysiology of these patients. Moreover, the level of aberrant proteins may parallel the clinical severity.

We previously showed that the exon-skipping mutant channel subunits (Δ7, Δ7-8, or Δ8) are non-functional, and they have mutant-specific degree of dominant-negative effect on WT channels, by trapping WT intracellularly and thereby interfering its translocation to the plasma membrane [10]. On the assumption that as the result of splicing error the reduction in potassium current would occur in the mutant carriers with similar degrees evaluated by the real-time RT-PCR (Fig. 4b), we estimated functional consequences of these splicing mutation (IVS7 +3A>G and c.1032G>A). Ratios simulating the proportions of various transcripts of KCNQ1 in affected individuals resulted in a pronounced reduction in the whole-cell potassium current in *Xenopus* oocytes, compared with ratios simulating those in normal individuals; 64.8% for IVS7 +3A>G, and 41.4% for c.1032G>A (Fig. 5b).

The computer simulation study (Fig. 6) incorporating these quantitative results demonstrated the pronounced QT prolongation under beta-stimulation in both IVS7 +3A>G and c.1032G>A, and the occurrence of tachyarrhythmias only in c.1032G>A. Our computer model was human ventricular model [15] and the parameters for beta-stimulation were based on physiological data reported

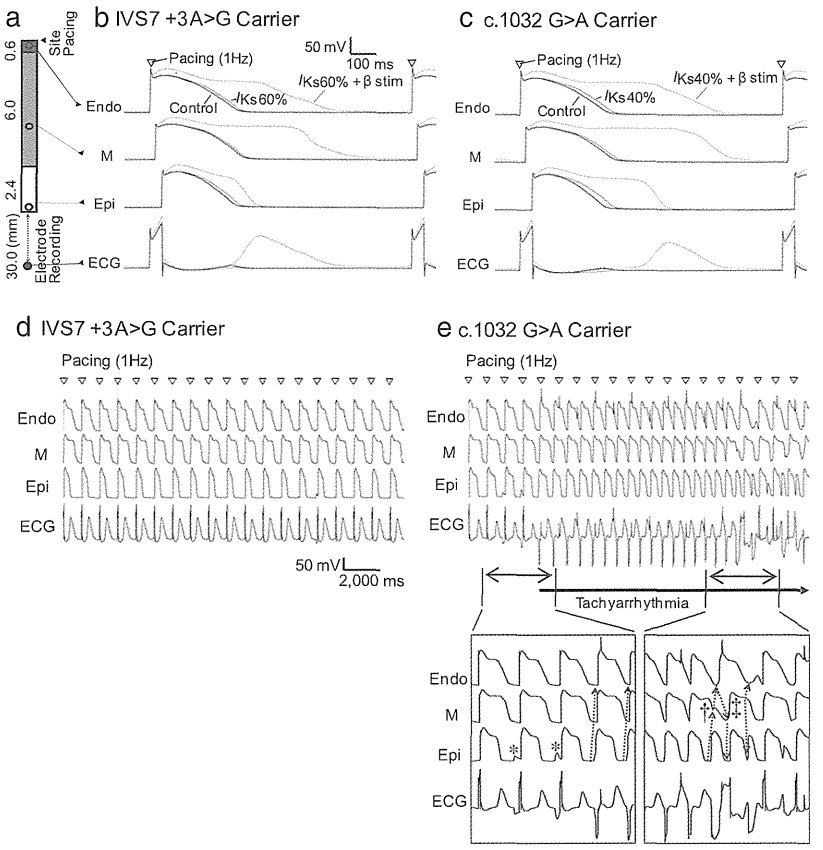

Fig. 6. Computer simulation. (a) One-dimensional myocardial simulated tissue, representing the electrical behaviors of left ventricular free wall. Endo, endocardium; M, mid-myocardium; and Epi, epicardium. Open circles indicate recording sites for regional action potentials, whereas the filled circle represents a unipolar recording electrode for ECG. (b and c) Regional action potentials and simulated ECG during 1 Hz pacing for IVS7 +3A>G carrier and c.1032G>A carrier, respectively. Open triangles denote timings of 1 Hz pacing. (d and e) No arrhythmia was induced for IVS7 +3A>G carrier, whereas tachyarrhythmia was induced for c.1032G>A carrier. See text for details.

previously [20–22]. These observations appear to parallel with clinical phenotypes of the mutation carriers; syncopal episodes mainly during exercise in c.1032G>A but not IVS7 +3A>G patients, whereas asymptomatic IVS7 +3A>G carriers. Thus, the quantitative assessments of splicing abnormality could be useful in the risk stratification of LQTS patients associated with KCNQ1 splicing mutations.

4.4. Study limitations

Compared to the clinical data on c.1032G>A, those on IVS7 +3A>G were gathered from two patients, because we failed to conduct the genetic test and clinical tests in their remaining relatives. Because of a relative mildness of their phenotypes, carriers of IVS7 +3A>G mutation may be less frequently recommended for the genetic test, and this may partially explain why the number of identified IVS7 +3A>G family was small.

4.5. Conclusions

A novel KCNQ1 splicing mutation IVS7 +3A>G generates exon-skipping mRNAs, and manifests a mild phenotype of LQTS. The amount of these mRNAs and its functional consequences may determine the clinical severity of the disease.

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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 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

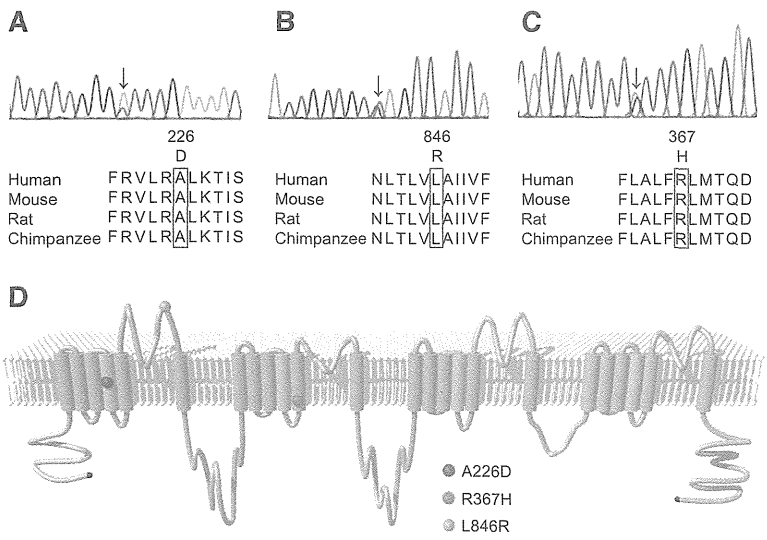

Figure 1. Mutations in *SCN5A* identified in patients with idiopathic ventricular fibrillation associated with early repolarization. **A**, The c.677C→A mutation in *SCN5A* resulting in p.A226D found in patient 1. **B**, The c.2537T→G mutation in *SCN5A*, resulting in p.L846R found in patient 2. **C**, 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

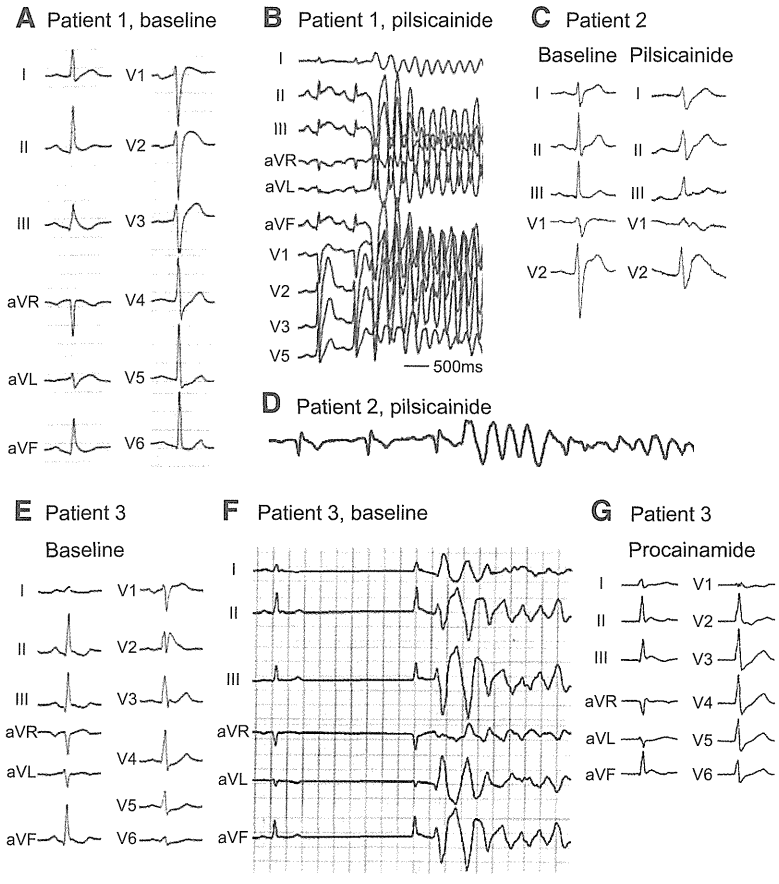

Figure 2. Electrocardiograms of patients with idiopathic ventricular fibrillation and a mutation in *SCN5A*. **A**, Early repolarization was present in the inferior and right precordial leads in patient 1. **B**, After administration of pilsicainide, early repolarization was augmented and ventricular 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]

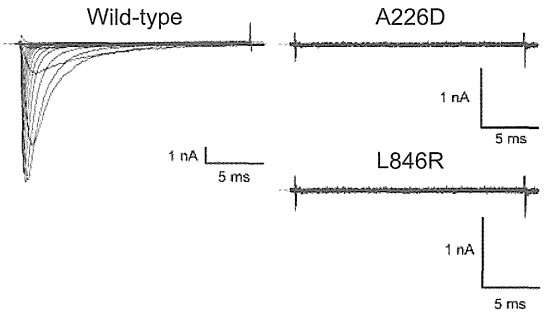

Figure 3. Electrophysiological characteristics of the *SCN5A* mutants. Representative traces of sodium current demonstrating that all of the mutant channels failed to generate any currents. We previously reported that R367H mutant fails to generate any currents.[27]

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.

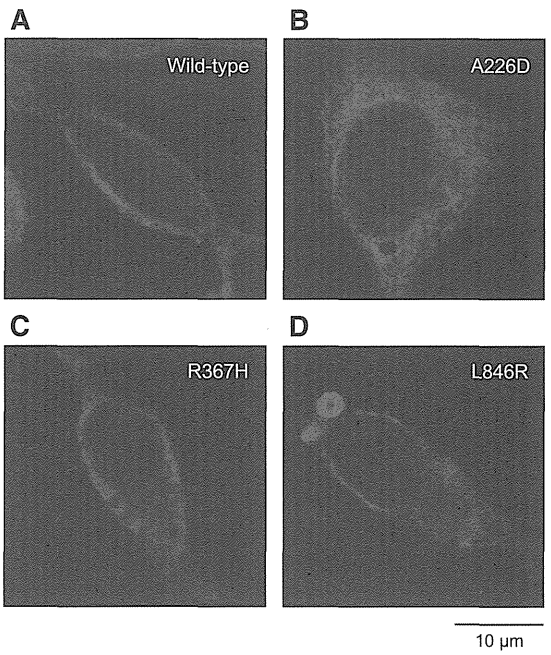

Figure 4. Representative confocal microscopy images. **A**, Cells expressing wild-type SCN5A channels showed marked peripheral fluorescence. **B**, Cells expressing A226D channels showed cytoplasmic fluorescence. **C** and **D**, Cells expressing R367H channels and those expressing L846R channels showed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

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 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.

Original Article

Carvedilol, a Non-Selective β-with α_1-Blocker is Effective in Long QT Syndrome Type 2

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Background: β-blockers offer the first line therapy in congenital long QT syndrome (LQTS), and are more effective to prevent the cardiac event in LQTS type 1 than in type 2 or 3. In contrast, left cardiac sympathetic denervation (LCSD) was shown to be highly effective in patients refractory to β-blockers. Total sympathetic ablation by LCSD indicates the addititional involvement of α-adrenoceptor-mediated pathway. In genotyped LQT2 patients, we therefore hypothesized that blockade of α-adrenoceptor in addition to β-adrenoceptor by carvedilol could reduce cardiac events more efficiently than other types of β-blockers.

Methods and Results: The study population consisted of 51 genotyped LQT2 patients (18 males, 23 ± 11 years old). They were divided into 2 groups (group 1: 43 patients treated with selective β-blockers, group 2: 8 patients with carvedilol) and retrospectively analyzed the efficacy of the respective β-blocker therapy in suppressing cardiac events. Cardiac events were observed in 11 patients of group 1 (26%) but none in group 2 during a follow-up period of 83 ± 80 months (P = 0.098).

Conclusions: Carvedilol may be a potentially beneficial therapy for genotyped LQT2 patients who are refractory to other β selective blockers.
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Key words: Long QT syndrome, β-blocker therapy, carvedilol, α-adrenoceptor

Introduction

Long QT syndromes (LQTS) are heterogeneous inherited ion channelopathies characterized by prolonged ventricular repolarization, syncope, ventricular arrhythmias, and sudden cardiac death with normal cardiac structure.[1)] Sympathetic activation and arrhythmogenesis are uniquely associated with

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LQTS. For example, in LQTS type 1 (LQT1), exercise produces the QT prolongation and subsequent arrhythmic episodes.[2,3] β-blockers are therefore routinely prescribed in symptomatic LQT1 patients and reported to prevent first cardiac events in 74–80% of patients.[4,5] In contrast, in LQT2, cardiac symptoms mainly develop at rest, while sleeping or by auditory stimuli,[6] and the efficacy of β-blockers is limited compared to that in LQT1. In a recent report[7] with large cohort of LQT2, β-blocker use reduced the risk of first cardiac events by 63%, however it was associated with less protection (29%) in the prevention of lethal cardiac events.

As to the additional therapy for patients refractory to β-blockers, left cardiac sympathetic denervation (LCSD) has been shown to be highly effective.[8,9] Total sympathetic ablation by LCSD indicates the additional involvement of α-adrenoceptor-mediated pathway. Labetalol, an α_1-and non selective β-blocker, was reported to be effective to suppress cardiac events in LQTS, although patients in this study were not genotyped.[10] As an anecdotal case, we experienced that a LQT2 patient (S871fs+31X) whose repetitive syncope due to TdP was not suppressed by propranolol (30 mg per day), but subsequent carvedilol (10 mg per day), an α_1-and non selective β-blocker, was fully effective.

Indeed, the difference in response to β-blockers may result from the distinct sympathetic response of I_{Ks} currents (encoded by *KCNQ1* gene that underlines LQT1[11,12]) and I_{Kr} currents (encoded by *KCNH2* gene that underlines LQT2[13]), which are both critically responsible for ventricular repolarization. We recently demonstrated both in CHO cells and HL-1 cardiomyocytes that Kv 11.1 channels encoded by *KCNH2* are acutely downregulated by α_1-adrenergic stimulation.[14] In the presence of reduced function with Kv11.1 mutants (Y43D and K595E), additional α_1-adrenergic stimulation led to a further decrease in residual channel currents and thereby producing an extreme delay in repolarization. Thus, α_1-adrenoceptor blockade might serve as a promising medication and improve the symptoms in LQT2 patients. We therefore retrospectively surveyed the efficacy of carvedilol for suppressing cardiac events compared to other β-selective blockers in genotyped LQT2 patients.

Methods

Study population (Figure 1) and genetic analysis

The study cohort consisted of 82 LQT2 probands and their 51 family members, who were referred as inherited cardiac arrhythmia subjects from 36 institutes in Japan, and genotyped from June 1996 to December 2009 in Shiga University of Medical Science (Otsu) or Kyoto University Graduate School of Medicine (Kyoto). The patients with LQT1, 3, 5, 6, 7 or compound mutations[15] were excluded. In the total of 133 LQT2 patients, the β-blocker therapy was introduced in 51 patients, which consisted of our final study population (**Figure 1**).

DNA sequence analyses of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ2* were performed as described previously.[16,17] Mutation screening was performed using polymerase chain reaction (PCR) or denatured high-performance liquid chromatography analyses (dHPLC, WAVE system; Transgenomic Inc., Omaha, NE, USA).[18] For aberrant PCR products, DNA sequencing was then conducted with a DNA sequencer (ABI 3130 DNA Sequencer; Perkin Elmer, Foster City, CA, USA). When a mutation was detected, the result was

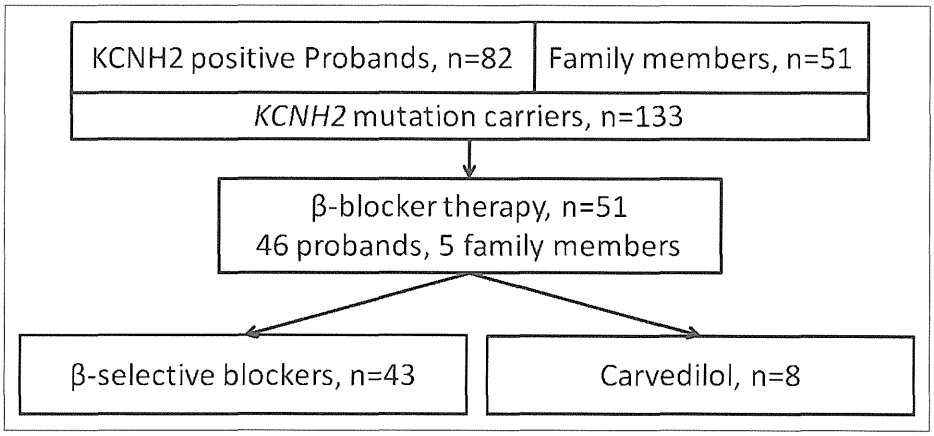

Figure 1 Schematic representation of the positive-mutation carriers in this study.

compared to >200 Japanese control subjects, and single nucleotide polymorphisms were excluded from this study.

The protocol for genetic analysis complied with the Declaration of Helsinki and was approved by the institutional ethics committees and performed under their guidelines. All individuals or their guardians gave written informed consent to genetic and clinical data analyses.

Clinical characteristics

In 51 LQT2 patients receiving the β-blocker therapy, baseline clinical characteristics were collected including age at diagnosis, age at the first cardiac event, age at β-blocker therapy started, and Schwartz score.[19] With regard to family history, we defined it as positive if a subject had a family member who had Schwartz score of ≥4. Triggers of symptom were defined as follows: 1) at rest/during sleep, 2) arousal, 3) auditory stimuli, 4) pregnancy/post delivery and 5) during exercise.

ECG parameters used for analyses were baseline heart rate (HR) and QT intervals. Measurements were performed in 3 successive sinus beats in lead II (if not possible, in lead V5) and averaged. QT was manually measured as the time interval between QRS onset (Q) and the point at which the isoelectric line intersected a tangential line drawn at the maximal down slope of the positive T wave or the upslope of the negative T wave (QT), and corrected using Bazett's formula.[20]

LQTS-related cardiac events included unexplained syncope, aborted cardiac arrest requiring cardiac resuscitation, appropriate ICD shock and unexpected sudden death exclusive of a known cause before age 45 years. Those data were compared between the group treated with β-selective blockers and the group with carvedilol.

Treatment including β-blocker therapy

The specific β-blocker used, as well as dose, was at the discretion of the treating physician. The type and dose of β-blocker used, cardiac event rate on each β-blocker, adjunctive therapy and follow-up period were collected and compared between the β-selective blockers group and the carvedilol group. If patients were treated with β-selective blockers and thereafter carvedilol due to ineffective β-blocker therapy, they were included in the carvedilol group. Follow-up period was calculated from the starting date of β-blocker to the day of a cardiac event. We excluded patients from analysis who were non-compliant to a prescribed β-blocker.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for continuous variables. Comparisons were performed by chi-square test for categorical variables and *t*-test for continuous variables. The Kaplan-Meier estimator was used to assess the time to a first event and the cumulative event rates by groups were compared using the log-rank test. $P < 0.05$ was considered statistically significant. The statistical software used for the analyses was JMP version 7.0.1 (SAS institute Inc., NC, USA).

Results

Characteristics of the study population

In our LQTS cohort, 133 patients from 82 unrelated families were identified as sole *KCNH2* mutation carriers. In those LQT2 patients, 51 patients (46 probands and 5 family members) received the β-blocker therapy. To compare the clinical characteristics, we divided the study population into 2 groups: the β-selective blockers group (43 patients, mean age at diagnosis 22 ± 11 years) and the carvedilol group (8 patients, mean age at diagnosis 26 ± 11 years) (**Figure 1**).

Table 1 shows the clinical characteristics of the two groups. Regarding triggers of symptoms, sudden arousal was more frequent in the carvedilol group ($P = 0.0313$). There was no statistical difference between the two groups including age, gender, Schwartz scores, ECG parameters and cardiac events before therapy. Distribution of mutations was not significantly different between the two groups.

Treatment

As an adjunctive therapy, β-selective group received mexiletine more frequently than the carvedilol group (10/43 β-selective group vs. 0/8 carvedilol group, $P = 0.0473$) (**Table 1**). Ten patients (9/43 in the β-selective group, 1/8 in the carvedilol group) were implanted with an implantable cardioverter defibrillator (ICD). No patients underwent LCSD.

β-Blocker therapy (Table 2)

The β-blocker therapy was started at age of 19 ± 12 (0–63) years in total, 17 ± 11 years in the β-selective group and 26 ± 12 years in the carvedilol group, respectively (**Table 1**). The mean age of carvedilol start was significantly ($P = 0.0372$) older than that of β-selective blockers. We experienced three patients in whom β-selective blockers first failed but subsequent carvedilol was successful to prevent cardiac events. Those cases were included

Table 1 Clinical characteristics

	All patients n = 51	β-selective blockers n = 43	Carvedilol n = 8	*P* value
Demographics				
Proband, n (%)	46 (90)	40 (93)	6 (75)	N.S.
Male, n (%)	18 (35)	16 (37)	2 (25)	N.S.
Age at diagnosis, mean ± SD, years	23 ± 11	22 ± 11	26 ± 12	N.S.
Age at first cardiac event, mean ± SD, years	15 ± 10	14 ± 9	18 ± 10	N.S.
Age at β-blocker started	19 ± 12	17 ± 11	26 ± 12	0.0372*
Schwartz score, mean ± SD	5.2 ± 1.7	5.2 ± 1.7	5.6 ± 1.3	N.S.
Electrocardiogram				
HR, mean ± SD, bpm	63 ± 10	61 ± 7	64 ± 11	N.S.
QTc, mean ± SD, ms	515 ± 63	513 ± 62	525 ± 65	N.S.
Location of mutation, n (%)				
N terminal	10 (17)	8 (17)	2 (25)	N.S.
C terminal	18 (35)	16 (37)	2 (25)	N.S.
Pore	11 (22)	10 (23)	1 (13)	N.S.
Trans membrane	12 (24)	9 (21)	3 (38)	N.S.
Non-missense mutation, n (%)	22 (43)	17 (40)	5 (62)	N.S.
Cardiac events before β-blocker therapy				
Syncope, presyncope, n (%)	48 (94)	40 (93)	8 (100)	N.S.
Documented TdP, n (%)	26 (51)	22 (51)	4 (50)	N.S.
Documented VF, n (%)	5 (10)	5 (12)	0 (0)	N.S.
Trigger of symptoms				
At rest, during sleep, n (%)	24 (50)	22 (55)	2 (25)	N.S.
Arousal, n (%)	10 (20)	6 (15)	4 (50)	0.0313*
Auditory stimuli, n (%)	7 (14)	6 (15)	1 (12)	N.S.
Pregnancy, post delivery, n (%)	3 (6)	2 (5)	1 (13)	N.S.
Exercise, n (%)	2 (4)	2 (5)	0 (0)	N.S.
Adjunctive LQTS therapy (any time)				
Mexiletine, n (%)	10 (20)	10 (23)	0 (0)	0.0473*
Pacemaker, n (%)	0	0	0	N.S.
LCSD, n (%)	0	0	0	N.S.
ICD, n (%)	10 (20)	9 (21)	1 (13)	N.S.
Follow-up duration after β-blocker, month ± SD	83 ± 80	84 ± 86	73 ± 43	N.S.

*$P < 0.05$ vs β-selective blocker group

TdP: Torsade de pointes, VF: ventricular fibrillation, LCSD: left cardiac sympathetic denervation, ICD: implantable cardioverter defibrillator

into the carvedilol group because they recurred shortly after β-selective blocker treatment and the period for carvedilol therapy was sufficiently long. β-selective blockers used in 43 LQT2 patients were atenolol in 7 patients (75.0 ± 61.2 mg, 25–200 mg), bisoprolol in 3 (4.2 ± 1.2 mg, 2.5–5.0 mg), carteolol in 1 (20 mg), metoprolol in 5 (68.0 ± 33.1 mg, 30–120 mg), nadolol in 1 (60 mg), and propranolol in 26 (34.0 ± 13.3 mg, 10–60 mg). Eight patients were treated with carvedilol (16.9 ± 10.3 mg, 5–40 mg). Unfortunately, we have no data of patients' body weight, and the data of mg per kg were not shown.

Cardiac events during follow-up

Mean follow-up durations without any cardiac events after drug introduction were 84 ± 86 months in the β-selective blocker group and 73 ± 43 months in the carvedilol group. Eleven patients (26%) had cardiac events while receiving β-blocker therapy, but no one (0%) experienced arrhythmic episodes in the carvedilol group (**Table 3**). Kaplan-Meier survival curve showed a tendency of superior efficacy of carvedilol ($P = 0.098$, log-rank test) (**Figure 2**). Three of 11 recurrence cases had pore mutations (27%), and four had non-missense mutations (36%),

Table 2 Dose and variety of β-blockers

	n, (%)	Dose per day, mean ± SD (range)
β-selective blockers, n = 43		
Atenolol	7 (14)	75.0 ± 61.2 (25–200) mg
Bisoprolol	3 (6)	4.2 ± 1.2 (2.5–5) mg
Carteolol	1 (2)	20 ± 0 mg
Metoprolol	5 (10)	68.0 ± 33.1 (30–120) mg
Nadolol	1 (2)	60 ± 0 mg
Propranolol	26 (51)	34.0 ± 13.3 (10–60) mg
Non-selective β- and α_1-blocker, n = 8		
Carvedilol	8 (16)	16.9 ± 10.3 (5–40) mg

Table 3 Cardiac events on β-blocker therapy

	β-selective blocker, n = 43	Carvedilol n = 8
Total of cardiac events, pt n, (%)	11 (26)	0
Syncope, n, (%)	11 (26)	0
Documented TdP, n, (%)	4 (9)	0
Aborted sudden cardiac arrest, n, (%)	1 (2)	0
Appropriate ICD shock, n, (%)	2 (5)	0

TdP: Torsade de pointes, ICD: implantable cardioverter defibrillator

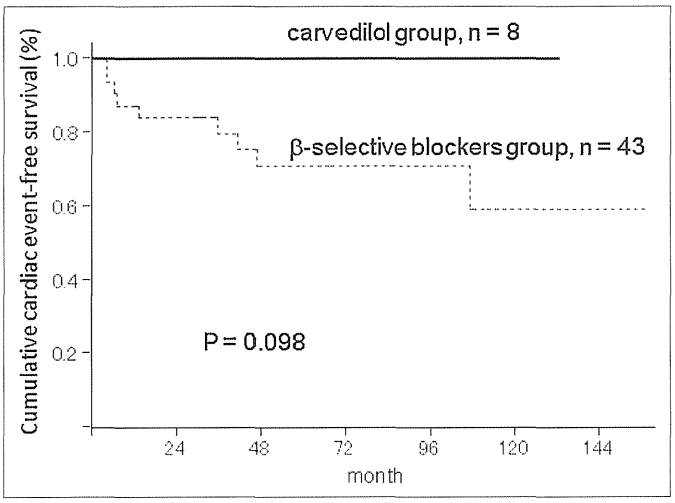

Figure 2 Kaplan-Meier analysis of cumulative cardiac event-free survival from each β-blocker therapy started to 150 months.

and therefore these incidences were not significantly different irrespective of β-selective blocker effectiveness.

Two of eleven patients (18%) in the β-selective blocker group experienced appropriate ICD shocks: one patient treated with propranolol (30 mg per day) and another treated by nadolol (60 mg per day), who showed an electrical storm. In two patients with ineffective propranolol therapy, recurrent syncope was controlled by diazepam as an adjunctive therapy.

Three of eight patients in the carvedilol group were first treated with propranolol, but experienced recurrent cardiac events, and the treatment was changed to carvedilol. In two cases, carvedilol was introduced because it was longer acting than propranolol. In the one remaining case, carvedilol was prescribed because of its additional α-blocking action.

Figure 3A shows a family tree showing a typical case that was refractory to propranolol but successfully controlled by carvedilol. The proband was a 9-year-old girl (indicated by arrow in **Figure 3A**), and genetic test identified a heterozygous *KCNH2* mutation (S871fs+31X). She was diagnosed as long QT syndrome at age of 6 years (12-lead ECG in **Figure 3B**, QTc = 500 ms) but remained asymptomatic. At age 9, she had loss of consciousness twice just after waking to an alarm clock in the morning. Her Holter ECG (**Figure 3C**) revealed repetitive TdP triggered by auditory stimuli, and she complained of uncomfortable chest distress. Propranolol (30 mg per day) was then prescribed, but her clinical complaints continued. Medication was finally switched to carvedilol (10 mg per day), which completely relieved her symptoms though the drug did not alter her QTc and HR. Her mother also collapsed at the delivery of the proband at age of 32 and genetic test revealed the same *KCNH2* mutation. Carvedilol was also started to prescribe for this proband's mother.

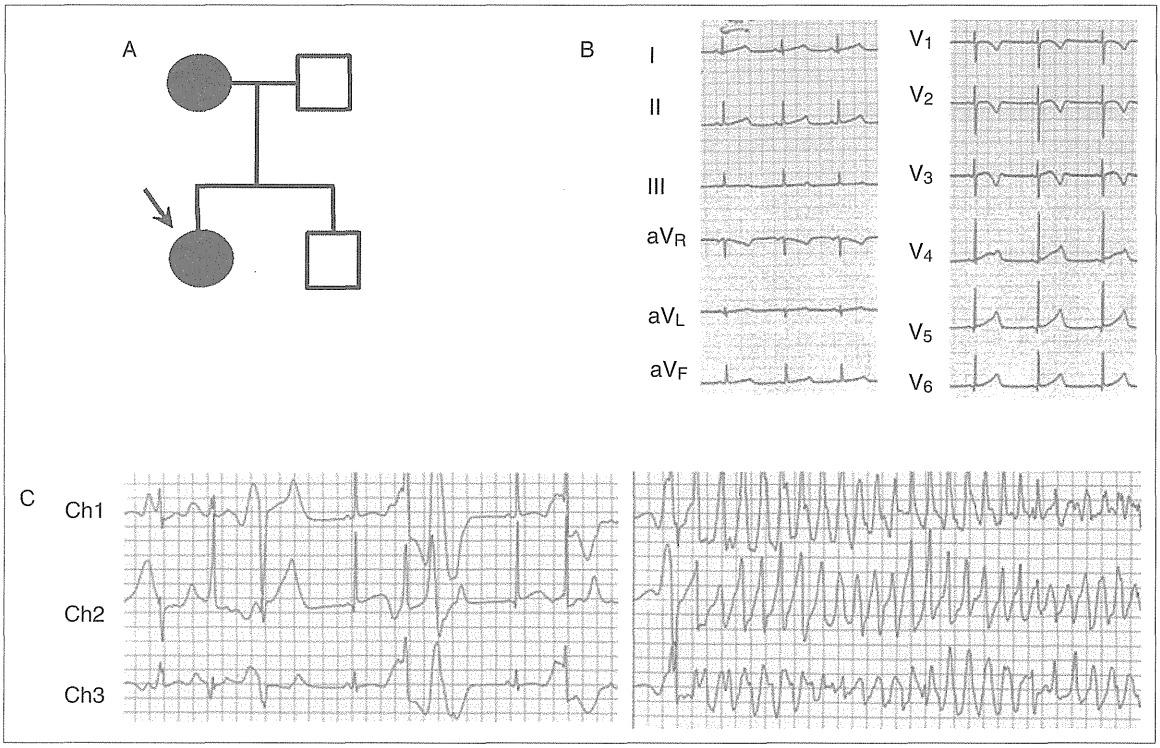

Figure 3 A typical case that was refractory to propranolol but controlled by carvedilol.
A. A pedigree of *KCNH2*-S871fs+31X. Circle indicates female, square indicates male. Closed symbol indicates mutation positive subject. Black arrow indicates proband of this family, a 9-year-old girl. **B.** 12 lead ECG at resting state of proband. QTc = 500 msec (V5). **C.** A Holter ECG of proband. TdP was detected during an episode of syncope triggered by alarm clock early in the morning. Left panel: just before the TdP. Right panel: TdP following left panel.

Discussion

In our study cohort of LQT2 patients, carvedilol, which blocks both α_1-and β-adrenoceptors, was more effective than other β-selective blockers in suppressing cardiac events without major complications. As widely recognized, β-blockers are the first line therapy for the prevention of cardiac events in the long QT syndrome. The studies on genotype-phenotype relationships of the syndrome in the last decade, however, have confirmed that β-blockers are most effective in LQT1, but less in LQT2 or LQT3 patients.[4] For example in 2004, Priori and colleagues[21] reported nearly a 3-fold increase in the risk of cardiac events during β-blocker treatment in LQT2 as compared to LQT1 patients, although there is a conflicting report by Goldenberg and colleagues.[22] They demonstrated that a β-blocker was sufficiently effective in high risk patients with both LQT1 and LQT2. As to α_1-and β-blockers, there are a few reports which demonstrated that α_1-and β-adorenoceptor blockade was effective to shorten QTc interval in the upright position before exercise and early recovery phase after exercise,[23] or suppress arrhythmic events in LQTS with unknown genotype.[10] There is another suggestive study of Khositseth et al[24] that phenylephrine-induced bradycardia increased transmural dispersion of repolarization (TDR) in symptomatic LQT2 but not in LQT1 patients. Therefore, α_1-adrenoceptor blockade (in addition to β-adrenoceptor blockade) in LQT2 patients may actually lead to suppression of QT prolongation and/or TDR, and eventually TdP. The results in the present study were in line with the above-mentioned hypothesis, and carvedilol actually appeared to be beneficial in suppressing cardiac events in LQT2.

As Moss et al. reported in 1971,[25] LCSD offers an alternative therapy to block sympathetic activation for LQTS patients without reducing heart rate. Antiarrhythmic action of LCSD is due largely to the electrophysiological consequences of reduced release of norepinephrine at the ventricular level and includes prevention/suppression of early afterdepolarization (EAD). Although it does not entirely control all cardiac events, the operation significantly reduces the number of symptoms and ICD shocks.[9] As the LCSD suppresses both β-and α-adrenergic

contribution of sympathetic control, blockade of α_1-adrenoceptors in addition to β-adrenoceptors by medication would also be useful to suppress the severe form of cardiac events.

More recently, we reported that α_1-adrenergic stimulation acutely reduced Kv 11.1 channel activities via membrane PIP_2 pathway.[14] Sudden α_1-adrenoceptor-mediated reduction in I_{Kr} at a lower HR (for example, during sleep) would act additionally to prolong action potential durations and may enhance inward current through Na/Ca exchanger, both contributing to the occurrence of EAD.[26] These observations may partially explain why sudden auditory stimulation by an alarm clock induces cardiac events in LQT2 patients.[6] In this connection, more recently Kim and colleagues[27] demonstrated in a large cohort of genotyped LQT2 patients that β-blockers were less effective in patients with arousal or non-exercise triggered events than those with exercise triggered events to prevent the recurrence. Although they did not mention the detailed species of β-blockers used for their patients, in our cohort, 11 recurrence cases in spite of β-selective blockers were all triggered by arousal or in a non-exercise resting state, and carvedilol prevented the cardiac event in three who were initially refractory to propranolol.

Study limitations

Because this study was conducted in a retrospective manner, we were not able to adjust the selection of patients between the two groups. Our study cohort consisted of a relatively small number of LQT2 patients. In the carvedilol group, there were no recurrent cases, which made further statistical analyses, such as multivariate correlation study, difficult. A further study with a larger number of patients will be awaited. In conclusion, in our genotyped LQT2 cohort, carvedilol was effective to suppress cardiac events, whereas 26% of the patients treated with other β-selective blockers experienced cardiac events, suggesting that the simultaneous blockade of α_1-adrenoceptors may offer an additional therapy for LQT2 patient with non-exercise triggers.

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