Figure 3   J Waves After the Pause

(A) Electrocardiogram (ECG) of the same patient discussed in Figure 1. Pause-induced augmentation of the J-wave was followed by ventricular fibrillation (VF) in this patient with idiopathic VF. J-wave augmentation was accompanied by ST-T displacement (lead II). The pause was produced by a premature ventricular beat. (B) ECG of a 49-year-old male with idiopathic VF. The premature ventricular beat produced a pause, but J-wave augmentation was not seen (lead II). VF was initiated by a premature beat that developed following a long R-R interval. (C) ECG of a 43-year-old male in the control group. There was no familial history of sudden cardiac death or cardiac disease. Premature ventricular contraction resulted in a pause but without a change in the J-wave (lead II).

occurred in 5 (33.3%) of 15 patients in storms after a short-long sequence. Isoproterenol was effective in controlling VF (Fig. 2).

In the remaining 12 (44.4%) of 27 patients, the J-wave remained unchanged (<0.05 mV), as shown in Figure 3: 0.192 ± 0.079 mV versus 0.196 ± 0.080 mV (p = 0.8377)
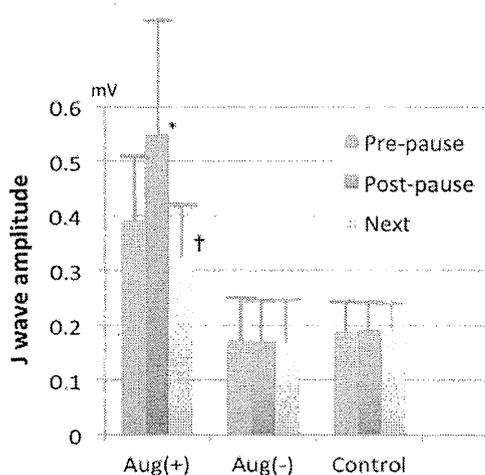

Figure 4   Comparisons of Pause-Dependent Changes in the J Waves Among the 3 Groups

In the subgroup with pause-dependent augmentation [Aug(+)], J-wave amplitude increased significantly among the post-pause beats (n = 15). In the beats coming just after the post-pause beats, the J-wave was diminished in amplitude (n = 6). No augmentation was induced by pauses in J waves in the subgroup without post-pause augmentation [Aug(−)] or in the control group. *p < 0.001 pre- versus post-pause. †p = 0.0406 and p = 0.0065 versus pre- and post-pause, respectively.

when the R-R interval was prolonged from 809 ± 137 ms to $1,156 \pm 175$ ms, as summarized in Table 2 (p < 0.0001). In 4 (33.3%) patients, VF developed and was controlled by isoproterenol.

The patients with pause-dependent augmentation of the J-wave amplitude were similar in age, sex, and J-wave locations to those without (Table 2). The baseline R-R intervals and their changes were similar between the 2 groups, but the pre- and post–J-wave amplitudes were larger in the patients with pause-dependent augmentation of the J-wave compared with those without (p < 0.0001).

Control group. In the 76 control subjects, sex and age were comparable to the 40 patients (Table 1). The locations of J waves were as follows: 65 (85.5%) in the inferior region, 25 (33.9%) in the left precordial region, 10 (13.2%) in the right precordial region, 12 (15.8%) in the high lateral region, and 36 (47.4%) at >1 site. The distribution pattern did not differ between the 2 groups.

The baseline R-R interval and the J-wave amplitude were different from the patient group (Table 1). When the R-R interval was prolonged from 941 ± 138 ms to $1,352 \pm 342$ ms by arrhythmias (n = 17), there was no augmentation of the J-wave amplitude (Figs. 3 and 4).

Sensitivity, specificity, and predictive values. Pause-dependent augmentation of the J waves was observed in 15 of 27 VF patients with a sensitivity of 55.6%, or 37.5% of the original 40 patients with J waves. Pause-dependent augmentation of J waves was observed only in patients with idiopathic VF. Both the specificity and the positive predictive values were 100%: the negative predictive value was 86.4% of the 27 patients, or 75.2% of the original 40

patients. The presence of pause-dependent augmentation of J waves was highly diagnostic for idiopathic VF.

Discussion

Fifty-four patients with idiopathic VF were admitted to Niigata University Hospital, and J waves were observed in 24 (44.4 %); another 16 similar patients were recruited from 8 other institutions.

Of the 40 patients, we were able to assess the instantaneous dynamicity of J waves after pauses in 27 patients (67.5%), and pause-dependent augmentation of the J waves was observed in 15 (55.6%) of 27 patients but not in any of the control patients. Augmentation of J waves was associated with depression of the ST-segment or inversion of the T-wave, and the beats just after the post-pause beat revealed attenuated J waves. The pause-dependent augmentation of J waves was highly specific and had highly predictive value. Effects of isoproterenol were reconfirmed.

The age and sex of the patients with idiopathic VF were similar to those reported previously (8–10). Prevalence of the J-wave was also similar to that reported by earlier researchers varying from 31% to 65%. This prevalence was higher than that of the Japanese (11% to 27%) (19) compared with the non-Japanese control subjects (5.0% to 34%) (5,8–12). Because of the frequent presence of J waves in the general population, it is urgent to establish a standard to distinguish "benign J waves" from "malignant" ones. So far, several characteristics of J waves involving idiopathic VF have been reported.

Extensive location or a large amplitude of J waves was statistically shown to be a risk factor for arrhythmic death (11); however, overlapping was remarkable between the patients with idiopathic VF and the control.

In addition, marked fluctuation of the J-wave might be observed in idiopathic VF especially before VF episodes (8,10,20–22), and the J-wave might develop new or disappear over time. This seems to be in contrast to ER or J waves in healthy individuals: ≥80% subjects exhibited the same type of J-wave after an interim of 5 years (11,23,24). Whether attenuation of the J-wave amplitude by isoproterenol or quinidine is specific to J waves with idiopathic VF needs be established (8,10,25–27).

Another striking feature of the J-wave in patients with idiopathic VF is an accentuation of J waves after a sudden prolongation of the R-R interval: that is, the pause dependency. This phenomenon was described in our first report in 1992 (7). Since then, data from similar cases have been collected. Among the 27 patients who were able to be analyzed with respect to pause-dependency, the pause-dependent augmentation of J waves was confirmed in 37.5% (15 of 40) patients with J waves, but such pause-dependent augmentation of J waves was not observed in any of the non-VF subjects. Notably, we would be able to provoke pauses by programmed stimulation during electrophysiological study and analyze the pause-dependency (15,28).

To date, only a few such cases have been reported (22,25). Although rare, a striking pause-dependent augmentation of J waves in the inferolateral leads has been observed in a patient with Brugada syndrome (29). During the follow-up of 2 years, he developed electrical storms from VF.

The presence of early repolarization with a horizontal/descending ST-segment was found to be able to predict arrhythmic death in a large population study (13). The presence of J waves was associated with a history of idiopathic VF with an odds ratio of 4.0, but the combination of J waves and a horizontal/descending ST-segment yielded an odds ratio of 13.8 for patients with idiopathic VF (14). In the present study, the ST-segment or T waves became more negative when J waves were accentuated after a pause (Fig. 2). Regarding the discordant relationship between the J-wave amplitude and ST-segment, delayed epicardial repolarization causing the epicardium to repolarize after endocardium seems to be responsible (30,31).

Experimentally, J waves are well explained by the transmural voltage gradient of the myocardial cells in phase 1 of the action potential of myocardial cells, which is created by transient outward currents (I_{to}) (32,33). Accentuated I_{to} is shown to result in accentuation of J waves on the surface ECG and I_{to} is known to be augmented at a slower rate. The bradycardia-dependent augmentation of the J-wave amplitude in idiopathic VF could be well explained by I_{to} (32,33). Delayed activation due to phase 4 block (block at a slow rate) may mimic J waves appearing at a slower rate and can be differentiated from the J-wave (34,35). Phase 4 block can be affected by the recovery characteristics of I_{to}, which permit a greater action potential overshoot and amplitude, and therefore a greater source current early in diastole (36).

At this point, we may be able to characterize the J-wave associated with idiopathic VF as follows: 1) large amplitude (often >0.2 mV) (11); 2) recent appearance (7,8); 3) remarkable fluctuation without any apparent cause (8,10); 4) extensive distribution (8); 5) response to isoproterenol or quinidine (8,10,24–27); 6) a concomitant horizontal/descending ST-segment (13,14); and 7) pause-dependent augmentation (7).

Study limitations. This was a small case series, and our conclusions must be confirmed in a larger number of patients. However, this study represents 20 years of experience. A worldwide survey is needed for further elucidation.

The response of the J-wave was analyzed in one-half of the patients because some did not have their situation complicated by benign arrhythmias. However, the pause-dependency of J waves might be evaluated in a systematic manner during electrophysiological study after giving electrical stimuli to either the atrium or the ventricle, inducing pauses (29). The causes of the waxing and waning nature of the J-wave were not determined. The possible role of a latent pathological process such as myocarditis as the cause of the J-wave appearing for weeks or longer should be excluded.

Finally, genetic abnormalities have been noted in some patients with idiopathic VF (37–40), but the present study did not include genetic screening. A systematic survey is of great importance in this respect.

Conclusions

Regarding the dynamicity of the J-wave in idiopathic VF, the pause-dependent augmentation was highly specific with high predictive values. This simple phenomenon may be used for the risk stratification of J waves.

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Time Course and Prognostic Implications of QT Interval in Patients with Coronary Artery Disease Undergoing Coronary Bypass Surgery

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Course and Prognostic Implication of QT After CABG. *Introduction:* The aim of the present study was to determine the prognostic implication of preoperative QT interval in relation to overall death and sudden cardiac death after coronary bypass surgery and to investigate the course of QT interval after surgery.

Methods and Results: Of 812 consecutive patients undergoing isolated off-pump coronary surgery, 656 were retrospectively analyzed after excluding the 48 patients who were taking QT prolonging drugs and the 108 patients who had any of the following electrocardiographic findings: atrial fibrillation, pacemaker, QRS of >120 milliseconds, bundle branch block. QT intervals were corrected for heart rate (QTc) using Bazett's formula. Prolonged QTc was defined as QTc of ≥450 milliseconds in men (n = 144) and ≥470 milliseconds in women (n = 36). The 5-year cumulative rate of sudden cardiac death in patients with prolonged QTc was 25% against 4% for those with normal QTc (P = 0.01). The risk-adjusted hazard ratio (95% confidence interval) for the association between preoperative QTc and overall death was 1.47 (1.21–1.74) per 1-SD increase in QTc; and 2.38 (1.50–3.45) for prolonged versus normal QT. For sudden cardiac death, the respective ratios were 1.63 (1.32–2.25) per 1-SD increase in QTc; and 3.32 (2.14–4.23). QTc interval did not change during the first year after surgery, but increased significantly during the subsequent years. Patients with prolonged QTc before surgery had consistently longer QTc even after revascularization than those with normal QTc.

Conclusion: Preoperative QT interval was an independent predictor of overall death and sudden cardiac death after isolated coronary bypass surgery. *(J Cardiovasc Electrophysiol, Vol. 23, pp. 645-649, June 2012)*

coronary artery bypass graft, coronary artery disease, heart rate, QT prolongation, revascularization, sudden cardiac death

Introduction

Sudden cardiac death is associated with coronary artery disease.[1,2] In a recent population-based study, QT prolongation was identified as an independent predictor of sudden cardiac death among patients with coronary artery disease.[3] Preoperative QT interval has been reported to be predictive of overall mortality after coronary artery bypass surgery.[4,5] However, little is known about its significance as a risk predictor for sudden cardiac death after coronary bypass surgery. Although surgical revascularization may affect the QT interval, there is almost no information on the time course of QT interval after surgery. The aim of the present study was to investigate the influence of surgical revascularization on the time course of QT interval after surgery and to determine the prognostic implication of preoperative QT interval in relation to the overall death and sudden cardiac death in patients undergoing isolated coronary artery bypass surgery.

Methods

Between January 2002 and January 2010, 818 consecutive patients underwent isolated coronary artery bypass surgery by a single surgeon at our institution. Of these, 6 patients underwent emergent percutaneous cardiopulmonary bypass at other hospitals during cardiopulmonary resuscitation for cardiac arrest caused by acute myocardial infarction and were then transferred to our hospital, where they underwent emergent coronary bypass surgery with percutaneous cardiopulmonary bypass. The remaining 812 patients underwent myocardial revascularization using the off-pump technique without emergent conversion to cardiopulmonary bypass during the operation. Of this cohort, 656 patients were retrospectively analyzed after excluding the 48 patients who were taking QT-prolonging drugs: amiodarone or sotalol (n = 8), disopyramide, flecainide, procainamide, or cibenzoline (n = 28), or antipsychotics (n = 12), and the 108 patients who had any of the following electrocardiographic findings: chronic atrial fibrillation or atrial flutter (n = 20), pacemaker rhythm (n = 5), QRS of >120 milliseconds (n = 65), bundle branch block (n = 24). All patients had previously granted permission for use of their medical records for research purposes. The study was approved by the institutional review board.

The off-pump technique was used for all patients. The details of the revascularization strategy were described previously.[6]

Electrocardiography

Standard 12-lead resting electrocardiograms tracing at 25 mm/s paper speed and 10 mm/mV amplitude (on average 6–8 beats) were performed on admission with a MAC-5500 digital ECG system (GE Medical Systems, Milwaukee, WI, USA) and stored in a MUSE System (GE Medical Systems). Electrocardiogram analysis was performed using a Marquette 12SL ECG Analysis Program (GE Medical Systems). The QT interval was measured from the beginning of the earliest onset of the QRS complex to the end of the T wave. The end of the T wave was defined as the return of the descending limb to the isoelectric line. When a discrete U wave was present after the T wave, the T wave offset was defined as the nadir between the T and U waves. When the T and U waves were fused, the U component was calculated as the T wave. The QT measurement was made in leads II and V5 or V6, with the longest value being used. QT interval was corrected for heart rate (QTc) using Bazett's formula.[7] Data were analyzed using gender-specific QTc categories (men: normal <450 milliseconds, prolonged ≥450 milliseconds; women: normal <470 milliseconds, prolonged ≥470 milliseconds). Sokolow-Lyon voltage was calculated by addition of the amplitude of the S wave in lead V1 to the amplitude of the maximum R wave in lead V5 or V6.[7] Cornell voltage was calculated by addition of the amplitude of the R wave in lead aVL to the amplitude of the S wave in lead V3.[8,9]

Endpoints and Definitions

The primary endpoint was overall death. The secondary endpoint was sudden cardiac death. Sudden cardiac death was defined as a sudden unexpected pulseless condition of likely cardiac origin. If unwitnessed, sudden cardiac deaths were those in which the patient was found dead within 24 hours of having last been seen alive and in a normal state of health. Documentation of the cause of death was based on information obtained from witnesses, family members, death certificates, hospital records, and autopsy records. All patient data were collected prospectively and entered in our database. Chronic kidney disease was defined as glomerular filtration rate <60. Glomerular filtration rate was estimated from the Modification of Diet in Renal Disease equation: estimated glomerular filtration rate mL/min/1.73 m^2 = 194 × serum creatinine level (mg/dL)$^{-1.094}$ × age (year)$^{-0.287}$ × 0.739 (if female).[10] Preoperative serum creatinine was measured at admission.

Statistical Analysis

Comparisons between the groups were performed with Pearson's chi-square test for categorical variables and with unpaired t-test or Mann-Whitney's U test for continuous variables. Interval-to-interval comparisons were carried out with paired t-test for normally distributed variables. Linear relations were checked with a standard least-squares linear regression analysis. The cumulative risks of overall death and sudden cardiac death were estimated separately for each group using the Kaplan–Meier method and were compared between groups using the log rank test. The association between preoperative QTc and overall death or sudden cardiac death was estimated using the Cox proportional haz-

ard model. Hazard ratio (HR) with 95% confidence interval (95% CI) was calculated in 2 ways: by modeling QTc as a continuous variable (per 1-SD increase) and by categorizing QTc into 2 groups (normal QTc group vs prolonged QTc group). To determine whether QTc remained predictive after adjustment for possible confounders of this relationship, we constructed 3 models. The first model included QTc alone, the second QTc and the logistic European system for cardiac operative risk evaluation (Euro SCORE), and the third QTc and potential risk factors for mortality: age, female gender, body mass index, hypertension, diabetes mellitus, chronic kidney disease, peripheral arterial disease, previous myocardial infarction, triple vessel disease, and ejection fraction. The proportional hazards assumption was checked by inspecting the log-minus-log survival curves. No violation of this assumption was detected. To evaluate the impact of preoperative QTc in explaining each outcome, we constructed receiver operating characteristic (ROC) curves and determined the area under the curve. All statistical testing was 2-sided. Results were considered statistically significant at a level of P < 0.05. All analyses were performed with the SPSS statistical package version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient Characteristics

The mean value (± standard deviation) of the QTc interval was 427 ± 33 milliseconds in males (n = 519) and 439 ± 37 milliseconds in females (n = 137). Of the 656 patients enrolled, 144 males (27.7%, 144/519) had QTc of ≥450 milliseconds while 36 females (26.3%, 36/137) had QTc of

TABLE 1

Baseline Characteristics

	Normal QT n = 476	Prolonged QT n = 180	P
Age (years)	68.0 ± 9.8	68.4 ± 10.9	0.59
Female gender	101 (21.2)	36 (20.0)	0.73
Body mass index (kg/m^2)	23.6 ± 3.1	23.2 ± 3.3	0.21
Hypertension	335 (70.4)	120 (66.7)	0.38
Diabetes mellitus	238 (50.0)	94 (52.2)	0.53
Hemoglobin A1c (%)	6.3 ± 1.2	6.4 ± 1.3	0.21
Chronic kidney disease	270 (56.7)	118 (65.6)	0.03
Peripheral arterial disease	60 (12.6)	25 (13.9)	0.49
Triple vessel disease	340 (71.4)	132 (73.3)	0.38
Ejection fraction, %	58 ± 11	56 ± 13	0.23
Previous myocardial infarction	198 (41.6)	88 (48.9)	0.04
History of ventricular tachycardia	19 (4.0)	8 (4.4)	0.36
Serum potassium levels	4.0 ± 0.5	4.1 ± 0.6	0.33
Electrocardiography			
PR duration (milliseconds)	169.7 ± 24.5	168.3 ± 31.5	0.75
QRS duration (milliseconds)	94.8 ± 10.1	95.9 ± 9.7	0.49
Sokolow-Lyon voltage (mV)	29.0 ± 10.9	28.9 ± 12.3	0.95
Cornell voltage (mV)	18.9 ± 7.8	20.7 ± 11.2	0.22
Medication on admission			
Renin angiotensin system inhibitor	263 (55.3)	101 (56.1)	0.80
Digitalis	22 (4.6)	9 (5.0)	0.51
β-blocker	269 (56.5)	98 (54.4)	0.46
Statin	297 (62.4)	114 (63.3)	0.76

Continuous variables are presented as mean ± standard deviation, categorical variables as number (percentage).
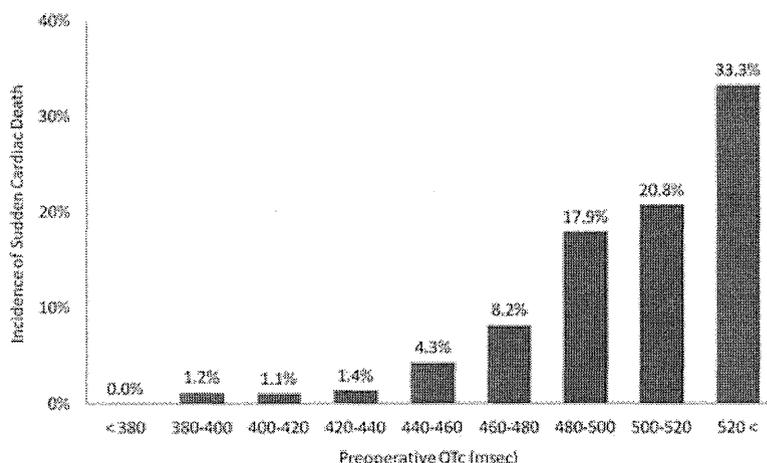
Figure 1. *Incidence of sudden cardiac death by preoperative QTc interval.*

$\geq$470 milliseconds (prolonged QT group, n = 180). The remaining 375 males and 101 females had QTc of <450 milliseconds and <470 milliseconds, respectively (normal QT group, n = 476). Table 1 presents a comparison between the two groups of baseline characteristics, electrocardiographic findings, and medication on admission. No significant difference was found in age, gender, body mass index, hemoglobin A1c, ejection fraction, serum potassium levels, history of ventricular tachycardia, or prevalence of hypertension, diabetes mellitus, or peripheral arterial disease. However, the prolonged QT group had significantly higher prevalence of chronic kidney disease and previous myocardial infarction than the normal QT group. Electrocardiographic findings and medication on admission were similar between the groups.

Operative Procedures, Postoperative Complications, and Medication on Discharge

The left anterior descending artery was revascularized using the *in situ* internal thoracic artery in all patients. There was no significant difference between the normal QT group and the prolonged QT group in the number of distal anastomoses (3.4 $\pm$ 1.2 and 3.4 $\pm$ 1.1, P = 0.43) or the rates of bilateral internal thoracic artery grafting (66.7% and 60.5%, P = 0.20), gastroepiploic artery grafting (41.3% and 39.9%, P = 0.71), or complete revascularization (98.6% and 98.5%, P = 0.98). There was no significant difference between the normal QT group and the prolonged QT group in the incidence of 30-day mortality (1.7% vs 2.2%, P = 0.27), perioperative myocardial infarction (0.8% vs 1.0%, P = 0.66), and postoperative atrial fibrillation (22.5% vs 18.7%, P = 0.30). No significant difference was found between the 2 groups in medication on discharge including renin angiotensin system inhibitors (78.5% and 79.6%, P = 0.43), digitalis (15.7% and 15.1%, P = 0.83), β-blockers (84.3% and 82.4%, P = 0.57), statins (87.3% and 82.4%, P = 0.43), cibenzoline succinate (5.6% and 5.0%, P = 0.85), and amiodarone (3.0% and 2.7%, P = 0.80).

Cumulative Risk of Death

The follow-up rate of the patients was almost complete (99.3%). The mean duration ($\pm$ standard deviation) of the observation period was 4.1 $\pm$ 1.6 years. The preoperative QTc interval was significantly longer in patients who died than in survivors (overall death 446 $\pm$ 43 vs 427 $\pm$ 32,

P = 0.01; sudden cardiac death 465 $\pm$ 50 vs 428 $\pm$ 32, P = 0.01). The incidence of sudden cardiac death increased with increasing preoperative QTc interval (P = 0.01, trend test using a logistics regression, Fig. 1). Kaplan–Meier estimates of the cumulative risk of overall death and sudden cardiac death in the 2 groups are shown in Figure 2. The 5-year cumulative rates of overall death in the prolonged QT group versus the normal QT group were 33% versus 12% (P = 0.01). For sudden cardiac death, the 5-year cumulative rates were 25% versus 4% (P = 0.01). The 2 curves continued to diverge through the 7 postoperative years. No significant difference
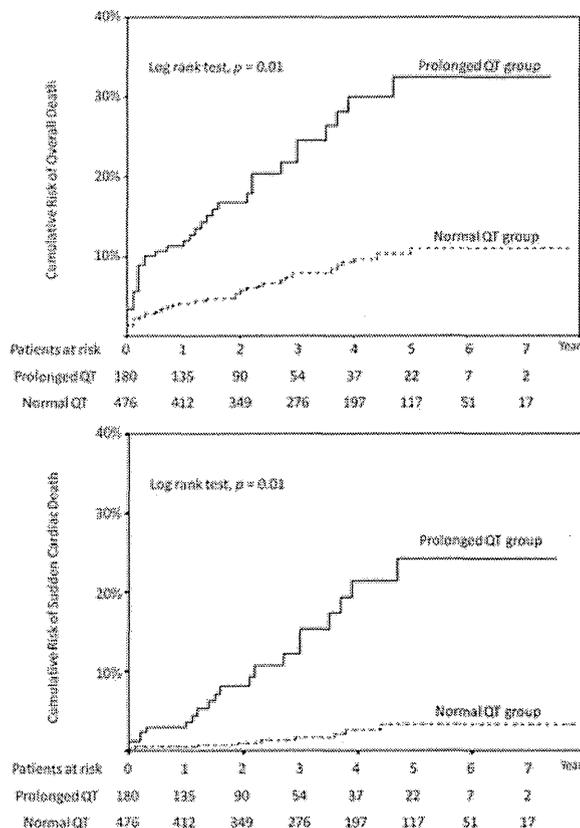

Figure 2. *Kaplan–Meier estimates of cumulative risk of overall death and sudden cardiac death.*

TABLE 2

Hazard Ratio and 95% Confidence Interval for Association Between Preoperative QTc and Overall Death or Sudden Cardiac Death

	Model 1	Model 2	Model 3
Overall death			
Per 1-SD increase	1.57 (1.32–1.87)	1.35 (1.13–1.72)	1.47 (1.21–1.74)
Normal QTc	1 (Ref)	1 (Ref)	1 (Ref)
Prolonged QTc	2.88 (1.75–3.98)	2.24 (1.57–3.35)	2.38 (1.50–3.45)
Sudden cardiac death			
Per 1-SD increase	1.93 (1.56–2.38)	1.56 (1.25–1.97)	1.63 (1.32–2.25)
Normal QTc	1 (Ref)	1 (Ref)	1 (Ref)
Prolonged QTc	3.95 (2.45–5.22)	3.13 (2.09–4.18)	3.32 (2.14–4.23)

Values are hazard ratio (95% confidence interval). Model 1 includes QTc alone, Model 2 QTc and the logistic European System for Cardiac Operative Risk Evaluation (Euro SCORE), and Model 3 QTc, age, female gender, body mass index, hypertension, diabetes mellitus, chronic kidney disease, peripheral arterial disease, previous myocardial infarction, triple vessel disease, and ejection fraction. Prolonged QTc was defined as QTc of ≥450 milliseconds in men and ≥470 milliseconds in women.

was found in the prolonged QT group versus the normal QT group in the 5-year cumulative rates of noncardiovascular death (5.1% vs 5.9%, P = 0.40).

Multivariate Predictors of Overall Death and Sudden Cardiac Death

Table 2 shows the unadjusted and adjusted HR (95% CI) for the association between preoperative QTc and overall death or sudden cardiac death. Prolonged QTc was independently associated with a higher risk of overall death and sudden cardiac death. Adding the logistic Euro SCORE or the potential risk factors into the models attenuated this association, but prolonged QTc remained an independent risk factor for overall death and sudden cardiac death. Other variables statistically significant in the multivariate model for overall death were age (HR 1.64 per 10-year increase, 95% CI 1.21–2.23, P = 0.01), peripheral arterial disease (HR 2.54, 95% CI 1.56–3.34, P = 0.01), and ejection fraction (HR 1.18 per 5% decrease, 95% CI 1.08–1.29, P = 0.01). For sudden cardiac death, significant variables were diabetes mellitus (HR 2.26, 95% CI 1.46–2.88, P = 0.01), peripheral arterial disease (HR 2.13, 95% CI 1.33–2.98, P = 0.01), and ejection fraction (HR 1.23 per 5% decrease, 95% CI 1.10–1.40, P = 0.01).

Receiver Operator Characteristic Analysis

The strong association of preoperative QTc with overall death and sudden cardiac death was reflected by the area under the receiver operator characteristic curve of 0.66 for overall death (95% CI 0.59–0.72, P = 0.01) and 0.78 for sudden cardiac death (95% CI 0.70–0.85, P = 0.01).

Time Course of QTc after Surgery

Table 3 summarizes the differences between preoperative QTc and postoperative QTc. QTc interval measured in postoperative studies at 3 months and 1 year was similar to the preoperative QTc interval. However, the QTc interval increased significantly during the subsequent years. Figure 3 compares the QTc interval between the prolonged QT group and the normal QT group. The prolonged QT group had significantly longer QTc interval than the normal QTc group during the entire follow-up period. In the prolonged

TABLE 3

Comparison of Differences Between Preoperative QTc and Postoperative QTc

Pair	n	Mean Difference (95% Confidence Interval)	P
Preop. to 3 months	613	1.0 (−1.4–3.4)	0.42
Preop. to 1 year	547	2.2 (−0.6–5.1)	0.13
Preop. to 2 years	439	4.0 (0.8–7.3)	0.01
Preop. to 3 years	330	10.0 (5.9–14.1)	0.01
Preop. to 4 years	234	14.2 (9.1–19.3)	0.01
Preop. to 5 years	139	16.5 (11.0–22.0)	0.01
Preop. to 6 years	58	17.0 (10.8–23.1)	0.01

Comparison was carried out using paired *t*-test as differences were normally distributed.

QT group, a significant decrease in the QTc interval from the preoperative QTc was observed in the first 3 months after surgery (mean difference −15.1, 95% CI −21.3 to −8.9, P = 0.01), in contrast with the marked increase in the QTc interval in the normal QT group (mean difference 6.8, 95% CI 4.7 − 9.0, P = 0.01). Both groups had a stable level in the period between 3 months and 2 years postoperatively and experienced a moderate increase during the subsequent years.

Discussion

The major findings of the present study were that the incidence of overall death and sudden cardiac death was significantly higher in patients with prolonged QTc than in those with normal QTc; that preoperative QTc was an independent risk predictor of overall death and sudden cardiac death; that the QTc interval did not change during the 1st year after surgery, but increased significantly during the subsequent years; and that patients with prolonged QTc had significantly higher postoperative QTc than those with normal QTc during the entire follow-up period.

Several investigators have reported on the association between preoperative QT interval and mortality after coronary artery bypass surgery. In a follow-up study 1 month postoperatively, Vrtovec *et al.* compared cardiac death in 567 ischemic heart failure patients with or without prolonged
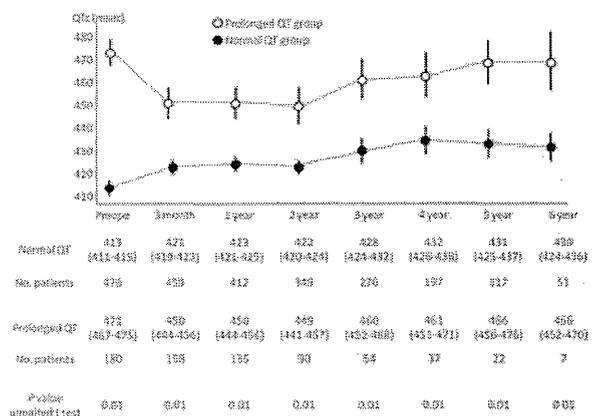

Figure 3. *Comparisons of postoperative QTc interval between prolonged QT group and normal QT group. Error bars represent 95% confidence intervals of mean value.*

QTc who had undergone coronary artery bypass grafting and demonstrated that the preoperative QTc interval was significantly longer in patients who died than in survivors (476 ± 45 vs 434 ± 50, P < 0.001); preoperative QTc of >440 milliseconds was the only independent predictor of mortality in multivariate analysis (HR 4.97, P = 0.002).[4] Foroughi *et al.* assessed the prognostic value of QT interval for death due to low cardiac output in 198 patients undergoing coronary artery bypass grafting and reported that the mean QTc was significantly longer in patients who died than in the control group (480.7 ± 96.2 vs 425.4 ± 21.6, P = 0.039).[5] Our results concur with these findings. The present study indicates the significance of preoperative QT interval as a risk predictor for sudden cardiac death after coronary bypass surgery.

There have been several reports of change in the QT interval relatively early in the postoperative period after coronary bypass surgery: in the acute phase after surgery, a significant worsening of QT dynamicity[11] and increase in the QTc interval[12] have been observed; at 1 month after surgery, the QTc interval and QTc dispersion have been found to be significantly reduced.[13] The present study provides data on QTc interval obtained in the late postoperative period. Overall, the QTc interval stayed constant until 1 year after surgery and then gradually increased. Preoperative QTc correlated positively to postoperative QTc. Patients with prolonged QTc before surgery had consistently longer QTc even after revascularization than those with normal QTc. The prolongation of QT interval reflects the inhomogeneity of ventricular repolarization, leading to ventricular arrhythmia. Our results indicate that patients with preoperative QTc prolongation are at high risk of severe arrhythmia even after surgical revascularization.

The present study has a number of potential limitations. First, it was a retrospective study. Even with multivariate adjustment, we cannot eliminate the unobserved confounders and selection bias between patients with prolonged QTc and those with normal QTc. Second, the lack of data on the baseline characteristics, ejection fraction, and the changes in drug therapy during the follow-up period cannot allow us to determine whether postoperative QTc has any independent influence on risk of death. Third, Bazett's formula is well known to undercorrect the QT interval for slower heart rates and to overcorrect for faster heart rates. Therefore, it is possible that β-blockers were selectively withheld in patients with slower heart rates after discharge (who would have had spuriously high corrected QT intervals). This could confound the risk relationship between QT interval and sudden death, given the protective effect of β-blockers. Fourth, other markers of ventricular repolarization (QT dispersion, JT interval, and JT dispersion) were not studied. Fifth, all enrolled subjects were Japanese patients who underwent revascularization using the off-pump technique at a single center, which limits the generality of the findings. Sixth, the definition of sudden cardiac death was much more subjective. We have no information about time between the occurrence of symptom and death in patients who were categorized in sudden cardiac death. There was no independent review committee detailing the categorization of sudden cardiac death. Finally, the lack of coronary angiographic follow-up data did not allow us to evaluate the possible link between graft patency, QT interval, and the outcome.

Conclusion

Preoperative QTc interval was an independent predictor of overall death after isolated off-pump coronary artery bypass surgery. Although, the definition of the sudden cardiac death was much more subjective, preoperative QTc interval was similarly associated with sudden cardiac death after surgery. QTc interval did not change during the first year after surgery, but increased significantly during the subsequent years. Patients with prolonged QTc before surgery had consistently longer QTc even after revascularization than those with normal QTc. The lack of data on the baseline characteristics, ejection fraction, and the changes in drug therapy during the follow-up period cannot allow us to determine whether postoperative QTc has any independent influence on risk of death. In clinical practice, QT interval can be determined easily by computer and may be useful for noninvasive risk stratification of patients at high risk of sudden cardiac death. Further studies are necessary to identify strategies to improve outcomes for high-risk patients identified on the basis of prolonged QTc interval.

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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]

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 ≥ 0.1 mV ≥ 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
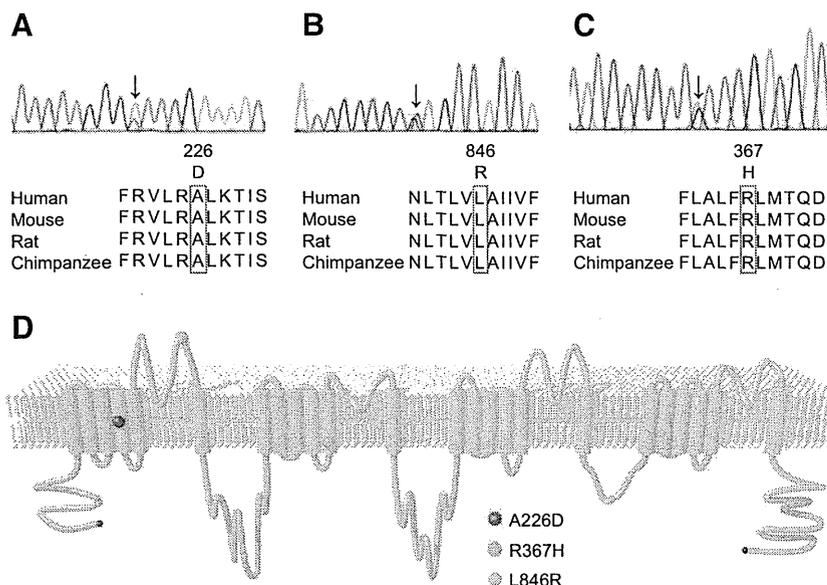

Figure 1. Mutations in *SCN5A* identified in patients with idiopathic ventricular fibrillation associated with early repolarization. **A**, The c.677C→A mutation in *SCN5A* resulting in p.A226D found in patient 1. **B**, The c.2537T→G mutation in *SCN5A*, resulting in p.L846R found in patient 2. **C**,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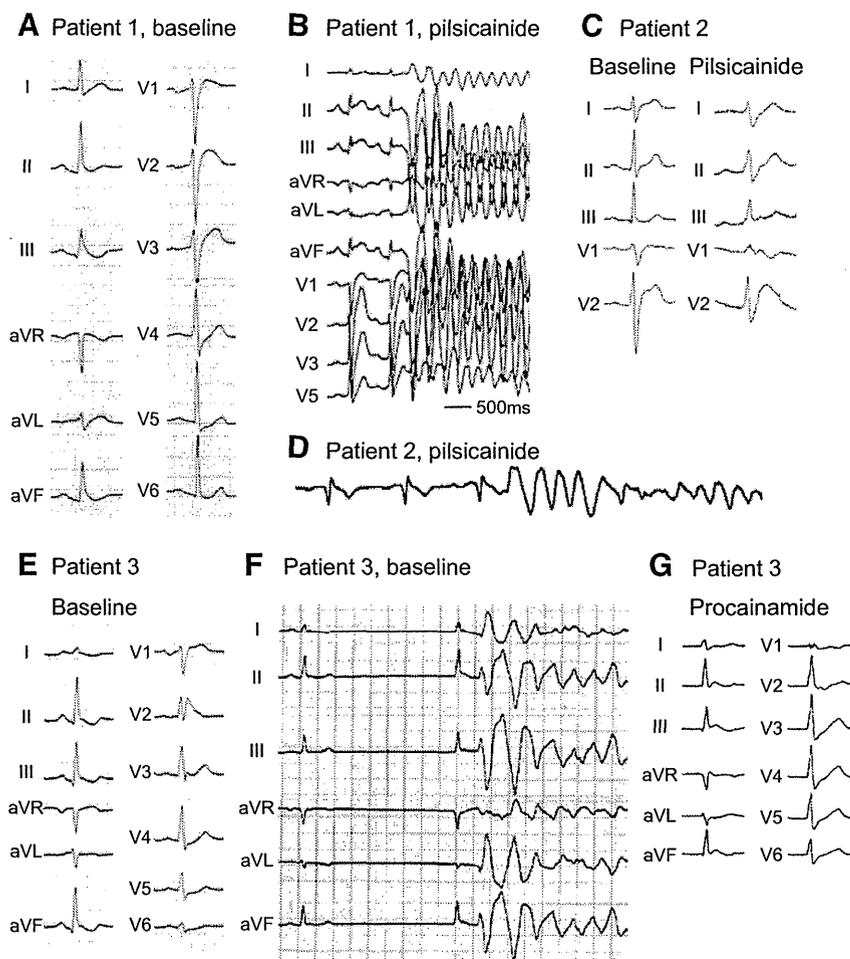

Figure 2. Electrocardiograms of patients with idiopathic ventricular fibrillation and a mutation in *SCN5A*. **A**, Early repolarization was present in the inferior and right precordial leads in patient 1. **B**, After administration of pilsicainide, early repolarization was augmented and ventricular 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]
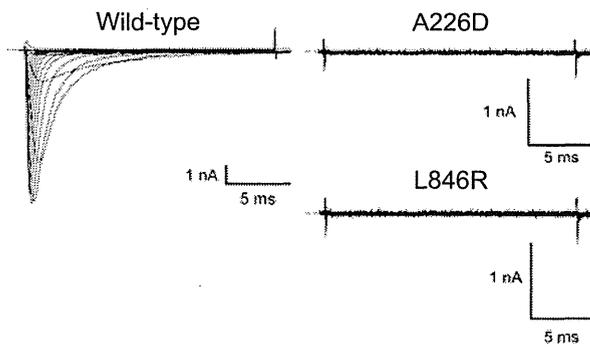
Figure 3. Electrophysiological characteristics of the *SCN5A* mutants. Representative traces of sodium current demonstrating that all of the mutant channels failed to generate any currents. We previously reported that R367H mutant fails to generate any currents.[27]

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
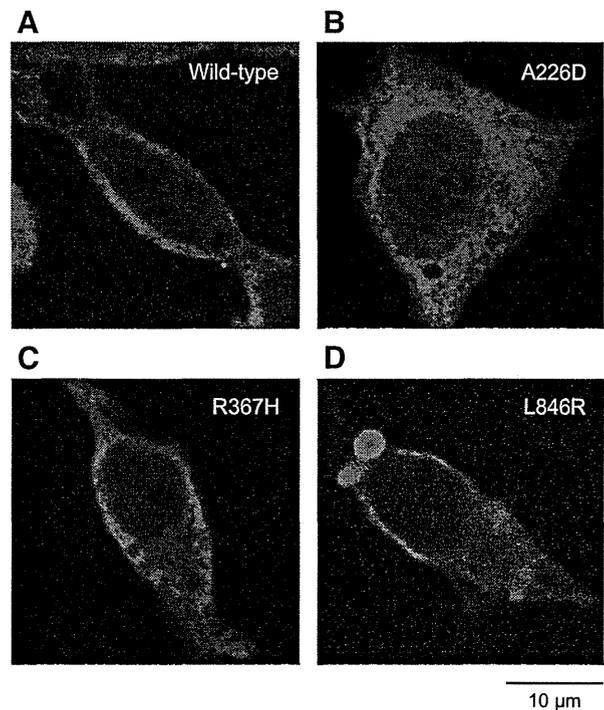

Figure 4. Representative confocal microscopy images. **A,** Cells expressing wild-type SCN5A channels showed marked peripheral fluorescence. **B,** Cells expressing A226D channels showed cytoplasmic fluorescence. **C** and **D,** Cells expressing R367H channels and those expressing L846R channels showed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

Phenotype Variability in Patients Carrying *KCNJ2* Mutations

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Background—Mutations of *KCNJ2*, the gene encoding the human inward rectifier potassium channel Kir2.1, cause Andersen-Tawil syndrome (ATS), a disease exhibiting ventricular arrhythmia, periodic paralysis, and dysmorphic features. However, some *KCNJ2* mutation carriers lack the ATS triad and sometimes share the phenotype of catecholaminergic polymorphic ventricular tachycardia (CPVT). We investigated clinical and biophysical characteristics of *KCNJ2* mutation carriers with "atypical ATS."

Methods and Results—Mutational analyses of *KCNJ2* were performed in 57 unrelated probands showing typical (≥ 2 ATS features) and atypical (only 1 of the ATS features or CPVT) ATS. We identified 24 mutation carriers. Mutation-positive rates were 75% (15/20) in typical ATS, 71% (5/7) in cardiac phenotype alone, 100% (2/2) in periodic paralysis, and 7% (2/28) in CPVT. We divided all carriers (n=45, including family members) into 2 groups: typical ATS (A) (n=21, 47%) and atypical phenotype (B) (n=24, 53%). Patients in (A) had a longer QUc interval [(A): 695±52 versus (B): 643±35 ms] and higher U-wave amplitude (0.24±0.07 versus 0.18±0.08 mV). C-terminal mutations were more frequent in (A) (85% versus 38%, *P*<0.05). There were no significant differences in incidences of ventricular tachyarrhythmias. Functional analyses of 4 mutations found in (B) revealed that R82Q, R82W, and G144D exerted strong dominant negative suppression (current reduction by 95%, 97%, and 96%, respectively, versus WT at −50 mV) and T305S moderate suppression (reduction by 89%).

Conclusions—*KCNJ2* gene screening in atypical ATS phenotypes is of clinical importance because more than half of mutation carriers express atypical phenotypes, despite their arrhythmia severity. (***Circ Cardiovasc Genet***. 2012; 5:344-353.)

Key Words: CPVT ■ ion channels ■ Andersen-Tawil syndrome ■ KCNJ2 ■ phenotype

Andersen-Tawil syndrome (ATS) represents a disease entity characterized by 3 features: (1) ventricular arrhythmias with Q(T)U prolongation, (2) periodic paralysis, and (3) dysmorphic features.[1,2] It is an autosomal-dominant inherited disease resulting from a heterozygous mutation of the *KCNJ2* gene. This gene encodes an inward rectifier K channel (Kir2.1), ubiquitously expressed in the myocardium, skeletal muscle, brain, and osteocytes.[3] Since the first discovery of a mutation in this disease in 2001,[4] we have extensively examined *KCNJ2* mutations in patients suspected of having ATS.[5–7] In 2007, we described both the clinical and genetic features of 23 patients (13 probands and 10 family members) and reported that the identification rate of *KCNJ2* mutation in this cohort was 100% if the patients satisfied ≥ 2 features of ATS.[8] On a closer inspection, however, we noticed that ≈30% of the *KCNJ2* mutation carriers lacked 2 of the ATS features: frequent PVC, bidirectional or polymorphic ventricular tachycardias (bVT or pVT), with QT or QU prolongation, without periodic paralysis or dysmorphic features. Recently, Tester et al[9] reported a possible phenotypic overlap between ATS and catecholaminergic polymorphic VT (CPVT). CPVT is a form of inherited cardiac arrhythmia,
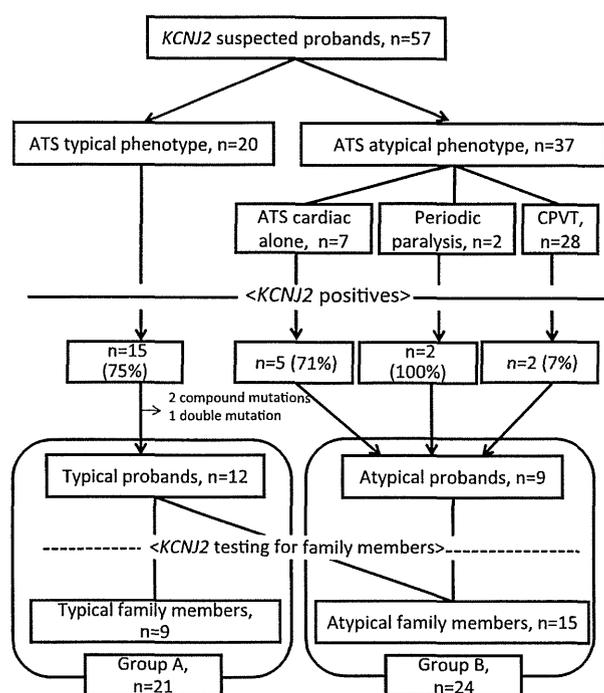
Figure 1. A Flow Chart of 57 probands suspected as carriers of *KCNJ2* mutations. ATS indicates Andersen-Tawil syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia.

characterized by exercise- and/or stress-induced p/bVTs with a normal cardiac structure.[10] However, the incidence and clinical characteristics of atypical ATS and *KCNJ2*-related CPVT remain unknown.

Clinical Perspective on p 353

In the present study, we conducted the genetic screening for *KCNJ2* mutation-related phenotypes—those fulfilling at least 1 of the ATS features or CPVT criteria. We compared the clinical and genetic features between typical ATS and atypical phenotype (only 1 of the ATS features or CPVT) patients. We hypothesized that the mutated channel function found in patients with atypical ATS phenotypes would show different I_{K1} current properties. Using the patch-clamp technique, we examined the functional features of four mutations found in the atypical phenotype group.

Methods

Study Subjects

Fifty-seven unrelated probands (65% females, age at diagnosis: 18 ± 12 years old) from 31 institutes in Japan were enrolled in the study. They were clinically diagnosed with either typical ATS (defined as patients with ≥2 ATS features) (n=20), mild ATS [those with 1 of the ATS features—cardiac arrhythmia alone (n=7), periodic paralysis with an abnormal U wave (n=2)], or CPVT (n=28) (Figure 1 and Table 1).

Three features of ATS were clinically determined as follows: (1) Cardiac involvement was determined by the presence of ventricular arrhythmias (frequent premature ventricular contractions (PVCs), bigeminy, bVT, pVT, or monomorphic VT), with prolongation of the corrected QU interval and/or a prominent U wave.(2) The presence of periodic paralysis was based on standard criteria.[11] (3) Dysmorphic features were defined by the presence of 2 or more of the following: (a) low-set ears, (b) hypertelorism (wide-set eyes), (c) a small mandible, (d) clinodactyly (permanent lateral or medial curve of a finger or toe), and (e) syndactyly (persistent webbing between fingers or toes).[12]

Twenty-eight of the 57 patients fulfilled the diagnostic criteria of CPVT—exertional syncope plus documentation of bVT or pVT during exercise or exercise tests. Patients with QT prolongation were excluded.[10]

ECG Manifestation

We measured QU intervals if there was a prominent U wave and QT intervals in cases showing no U wave. The QT interval was defined from the onset of QRS to the end of the T wave. The U wave was defined as an early diastolic deflection after the end of the T wave. The QU interval was defined from the onset of QRS to the end of the U wave. QT and QU intervals were corrected according to the Bazzett formula.[13,14] The end of the T or U wave was the point at which a tangent drawn to the steepest portion of the terminal part of the T or U wave crossed the isoelectric line.[15] Because a prominent U wave is often fused to the next PQ segment in some cases, we defined the isoelectric line as a segment connecting 2 points preceding consecutive QRS complexes. A diagnosis of QT prolongation was made if the QTc exceeded 440 ms for males and 460 ms for females, in accordance with the standard criteria.[16] Abnormal U waves were judged based on the following criteria: (a) wave amplitude ≥0.2 mV or (b) amplitude larger than preceding T wave.[8,17]

bVT was identified as a VT characterized by a beat-to-beat alternation of the QRS axis in most of the documented runs of ventricular tachycardia (>4 consecutive beats).[10] pVT was defined as the VT with an irregularly variable axis of the QRS.

DNA Isolation and Mutation Analysis

The protocol for genetic analysis was approved by the institutional ethics committee and performed under its guidelines. All patients

Table 1.   Demographic Characteristics of Different Patient Cohorts: *KCNJ2* Mutation Incidence Rates (57 Probands)

	Typical ATS (Group A)	Atypical ATS (Group B)			
		ATS Cardiac Alone	ATS Periodic Paralysis Alone	CPVT	Total
Probands, n	20	7	2	28	57
Female, n (%)	13 (65)	5 (71)	0	19 (68)	37 (65)
Age, y	17 ± 11	21 ± 13	19 ± 6	18 ± 13	18 ± 12
QT(U)c,* ms	660 ± 90	600 ± 106	548 ± 77	429 ± 65	
KCNJ2 positives, n (%)	15 (75)	5 (71)	2 (100)	2 (7)	24 (42)

ATS indicates Andersen-Tawil syndrome; CPVT, catecholaminergic polymorphic ventricular tachycardia.

*QUc interval was used in typical ATS, ATS cardiac phenotype alone, and ATS periodic paralysis alone. QTc interval was used in CPVT.

Table 2. *KCNJ2* Mutation-Carrying Probands (n=24)

	Case No.	KCNJ2-DNA	Protein	Age/Sex	ECG†	QTc/QUc, ms	Dysmorphism	Paralysis	Mutated Channel Function‡	Syncope/ACA	Treatment
Typical ATS phenotype (n=15)	1	118C>T	R40X*	15/F	1, 2	451/618	+	−	−	−/−	BB
	2	200G>A	R67Q	14/F	1, 2	360/626	+	+	d.n.[8]	−/−	Ib, BB
	3	244C>T	R82W KCNH2-P1093L	11/M	2	NA/NA	+	−	d.n.[9,27]	−/−	BB
	4	430G>A	G144S KCNQ1-A341V	32/F	1, 2	NA/NA	+	+	d.n.[8,12]	+/+	IV, BB
	5	436G>A	G146S	27/F	2	487/731	NA	+	d.n.[8]	−/−	BB
	6	574A>G	T192A	16/M	1	420/700	−	+	d.n.[5]	−/−	Az
	7	653G>A	R218Q	13/F	2	423/741	−	+	d.n.[8]	−/−	BB, Az
	8	653G>A	R218Q	12/F	2	434/616	+	+	d.n.[8]	−/−	Az
	9	653G>A	R218Q	12/M	1, 4	483/716	+	−	d.n.[8]	−/−	...
	10	652C>T	R218W	6/F	1	365/753	−	+	d.n.[4,8]	+/−	FL
	11	652C>T	R218W	11/F	3	508/701	+	−	d.n.[4,8]	−/−	Ib, BB
	12	652C>T	R218W	6/M	2	NA/NA	+	+	d.n.[4,8]	−/−	IV
	13	652C>T	R218W	19/F	2	468/681	+	−	d.n.[4,8]	+/−	BB
	14	652C>T	R218W	12/M	1, 2, 3	400/689	+	−	d.n.[4,8]	−/−	...
	15	238C>T 904G>A	R80C* V302 M	5/M	1, 2	468/681	+	+	R80C: − V302 M: d.n., tr[12]	−/−	...
ATS cardiac phenotype alone (n=5)	16	245G>A	R82Q	46/F	2, 6	553/680	−	−	d.n. Figures 4, 5	−/−	...
	17	244C>T	R82W	29/F	1, 2	433/672	−	−	d.n.[9,27] Figures 4, 5	−/−	BB
	18	652C>T	R218W	6/F	1, 3	427/617	−	−	d.n.[4,8]	−/−	BB
	19	652C>T	R218W	5/M	1, 3	392/707	−	−	d.n.[4,8]	−/−	IV
	20	683ins§	R228ins*‖	24/M	1, 4	498/...	−	−	−	−/−	Pil
Periodic paralysis alone	21	199C>T	R67W	24/M	...	425/625	−	+	d.n.[8]	−/−	Az
	22	1106C>A	S369X	13/M	...	472/600	−	+	tr[7]	−/−	...
CPVT	23	431G>A	G144D	32/F	3	465/NA	−	−	Figures 4, 5	+/+	BB, FL
	24	914C>G	T305S*	36/F	1, 4, 5	443/664	−	−	Figures 4, 5	+/+	BB, ICD

NA indicates not available; age, age at diagnosis; ACA, aborted cardiac arrest; CPVT, catecholaminergic polymorphic ventricular tachycardia; ICD, implantable cardioverter-defibrillator; BB, β-blocker; Ib, mexiletine; IV, verapamil; FL, flecainide; Pil, pilsicainide; and Az, acetazolamide.

*Novel mutation.

†PVC=1, bVT=2, pVT=3, VT=4, ventricular fibrillation=5, long-QT=6.

‡d.n. indicates dominant negative; tr, trafficking defect.

§683insGAAAAGCCACTTGGTGGAAGCTCATGTTCG.

‖R228insKSHLVEAHVR.

provided informed consent before the genetic analysis was carried out. Genomic DNA was isolated from leukocyte nuclei using a DNA purification kit (Maxwell Blood DNA Purification Kit, Promega, Madison, WI). Genetic screening was first performed using denaturing high-performance liquid chromatography (dHPLC WAVE System; Transgenomic, Omaha, NE).[18] Abnormal conformers were amplified via PCR, and sequencing was performed on an ABI 3130 DNA sequencer (Perkin Elmer, Foster City, CA). The cDNA sequence numbering was based on the GenBank reference sequence NM 000891.2 for *KCNJ2*. Regarding suspected CPVT probands, we also performed screening involving target mutation analysis for 34 *RyR2* gene exons (3, 8–16, 44–49, 83–84, 88–89, 91–97, and 99–105)[19,20] and all exons of the *CASQ2* gene. In addition to these 3 genes, we examined the entire coding sequence of *KCNQ1*, *KCNH2*, *SCN5A*, and *KCNE1-5* to exclude the unexpected presence of compound mutations related to primary electric diseases.[21,22] When a mutation was detected, we examined its presence in >200

Japanese control subjects to exclude the possibility of polymorphisms. When mutations were detected in probands, we also screened their family members.

Genotype-Phenotype Correlation

Baseline clinical characteristics collected were the age at diagnosis, symptomatic episodes, and treatment. As shown in Figure 1, we divided all *KCNJ2* mutation carriers into 2 groups—a typical ATS group (group A): carriers showing 2 or more ATS features, and an atypical ATS group (group B): those showing only 1 of the ATS features or CPVT. Compound mutation and *KCNJ2* double mutation cases were excluded from analysis.

In Vitro Mutagenesis

Regarding four *KCNJ2* mutations found in group B (R82W, R82Q, G144D, and T305S), site-directed mutagenesis was used to construct mutants, as described previously.[23] Briefly, human *KCNJ2* cDNA

was subcloned into the pCMS-EGFP plasmid (Clontech, Palo Alto, CA). We engineered *KCNJ2* mutants using a site-directed mutagenesis kit, QuickChange II XL (Stratagene, La Jolla, CA). The presence of mutations was confirmed by sequencing.

Electrophysiological Experiments and Data Analysis

To assess the functional modulation of *KCNJ2* channels, we used a heterologous expression system with CHO cells.[8,24] Briefly, the cells were transiently transfected using the Lipofectamine method (Invitrogen, Carlsbad, CA), using a 1.0 μg/35 mm dish of pCMSEGFP/*KCNJ2* (wild-type [WT] and/or mutant). For electrophysiological experiments, GFP-positive cells were selected 24 to 72 hours after transfection. Current measurement was conducted using the conventional whole-cell configuration of patch-clamp techniques at 37°C, using an EPC-8 patch-clamp amplifier (HEKA Electronik; Lambrecht, Germany). Currents were evoked by 150 ms square pulses applied in 10 mV increments to potentials ranging from −140 mV to +30 mV from a holding potential of −80 mV. Pipettes were filled with a solution containing (in mmol): K-aspartate, 60; KCl, 65; KH_2PO_4, 1; $MgCl_2$, 2; EDTA, 3; ATP (dipotassium salt), 3; and HEPES, 5 (pH adjusted to 7.2 with KOH), and had a resistance of 3.0 to 5.0 mol/LΩ. The bath solution contained (in mmol): NaCl, 140; KCl, 5.4; $MgCl_2$, 0.5; $CaCl_2$, 1.8; NaH_2PO_4, 0.33; glucose, 5.5; and HEPES, 5 (pH adjusted to 7.4 with NaOH).[25]

Immunocytochemistry

The hemagglutinin (HA) epitope (YPYDVPDYA) was introduced into the pCMS-EGFP/*KCNJ2* (WT and mutants) between Ala-115 and Ser-116 (extracellular lesion between TM1 and TM2), as previously described.[8] CHO cells were transfected with 1.0 μg of plasmid DNA in 35-mm, glass-bottom dishes. Forty-eight hours later, the cells were washed twice with phosphate-buffered saline (PBS), followed by incubation with a mouse anti-HA primary antibody (1:400) (Roche Diagnostics GmbH, Mannheim, Germany) overnight at 4°C. The cells were then washed twice with PBS and incubated with an anti-mouse antibody conjugated to Alexa 568 fluor (1:400) (Molecular Probes, Eugene, OR) as a secondary antibody for 120 minutes, at room temperature. Finally, cells were washed with and immersed in PBS, and confocal imagings were obtained with a Nikon C1si (Nikon Instruments, Tokyo, Japan).

Statistical Analysis

Clinical data are expressed as the mean±SD for continuous variables. Comparisons were performed using the χ^2 test (for counts ≥5) and Fisher exact test (for counts <5) for categorical variables and Wilcoxon test for continuous variables. All analyses of the 45 *KCNJ2* mutation-positive patients and families took into account the relatedness of patients, using a mixed model for continuous data and GEE for categorical data. The electrophysiological current data are shown as mean±SEM. A value of $P<0.05$ was considered significant.

Results

Incidence and Characteristics of *KCNJ2* Mutations in Probands

We identified 16 different *KCNJ2* mutations in 24 of 57 probands (42%) (Table 1 and Figure 1). The mean QUc intervals became longer in ATS with an increasing number of ATS features. Prevalences of *KCNJ2* mutation were 75% (15/20) in typical ATS, 71% (5/7) in mild ATS with a cardiac phenotype alone, 100% (2/2) in mild ATS with periodic paralysis alone, and 7% (2/28) in CPVT.

Table 2 summarizes the genotype/phenotype of *KCNJ2* mutation-positive probands. The mean age at diagnosis of all probands was 18±12 years old. It was significantly younger (14±12 years old) in probands with typical ATS compared
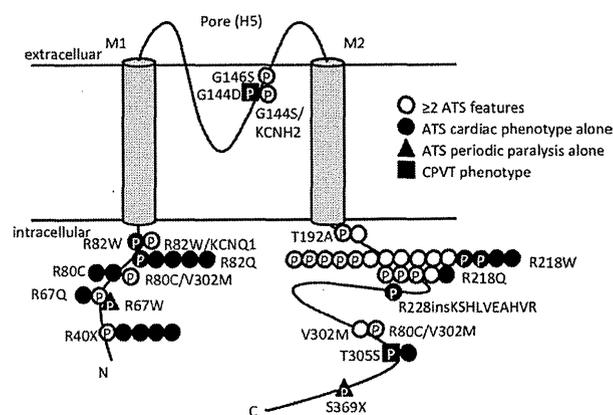

Figure 2. Topology of Kir2.1 channel showing Andersen-Tawil syndrome (ATS)-related mutation sites. **Open circles** indicate probands showing 2 or more ATS features (group A). **Closed symbols** indicate the probands in group B. **Closed circles** indicate those showing ATS with the cardiac phenotype alone; **closed triangles** are ATS with periodic paralysis alone; and **closed squares** represent a clinical diagnosis of CPVT. "P" in each symbol indicates proband.

with those with mild ATS (21±14 years old) or the CPVT phenotype (34±3.5 years old). In 16 *KCNJ2* mutations, 13 (81%) were missense, 2 (13%) nonsense, and 1 (6%) insertion (Table 2 and Figure 2). Six were located in the N terminus, 3 in the pore region, and 9 in the C terminus. Figure 2 depicts the phenotypes and mutation sites of probands and family members. Open circles indicate mutation carriers with 2 or more ATS features (group A), and closed symbols those with atypical ATS (group B); closed circles show the ATS cardiac phenotype alone, closed triangles show periodic paralysis alone, and closed squares show the CPVT phenotype. The letter P in each symbol indicates proband. A 5-year-old boy (case 15, Table 2) was found to have double *KCNJ2* mutations, paternal V302 M, and maternal R80C. Compound mutations were detected in 2 probands: *KCNJ2* R82W plus *KCNH2* P1093L (case 3) and *KCNJ2*-G144S plus *KCNQ1*-A341V (case 4). We excluded these 3 cases from further analyses.

Characteristics of *KCNJ2* Mutation Carriers

After excluding the compound mutation cases, 45 *KCNJ2* mutation carriers (27 females, 21 probands, and 24 of their mutation-positive family members) were enrolled (Table 3). Their mean age at diagnosis was 23±16 years, and the average QUc was 667±50 ms. Regarding arrhythmias, ECGs detected PVC in 30 (67%), bVT in 15 (33%), and pVT in 5 (11%) carriers. One patient had ventricular fibrillation, 4 patients (9%) had syncope, and 11 (24%) received β-blocker therapy.

Prevalence of 3 ATS Features

In 45 *KCNJ2* mutation carriers, ventricular arrhythmias (A) were found in 67% (n=30), periodic paralysis (P) in 40% (n=18), and dysmorphism (D) in 36% (n=16). Abnormal U wave (U) was positive in 88% (n=38 of 43) after excluding 2 cases whose U waves were not measured because of the presence of bigeminy. Twenty-one patients (47%) belonged