Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Atrioventricular Block-Induced Torsades de Pointes With Clinical and Molecular Backgrounds Similar to Congenital Long QT Syndrome

Yuko Oka, MD; Hideki Itoh, MD, PhD; Wei-Guang Ding, MD, PhD; Wataru Shimizu, MD, PhD; Takeru Makiyama, MD, PhD; Seiko Ohno, MD, PhD; Yukiko Nishio, MD; Tomoko Sakaguchi, MD, PhD; Akashi Miyamoto, MD; Mihoko Kawamura, MD; Hiroshi Matsuura, MD, PhD; Minoru Horie, MD, PhD

Background: Atrioventricular block (AVB) sometimes complicates QT prolongation and torsades de pointes (TdP).

Methods and Results: The clinical and genetic background of 14 AVB patients (57±21 years, 13 females) who developed QT prolongation and TdP was analyzed. Electrophysiological characteristics of mutations were analyzed using heterologous expression in Chinese hamster ovary cells, together with computer simulation models. Every patient received a pacemaker or implantable cardioverter defibrillator; 3 patients had recurrence of TdP during follow-up because of pacing failure. Among the ECG parameters, QTc interval was prolonged to 561±76 ms in the presence of AVB, but shortened to 495±42 ms in the absence of AVB. Genetic screening for *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* revealed four heterozygous missense mutations of *KCNQ1* or *KCNH2* in 4 patients (28.6%). Functional analyses showed that all mutations had loss of functions and various gating dysfunctions of I_{Ks} or I_{Kr}. Finally, action potential simulation based on the Luo-Rudy model demonstrated that most mutant channels induced bradycardia-related early afterdepolarizations.

Conclusions: Incidental AVB, as a trigger of TdP, can manifest as clinical phenotypes of long QT syndrome (LQTS), and that some patients with AVB-induced TdP share a genetic background with those with congenital LQTS. (*Circ J* 2010; **74:** 2562–2571)

Key Words: Atrioventricular block; Ion channels; Long QT syndrome; Torsades de pointes

The acquired form of long QT syndrome (LQTS) is a major cause of torsades de pointes (TdP),[1,2] which results from various factors, including drugs, bradycardia or hypokalemia. Regarding bradycardia, Kurita et al demonstrated that patients with bradycardia-induced TdP display abnormally prolonged QT intervals at slower heart rates (<60 beats/min) than those without TdP.[3] Some groups have reported the genetic background of bradycardia-induced TdP, as well as of congenital LQTS. In 2001, we reported a female with 2:1 atrioventricular block (AVB) and TdP, in whom the *KCNH2* A490T mutant was identified as heterozygous.[4] Subsequently, Lupoglazoff et al demonstrated that, in neonates, LQTS with 2:1 AVB is associated with *KCNH2* mutations whereas sinus bradycardia-related LQTS is associated with *KCNQ1* mutations.[5] Chevalier et al reported that among 29 patients with complete AVB and a QT interval >600 ms, 5 (17%) had mutations on genes encoding K^+ channels, and the expression test of these mutations showed functional changes compared with the wild-type (WT) K^+ current.[6]

Editorial p2546

In Japan, some papers on congenital LQTS have been published,[2,7–9] but the molecular pathogenesis of AVB-related TdP has not been fully examined, particularly with respect to the relationship between genotype and cellular electrophysiology. The aim of this study was to investigate gene mutations and clarify their functional outcome in con-

Received May 31, 2010; revised manuscript received July 30, 2010; accepted August 2, 2010; released online October 21, 2010 Time for primary review: 16 days

Department of Respiratory and Cardiovascular Medicine (Y.O., H.I., T.S., A.M., M.K., M.H.), Department of Physiology (W.-G.D., H.M.), Shiga University of Medical Science, Otsu; Division of Cardiology, Division of Arrhythmia and Electrophysiology, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Suita (W.S.); and Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto (T.M., S.O., Y.N.), Japan

Mailing address: Minoru Horie, MD, PhD, Department of Respiratory and Cardiovascular Medicine, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu 520-2192, Japan. E-mail: horie@belle.shiga-med.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-10-0498

secutive AVB patients complicated with TdP.

Methods

Study Population

The study cohort contained 14 consecutive probands, from unrelated families, who showed a prolonged QT interval and TdP associated with AVB. They were referred to 3 institutes in Japan; Shiga University of Medical Science (Otsu), National Cardiovascular Center (Suita), and Kyoto University Graduate School of Medicine (Kyoto) for LQTS genetic testing between 1996 and 2008.

Clinical Characterization

In each case, we recorded 12-lead electrocardiograms (ECGs) before and after AVB episodes, as well as gathering the results from other cardiovascular examinations and detailed clinical evaluations. Prolonged QT interval was diagnosed by the presence of prolongation of ventricular repolarization (corrected QT interval [QTc] >460 ms in lead V_5, according to Bazett's formula).[10] We excluded cases of TdP caused by AVB with drugs associated with QT prolongation, as well as those with active ischemia detected by noninvasive or invasive tests, including coronary angiography. We also investigated cardiac events in all 14 probands and their family members. Cardiac events were syncope, TdP, ventricular fibrillation (VF), aborted cardiac arrest (requiring defibrillation) or sudden cardiac death. We also followed the therapies and clinical prognoses of these patients.

Genetic Analysis

Genomic DNA was isolated from venous blood by QIAamp DNA blood midikit (Qiagen, Hilden, Germany). Established primer settings were used to amplify the entire coding regions of the known LQTS genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*). Denaturing high-performance liquid chromatography (WAVE system Model 3500, Transgenomic, Omaha, NE, USA) was performed as described elsewhere, and abnormal conformers were amplified by polymerase chain reaction (PCR), and sequenced with an ABI PRISM-3130 sequencer (Perkin-Elmer Applied Biosystems, Wellesley, MA, USA). If we detected mutations in these genes, family members associated with the probands were also genetically analyzed. Formal informed consent was obtained from each patient or their guardians according to standards approved by local institutional review boards.

Expression Plasmids

The expression plasmids, pIRES2-EGFP/*KCNQ1*(wild-type; WT/*KCNQ1*) and pRc-CMV/*KCNH2* (WT/*KCNH2*) were kindly provided by Dr Barhanin (Université de Nice, Sophia Antipolis, Valbonne, France) and Dr Sanguinetti (University of Utah, Salt Lake City, UT, USA), respectively. The mutations were introduced using overlap PCR. The mutant plasmids were constructed by substituting the 838-bp *Xho*I-*Bgl*II for the G272V mutant, 464-bp *Hin*dIII-*Bst*XI for the D111V mutant, 1458-bp *Bst*XI-*Bgl*II for the A490T mutant, or 592-bp *Fse*I-*Sbf*I fragments for the P846T mutant for the corresponding fragments of WT/*KCNQ1* or WT/*KCNH2*. The nucleotide sequence of the construct was confirmed prior to the expression studies.

Expression in Chinese Hamster Ovary (CHO) Cells

CHO cells were maintained in Dulbecco's modified Eagle's medium and Ham's F12 nutritional mixture (Gibco-BRL, Rockville, MD, USA) supplemented with 10% fetal bovine serum (Gibco-BRL) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) in a humidified incubator gassed with 5% CO_2 and 95% air at 37°C. CHO cells were transiently transfected using 1 μg of WT/*KCNQ1* or mutant/*KCNQ1*, and 1 μg of pIRES-CD8/*KCNE1* per 35-mm dish, using the LipofectAMINE method according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). In some experiments, 0.5 μg of WT/*KCNQ1* was transfected with or without mutant/*KCNQ1*, instead of 1 μg of WT/*KCNQ1*. Cells successfully transfected with both *KCNQ1* and *KCNE1* cDNA were selected by green fluorescent protein (GFP) and decoration with anti-CD8 antibody-coated beads (Dynabeads CD8; Dynal Biotech, Oslo, Norway). The cells were transiently transfected with either WT/*KCNH2* or mutant/*KCNH2*, using the LipofectAMINE method according to the manufacturer's instructions. For a 35-mm dish the amount of plasmid was 2 μg and 0.175 μg of GFP; only GFP-positive cells were used for the patch-clamp study.

Electrophysiological Experiments

Whole-cell patch-clamp recordings were conducted at 37.0±1.0°C using an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany) 48–72 h after transfection. No leak subtraction was used. The normal Tyrode solution contained (in mmol/L): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5 (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mmol/L): potassium aspartate 70, KCl 40, KH_2PO_4 10, EGTA 5, $MgSO_4$ 1, Na_2-ATP (Sigma, St Louis, MO, USA) 3, Li_2-GTP 0.1, and HEPES 5 (pH adjusted to 7.4 with KOH). A coverslip with adherent CHO cells was placed on the bottom of a glass recording chamber (0.5 ml in volume) mounted on the stage of an inverted microscope (TE2000-U, Nikon, Tokyo, Japan). Pipette resistance was 3–5 MΩ when filled with internal solution. Currents and voltages were digitized and voltage commands were generated through an LIH-1600 AD/DA interface (HEKA) controlled by PatchMaster software (HEKA). Current amplitude was divided by membrane capacitance (Cm) to obtain current densities (pA/pF) in each cell. The voltage-dependence of current activation was determined by fitting the normalized tail current (I_{tail}) vs test potential (V_{test}) to a Boltzmann function:

$$I_{tail} = 1/(1+\exp[(V_{0.5}-V_t)/k]),$$

where $V_{0.5}$ indicates the voltage at which the current is half-maximally activated and k is the slope factor.

Computer Simulation of Action Potential Duration (APD)

Ventricular action potentials were simulated by using the dynamic Luo-Rudy model with recent modifications.[11,12] The ratio of I_{Kr} and I_{Ks} conductance was set at 23:1, 17:1, and 19:1 in the epicardium, endocardium, and M cell layer, respectively. Based on the experimental data of voltage-clamp recordings of *KCNH2* channels heterologously expressed in CHO cells, we constructed Markov or Hodgkin-Huxley models for simulated mutant channels as compared with mutants associated with congenital LQTS. In order to construct mutant channel models, we decreased the conductance of each channel as appropriate for the decreased current density, and looked for adequate changes in mutant channels by changing each coefficient value, in turn, for gating states associated with impaired gating defects. The simulation for voltage-clamp experiments was calculated using the 4th-order Runge-Kutta method with a fixed-time step of

Table 1. Clinical Characteristics and Gene Mutations of Probands With Bradycardia-Induced Torsades de Pointes

Case no.	Age (years)	Sex (M/F)	Diagnosis	Cardiac events	Family history	ECG at AVB		ECG without AVB		Therapy	Follow-up		Mutation/Gene
						QTc (ms)	HR (beats/min)	QTc (ms)	HR (beats/min)		Period (months)	Arrhythmic events	
1	27	F	2:1 AVB	TdP	–	600	50	545	71	PM	41	None	A490T/KCNH2
2	74	F	CAVB	TdP	–	NA	NA	NA	NA	PM	22	None	
3	73	M	CAVB	TdP	–	635	39	NA	NA	PM	104	None	
4	57	F	CAVB	TdP	–	525	43	545	61	ICD, BB, Ib	96	None	D111V/KCNH2
5	69	F	2:1 AVB	TdP	–	452	45	476	86	PM, BB, Ib	84	None	
6	21	F	Wenckebach AVB	TdP	Sudden death	625	65	489	75	ICD, Ib	79	VF because of Wenckebach AVB	
7	76	F	2:1 AVB	TdP	–	578	50	424	83	PM, BB	59	None	G272V/KCNQ1
8	71	F	CAVB	TdP	–	729	57	489	72	ICD	46	None	P846T/KCNH2
9	73	F	CAVB	TdP	–	567	33	NA	NA	PM	129	None	
10	62	F	CAVB	TdP	–	473	29	NA	NA	PM	277	None	
11	68	F	CAVB	TdP	–	552	39	NA	NA	PM	207	TdP because of V pacing failure	
12	38	F	CAVB	TdP	Mother with LQTS	493	28	500	65	PM, BB	NA	NA	
13	76	F	CAVB	TdP	–	500	46	NA	NA	PM	57	TdP because of low back-up rate	
14	18	F	CAVB	TdP	–	570	47	NA	NA	PM	130	None	
Ave±SD	57±21					561±76	44±11	495±42	64±27		102±71		

AVB, atrioventricular block; TdP, torsades de pointes; PM, pacemaker; CAVB, complete atrioventricular block; NA, not available; ICD, implantable cardioverter defibrillator; BB, β-blocker; Ib, class Ib antiarrhythmic drugs; VF, ventricular fibrillation.

0.02 ms. The simulation programs were coded in C++ and implemented for personal computers.[12]

Statistical Analysis

Numerical data are presented as mean±standard error of the mean. Student's t-test was used to compare the data between different groups for electrophysiological measurement, and differences were considered significant at $P<0.05$.

Results

Clinical Characteristics of the Patients in This Study

The clinical characteristics of the 14 patients enrolled in the study are presented in Table 1. The mean age at the onset of AVB was 57±21 years, and 13 patients (92.8%) were females. All patients showed TdP with AVB: 10 had complete AVB, 3 had 2:1 AVB, and 1 had Wenckebach AVB. No patient had experienced syncope or ventricular arrhythmias prior to the appearance of TdP. One patient (case 6 in Table 1) with Wenckebach AVB had 2 family members who had suddenly died at the age of 1 year and 3 months, respectively. The mother of case 12 (Table 1) had atrial fibrillation, mitral regurgitation, and complete AVB with prolonged QT interval, but no TdP.

In most patients with AVB-related TdP, the tachyarrhythmia started from premature ventricular contractions after a long-pause interval following ventricular arrhythmias, so-called "TdP from short–long–short pattern" (Figure 1D).[13] The ECGs available at the time of AVB showed severely prolonged QT interval (heart rate 44±11 beats/min and QTc 561±76 ms). On the other hand, the ECGs without AVB available in 7 cases also showed a prolonged QT interval (heart rate 64±27 beats/min, $P<0.05$, and QTc 495±42 ms, P=NS, vs those in AVB). ECGs in sinus rhythm were obtained in 4 and 3 patients before and after AVB, respectively.

All patients underwent implantation of either an implantable cardioverter-defibrillator (ICD) or permanent pacemaker (PM), together with the administration of several drugs, including β-blockers (ICD n=3; PM n=11). Mean clinical follow-up during advanced therapy was 102±71 months. After the placement of a PM or ICD, 2 patients maintained own ventricular beats but the other 12 depended on ventricular pacing during the follow-up period. Three patients had recurrence of TdP even while receiving treatment. Patient no. 11 suddenly experienced repetitive TdP because of pacing failure and no. 13 also experienced TdP when her own ventricular beats had been set faster than the basal pacing rate. In patient 6, the reappearance of Wenckebach AVB without ventricular pacing caused ventricular tachycardia. In all 3 cases, no gene mutations were detected.

Molecular Genetics and Clinical Characteristics of Patients With Gene Mutations

The genetic analysis revealed different heterozygous mutations in 4 (28.6%) of 14 AVB-related TdP cases (Table 1): 1 *KCNQ1* mutation, G272V, and 3 *KCNH2* mutations, D111V, A490T and P846T (Figure 1A). All were located in the non-pore regions; G272V is located in the S5 domain for the *KCNQ1* channel; D111V, A490T, and P846T are located in the N-terminus, S2–S3 inner loop, and C-terminal domains for the *KCNH2* channel, respectively (Figure 1B). In the remaining 10 patients, we were unable to detect any mutations associated with the 5 major LQTS-related genes.

G272V in *KCNQ1* (Case 7 in Table 1) The G272V muta-

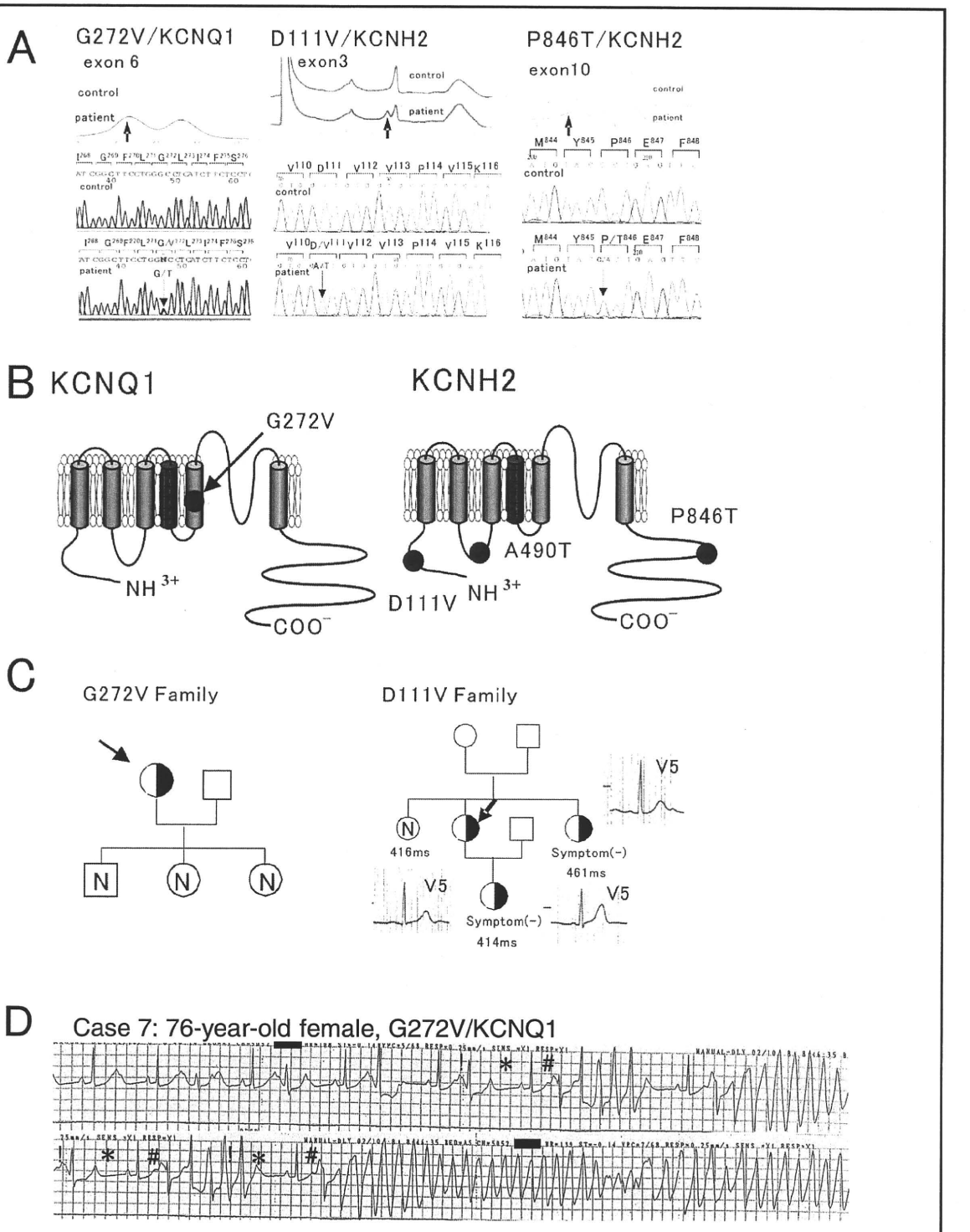

Figure 1. Molecular discovery and clinical data associated with *KCNQ1* and *KCNH2* mutations, and the initiation of atrioventricular block -related torsades de pointes (TdP). (**A**) Denaturing high-performance liquid chromatography patterns and DNA sequence data in normal controls and patients with G272V for *KCNQ1* (**Left**), D111V for *KCNH2* (**Middle**), and P846T for *KCNH2* (**Right**). (**B**) Schemes showing the topology of cardiac ion channel proteins for *KCNQ1* and *KCNH2* and the location of mutations identified in this study. (**C**) Two pedigrees of G272V and D111V families. Circles and squares represent female and male family members, respectively; probands are indicated by arrows. Heterozygous carriers are represented as half-filled symbols, family members in whom no genetic data were available are shown by open symbols, and non-carriers by open symbols with N. QTc intervals corrected by Bazzet's formula in lead V5 are given for each available family member. (**D**) Representative ECG recordings from case 7 (76-year-old female with G272V-KCNQ1 mutation). TdP during 2:1 AV block started with so-called "short (!)–long (*)–short (#) pattern" which resulted in a long pause (*).[13]

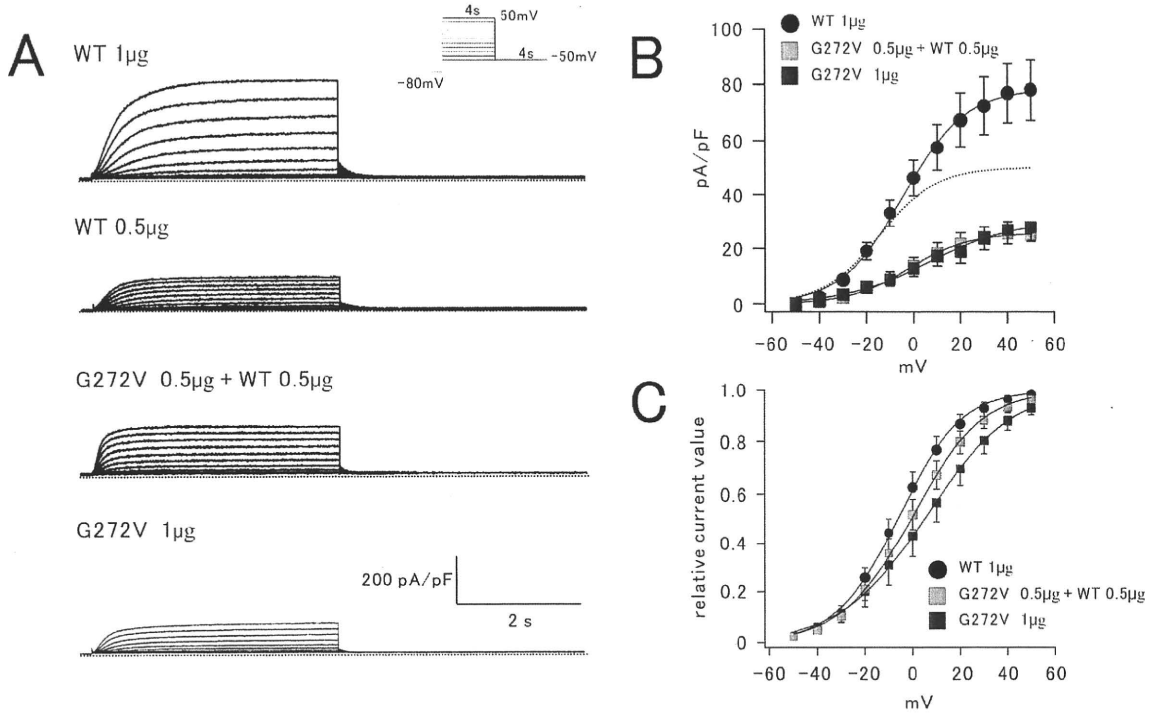

Figure 2. G272V KCNQ1 mutant channel shows loss of function associated with decreased density on I_{Ks}. Functional analyses on wild-type (WT) and G272V mutant channels expressed in Chinese Hamster ovary cells. (**A**) Representative current recordings for each channel. Currents were elicited from a holding potential of –80 mV, by depolarizing pulses (4-s duration) from –50 to +50 mV (with a 10-mV step increment) and subsequent repolarization to –50 mV for a 4-s duration (inset). Concentrations of cDNAs used for transfection are indicated near each graph. (**B**) Tail current-voltage relationships for 1 μg WT (closed circles; n=18), 0.5 μg WT (indicated by dotted line; n=14), 0.5 μg WT plus 0.5 μg G272V (green squares; n=19), 1 μg G272V (red squares; n=11). (**C**) Normalized activation curves by fitting to Boltzmann function.

tion was identified in a 76-year-old female who did not have a particularly relevant family history (**Figure 1A Left panel**). For approximately 10 years, she had taken nilvadipine and gliclazide because of hypertension and diabetes mellitus. Approximately 1 year before hospitalization, her QTc interval was within normal range (424 ms). When she was admitted to hospital because of syncope, her monitoring ECGs displayed 2:1 AVB (50 beats/min), prolonged QTc interval (578 ms), and repetitive TdP (**Figure 1D**). Her serum K^+ level was low (2.5 mEq/L). Because AVB persisted, she underwent DDD PM implantation. After correction of the serum K^+ level and PM therapy, her QTc interval shortened and TdP disappeared. She was free from cardiac events for the following 59 months. The genetic analysis revealed 3 children as non-mutation carriers (**Figure 1C Left panel**).

D111V in *KCNH2* (Case 4 in Table 1) The D111V mutation was identified in a 57-year-old female who did not have a particularly relevant family history (**Figure 1A Middle panel**). She experienced syncope after eating breakfast, and the monitoring ECG in the ambulance documented complete AVB (43 beats/min), prolonged QTc interval (525 ms) and TdP. After external PM therapy was initiated, TdP disappeared. She then underwent ICD implantation and started oral mexiletine hydrochloride (300 mg/day) and propranolol hydrochloride (30 mg/day); she has had no cardiac events over a follow-up period of 96 months. However, her QTc interval has remained prolonged even in the absence of AVB (545 ms, 4 years later). The genetic tests in her 3 relatives showed 2 mutation carriers (**Figure 1C Right panel**): a 51-year-old sister and 29-year-old daughter. Both these relatives were asymptomatic. Her daughter's QTc interval was within normal range (414 ms), but the sister's was prolonged (461 ms).

P846T in *KCNH2* (Case 8 in Table 1) The P846T mutation was found in a 71-year-old female who did not have a particularly relevant family history (**Figure 1A Right panel**). She experienced syncope after breakfast, and the monitoring ECG in the ambulance displayed complete AVB (45 beats/min) and repetitive TdP with prolonged QT interval. On admission, her AV conduction resumed at 57 beats/min, but her QTc interval remained prolonged (729 ms). After ICD implantation, she was free from cardiac events for 46 months, but her QTc interval remained prolonged (489 ms). We did not conduct a genetic analysis in this family.

A490T in *KCNH2* (Case 1 in Table 1) We have previously reported the clinical features of a A490T mutation identified in a 27-year-old female.[3] Briefly, her 12-lead ECG showed severe bradycardia because of 2:1 AVB (50 beats/min) with complete left bundle branch block and remarkable prolongation of QTc interval (600 ms). She fainted and collapsed while talking on the telephone, and the Holter ECG showed TdP associated with 2:1 AVB.

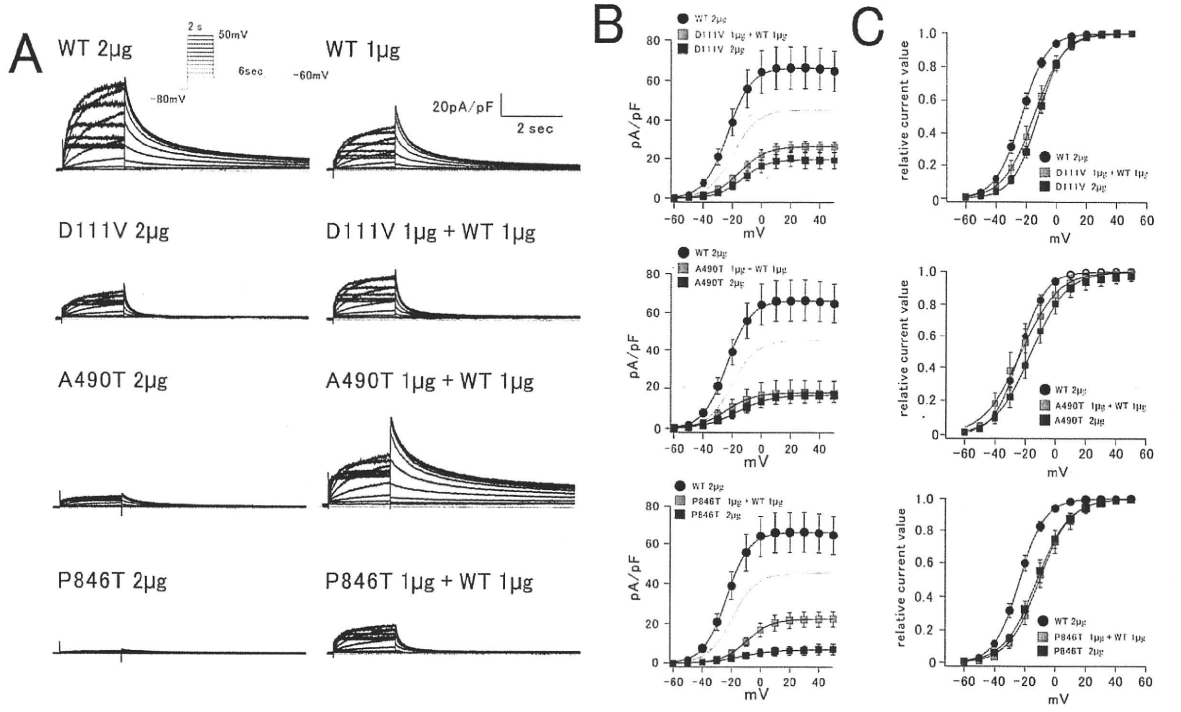

Figure 3. All *KCNH2* mutant channels show loss of function associated with decreased density on I_{Kr}. Electrophysiological characteristics of *KCNH2* mutant channels in Chinese Hamster ovary cells. (**A**) Families of representative current traces. Experimental protocol is shown in the inset. Concentrations of cDNAs used for transfection are indicated above each graph. (**B**) Tail current-voltage relationships for 2 μg wild-type (WT: closed circles; n=20), 1 μg WT (dotted line; n=14), 1 μg WT plus 1 μg mutant (green squares; n=10–13), 2 μg mutant (red squares; n=8–12). (**C**) Normalized activation curves by fitting to Boltzmann function.

Table 2. Parameters of Inactivation in WT and Mutant *KCNH2*

	WT (n=16)	WT/D111V (n=16)	D111V (n=15)	WT/A490T (n=17)	A490T (n=15)	WT/P846T (n=15)	P846T (n=16)
$V_{0.5}$ (mV)	−58.3±4.7	−40.1±4.1**	−47.4±7.0	−32.5±3.9*	−44.2±3.3	−38.7±2.4**	−55.5±3.5
Slope factor	29.2±1.4	33.9±1.3	35.3±1.7**	30.6±1.4†	34.9±1.1**	33.0±0.6†	37.5±1.7*

*P<0.001 vs WT, **P<0.01 vs WT, †P<0.05 vs WT.
WT, wild-type.

Expression Study

In order to clarify the functional consequences of the G272V mutation of *KCNQ1* and the D111V, A490T, and P846T mutations of *KCNH2*, we assessed the electrophysiological properties of the WT and mutant clones by using CHO cells.

Biophysical Assay of *KCNQ1* Mutant Channel Figure 2A shows representative examples of whole-cell currents recorded from CHO cells transfected with WT/*KCNQ1*, G272V/*KCNQ1* alone or WT co-expressed G272V/*KCNQ1* (WT/G272V) plus *KCNE1*. CHO cells transfected with WT/*KCNQ1* (1 or 0.5 μg) displayed outward currents with slow activation/deactivation kinetics on depolarization, which are typical of I_{Ks} currents, as previously reported.[14,15] In contrast, a cell transfected with G272V/*KCNQ1* (1 μg) displayed smaller I_{Ks} currents compared with that of the WT (1 μg). WT/G272V at an equimolar ratio (0.5 μg) also showed smaller I_{Ks} currents.

In Figure 2B, the tail current densities at −50 mV measured in multiple cells are plotted as a function of test pulse voltages (between −50 and +50 mV). The tail current densities at −50 mV after depolarizing test pulses to +40 mV were 77.0±11 pA/pF for 1 μg WT (n=18), 49.5±7.9 pA/pF for 0.5 μg WT (n=14), 25.4±4.5 pA/pF for 0.5 μg WT/G272V (n=19) (vs WT 1 μg, P<0.001), 26.7±4.9 pA/pF for 1 μg G272V (n=11) (vs WT 1 μg, P<0.01). Thus, compared with the WT I_{Ks} current, co-transfection of the mutant affected the expressed current densities.

Figure 2C represents the voltage-dependence of current activation. Tail current densities after each test potential were fitted to a Boltzmann function (see Methods). The parameters were $V_{0.5}$=−5.2±3.0 mV, k=11.1±0.6 for 1 μg WT, $V_{0.5}$=−1.0±3.7 mV, k=11.7±1.1 for 0.5 μg WT/G272V, $V_{0.5}$=5.7±5.4 mV, k=14.3±1.6 (vs WT 1 μg; P<0.05) for 1 μg G272V. Regarding half-activation voltages, WT plus G272V and G272V tended to shift to the depolarization side compared with WT but there was no statistical significance. In

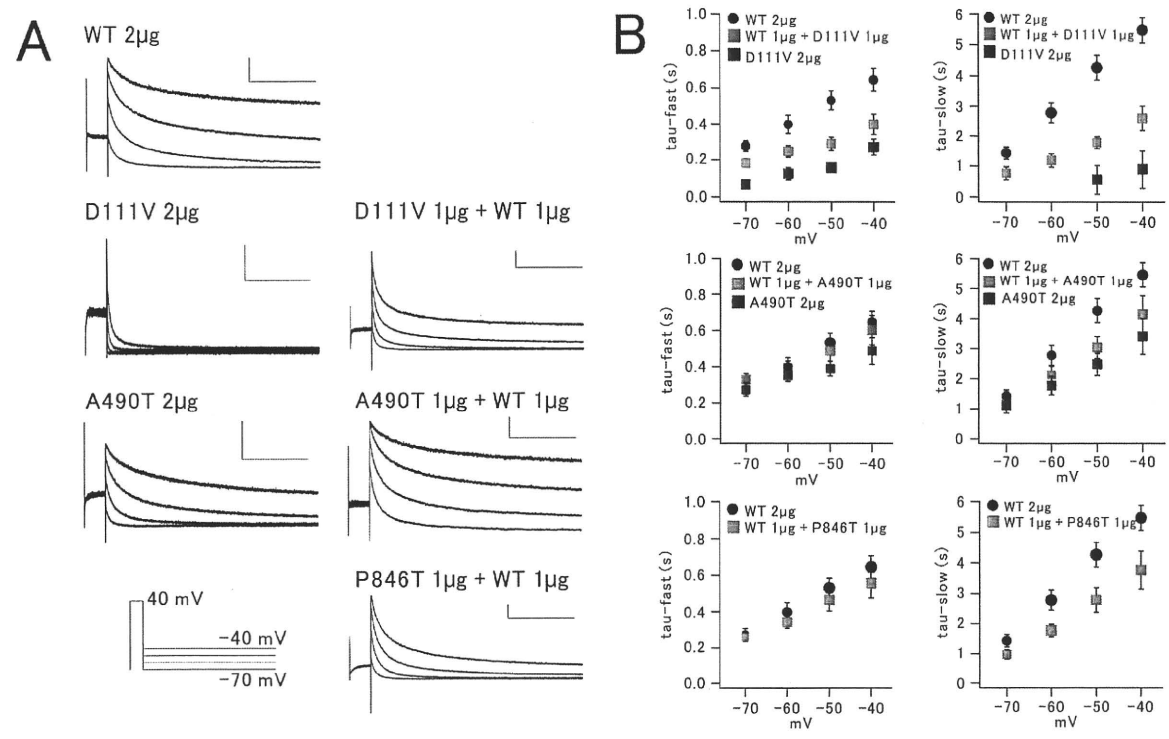

Figure 4. *KCNH2* mutant channels show a faster deactivation. (**A**) Representative tail currents elicited after depolarization to +40 mV (inset protocol). (**B**) Deactivation process was fitted to 2 components having different time constants, tau-fast and tau-slow. They were calculated in a pooled data from multiple cells transfected by 3 mutants (from top of panel B, D111V, A490T and P846T, respectively) and are plotted against membrane potentials (−70 to −40), for 2 μg WT (closed circles; n=15), 1 μg WT plus 1 μg mutant (green squares; n=7–14 for each channel), 2 μg mutant (red squares; n=6–8).

slope factors, G272V alone channel was larger than WT ($P<0.05$). Overall, the most important finding was the dominant-negative effect for the G272V channel.

Biophysical Assay of 3 *KCNH2* Mutant Channels Figure 3A shows representative examples of whole-cell currents recorded from CHO cells transfected with WT/*KCNH2* (2 and 1 μg), mutant/*KCNH2* (2 μg), or WT co-expressed mutant/*KCNH2* (WT/mutant) (1 μg each). CHO cells transfected with WT/*KCNH2* (2 or 1 μg, **Figure 3A Upper 2 panels**) displayed outward currents with inward rectifying properties, which are typical of I_{Kr} currents.[16] In contrast, the magnitude of currents from cells expressing all of the WT/mutants and mutant only were remarkably reduced (**Figure 3A Lower 6 panels**).

In **Figure 3B**, the tail current densities at −60 mV are plotted as a function of test pulse voltages (between −60 and +50 mV). The mean current densities after depolarizing test pulse to +20 mV in WT channels were 66.2±11 pA/pF for 2 μg (n=20) and 45.0±9.3 pA/pF for 1 μg (n=14). In contrast, those in the WT/mutant and mutant channels were 25.1±2.9 pA/pF in WT/D111V (n=13), 15.8±6.0 pA/pF in WT/A490T (n=10), 20.5±3.9 pA/pF in WT/P846T (n=12), 18.8±3.6 pA/pF for D111V (n=9), 15.2±3.4 pA/pF for A490T (n=12), 6.1±2.3 pA/pF for P846T (n=8), respectively. They were all significantly smaller than those of the 2-μg WT channels (vs WT 2 μg; $P<0.01$). **Figure 3C** shows that all WT/mutant and mutant channels tended to shift to the depolarization side compared with the WT. Overall, all mutant channels showed loss of function associated with a dominant-negative effect and shift of the activation curve to depolarization.

We then examined whether the mutations affected the inactivation kinetics of mutant channels using a double-pulse protocol. $V_{0.5}$ and the slope factor of steady-state inactivation differed between WT and WT plus mutant or mutant. All mutant *KCNH2* channels showed the shift of inactivation curves to depolarizing direction, and the differences were statistically significant (**Table 2**). Therefore, we also changed the parameters associated with inactivation states in the following simulation study.

Figure 4A depicts original current traces showing deactivation at 4 different repolarization potentials (from −70 to −40 mV) of WT and/or mutant/*KCNH2*. Deactivating currents were best fit with a double-exponential function, and are summarized in **Figure 4B**. At 4 different potentials, both time constants (Tau-fast and Tau-slow) for D111V and WT/D111V were smaller than those of the WT. Tau-slow of WT/P846T was also smaller than those of the WT. We could not assess that of P846T (2 μg), because it was too small to measure. In contrast, there were no significant changes between the WT and WT/A490T or A490T in the deactivation process.

Computer Simulation of APD

In order to compare how functional changes caused by mutations affect ventricular action potentials, a simulation study

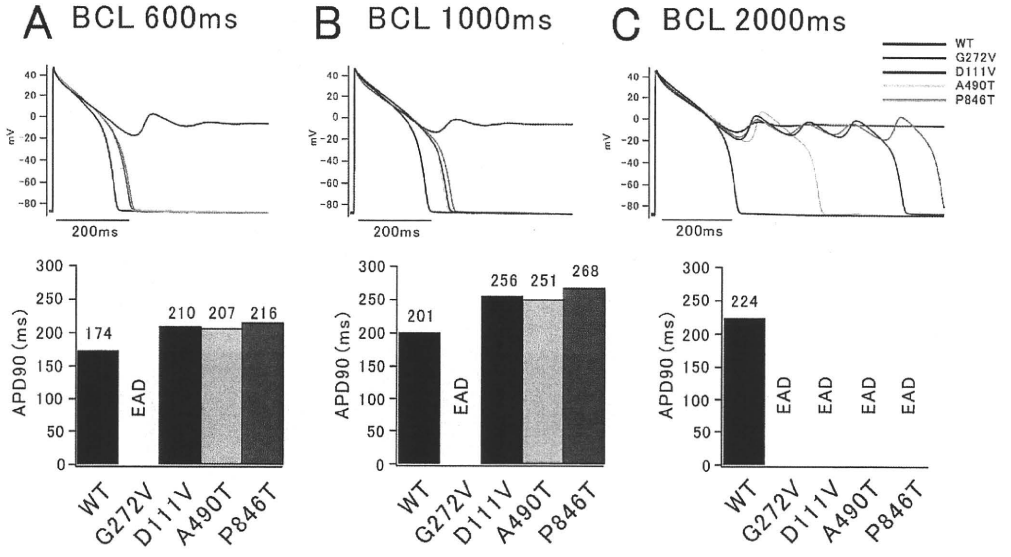

Figure 5. Computer simulations with Luo-Rudy myocardial model show bradycardia-induced early afterdepolarization (EAD). Each figure presents (**A**) action potential duration (APD) at basic cycle length (BCL) of 600 ms, (**B**) APD at BCL of 1,000 ms, and (**C**) APD at BCL of 2,000 ms. The longer the BCL in each model was, the more the APD was prolonged. EADs appeared in the models of D111V/WT, A490T/WT, and P846T/WT only at BCL of 2,000 ms, but appeared at all BCLs in the case of G272V/WT.

Table 3. Parameters of Simulation Data in Bradycardia-Induced Long QT Syndrome

Gene	Mutation	WT basal parameters	Mutant changed parameters
KCNQ1	G272V	gsk=0.202*(1+0.6/(1+pow(0.000038/cai),1.4)))	gsk=0.067*(1+0.6/(1+pow(0.000038/cai),1.4)))
		xs1 ss=1/(1+exp(-(v-1.5)/16.7))	xs1 ss=1/(1+exp(-(v-6.5)/16.7))
KCNH2	D111V	gherg=0.0135*pow(Kout,0.59)	gherg=0.331*0.0135pow(Kout,0.59)
		$\alpha\alpha$=65.5e-3*exp(0.05547153*(v-36))	$\alpha\alpha$=65.5e-3*exp(0.05547153*(v-69))
		αi=0.439*exp(–0.02352*(v+25))*4.5/Kout	αi=0.439*exp(–0.02352*(v+3))*4.5/Kout
		$\beta\beta$=2.9375e-3*exp(–0.02158*v)	$\beta\beta$=2*2.9375e-3*exp(–0.02158*v)
KCNH2	A490T	gherg=0.0135*pow(Kout,0.59)	gherg=0.1887*0.0135pow(Kout,0.59)
		αi=0.439*exp(–0.02352*(v+25))*4.5/Kout	αi=0.439*exp(–0.02352*(v-6))*4.5/Kout
KCNH2	P846T	gherg=0.0135*pow(Kout,0.59)	gherg=0.265*0.0135pow(Kout,0.59)
		$\alpha\alpha$=65.5e-3*exp(0.05547153*(v-36))	$\alpha\alpha$=65.5e-3*exp(0.05547153*(v-80))
		αi=0.439*exp(–0.02352*(v+25))*4.5/Kout	αi=0.439*exp(–0.02352*(v+3))*4.5/Kout
		$\beta\beta$=2.9375e-3*exp(–0.02158*v)	$\beta\beta$=1.3*2.9375e-3*exp(–0.02158*v)

was conducted using the Luo-Rudy model, which incorporated the Markov[13] or Hodgkin-Huxley[17] process gating for the mutant channels (**Figure 5**). **Table 3** shows the parameters of simulation that were changed to fit to experimental results. We simulated action potentials in all myocardial layers at 3 different basic cycle lengths (BCL 600, 1,000, 2,000 ms) (**Figures 5A–C**). In the endocardium and epicardium, APD of all mutant models was prolonged, but did not produce early afterdepolarizations (EAD) (data not shown in **Figure 5**). In contrast, in the simulated M cell layer, APD was lengthened significantly at a slower heart rate. In the lower half of **Figure 5**, below each simulated action potential, the corresponding bar graphs show APDs at 90% repolarization. Three APD models with D111V, A490T, and P846T displayed EADs at BCL of 2,000 ms, whereas G272V displayed it at all BCLs.

Discussion

There are 3 major findings in the present study. (1) In 4 of 14 consecutive AVB-associated TdP patients, 3 *KCNH2* and 1 *KCNQ1* heterozygous missense mutations were identified. (2) Electrophysiological analyses revealed loss of function associated with decreased current densities and various dysfunctions on I_{Ks} or I_{Kr} in 4 mutants. (3) Functional changes reconstituted by the computer simulation resulted in a prolonged APD and EAD under condition of bradycardia.

During AVB, our 14 patients showed a prolonged QT interval and TdP. Based on a comparison of ECGs available before and after AVB, we found the QT intervals were lengthened even in the absence of AVB. These clinical characteristics indicate that AVB-related TdP might share a similar genetic background with congenital LQTS: mutations on cardiac ion channel genes could be partially causative. Lupo-

glazoff et al[5] demonstrated that in neonates that, while LQTS with 2:1 AVB is associated with *KCNH2* mutations, sinus bradycardia-related LQTS is associated with *KCNQ1* mutations. In 9 of 10 cases, 2:1 AVB-induced LQTS could be caused by LQTS-related gene mutations. In contrast, Chevalier et al found 4 K^+ channel gene mutations in 5 of 29 adult patients with AVB-induced LQTS (17.3%).[6] Our cohort also consisted of adult LQTS patients, with a mutation rate of 28.6%. This prevalence rate was similar to Chevalier's report, but lower than that in the 2:1 AVB-related LQTS in neonates. These studies have shown that AVB-induced LQTS in neonates has a stronger genetic association than AVB-induced LQTS in adults. Regarding the diagnostic rate of genetic testing in general, no candidate mutations could be detected in 30–40% of congenital LQTS cases. In contrast, it has been shown recently that genetic polymorphisms modify the QT interval.[18–24] Although we did not check polymorphisms in the present study, it is possible that our subjects might have some modifier-gene mutations. Thus, it remains possible that the remaining 10 patients in our study without apparent genetic variants may have as yet unknown variants.

In our cohort, it was difficult to prove the efficacy of β-blockers because very few patients were taking these drugs. In order to investigate the efficacy of β-blockers it will be necessary to study more cases with AVB-induced TdP. The first step in the treatment of all patients with AVB-induced TdP is the implantation of a device. Although PM implantation as first-line therapy for AVB-induced TdP is not disputed, 3 of our patients had a recurrence of TdP after the device was implanted, because of inadequate ventricular pacing, suggesting that AVB patients with TdP require strict PM management. In cases of persistent QT prolongation, even after PM therapy, it might become necessary to consider ICD implantation.

Several AVB-related gene mutations have been functionally assayed:[6] 3 *KCNH2* mutations, R328C, R696C and R1047L, were shown to have no strong dominant-negative effects on I_{Kr}. Another *KCNE2* mutation (R77W), which was identified in an AVB patient while taking flecainide, exerted no effects on I_{Kr}. Overall, previous analyses of mutations have shown them to cause only mild functional change. Our study showed similar results; all 4 mutants displayed loss of function associated with decreased densities on I_{Ks} or I_{Kr}, which were basically similar to those in congenital LQTS. On average, our patients experienced TdP at 57 years of age, which is older than the mean age of onset reported for those with congenital LQTS. Mutation carriers, who remain asymptomatic well into adulthood, may incidentally have fatal events in the presence of additional triggers, such as AVB.[25]

Several mutations of *SCN5A*, coding the α-subunit of Na^+ channels, have been found in newborn and infant cases of long QT.[26–28] They showed functional 2:1 AVB caused by profound QT prolongation. Therefore, the pathological basis differs between those cases and ours. Irrespective of genetic testing results, our patients who developed TdP in the presence of AVB showed QT prolongation, even in sinus rhythm. Thus, AVB may not be directly associated with QT prolongation, but the bradycardia caused by AVB enhances it and eventually leads to TdP. Our computer simulation study showed that, at a slower heart rate, APD lengthened significantly, suggesting that AVB-related bradycardia could exacerbate QT prolongation.

Study Limitation

Female sex is a predisposing factor for the development of cardiac arrhythmic events in patients with congenital and acquired LQTS, as previous reports have demonstrated.[29–31] In our study, almost all patients (93%) were also female, and therefore it would be possible that not only AVB but female sex affected cardiac repolarization and ventricular irritability in our cohort.

Conclusion

This study showed that incidental AVB as a trigger of TdP could manifest as clinical phenotypes of LQTS, and that some patients with AVB-induced TdP could have genetic backgrounds associated with congenital LQTS-related genes.

Acknowledgments

We thank Ms Arisa Ikeda for excellent technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and the Biosimulation and Health Sciences Research Grant from the Ministry of Health, Labor, and Welfare of Japan, and grants from the Uehara Memorial Foundation (M.H.) and the Grant-in-Aid for Young Scientists from the Ministry of Education, Culture and Technology of Japan (H.I.).

References

1. Moss A, Kass R. Long QT syndrome: From channels to cardiac arrhythmias. *J Clin Invest* 2005; **115:** 2018–2024.
2. Shimizu W. Clinical Impact of Genetic Studies in Lethal Inherited Cardiac Arrhythmias. *Circ J* 2008; **72:** 1926–1936.
3. Kurita T, Ohe T, Marui N, Aihara N, Takaki H, Kamakura S, et al. Bradycardia-induced abnormal QT prolongation in patients with complete atrioventricular block with torsades de pointes. *Am J Cardiol* 1992; **69:** 628–633.
4. Yoshida H, Horie M, Otani H, Kawashima T, Onishi Y, Sasayama S. Bradycardia-induced long QT syndrome caused by a de novo missense mutation in the S2-S3 inner loop of HERG. *Am J Med Genet* 2001; **98:** 348–352.
5. Lupoglazoff JM, Denjoy I, Villain E, Fressart V, Simon F, Bozio A, et al. Long QT syndrome in neonates: Conduction disorders associated with HERG mutations and sinus bradycardia with KCNQ1 mutations. *J Am Coll Cardiol* 2004; **43:** 826–830.
6. Chevalier P, Bellocq C, Millat G, Piqueras E, Potet F, Schott JJ, et al. Torsades de pointes complicating atrioventricular block: Evidence for a genetic predisposition. *Heart Rhythm* 2007; **4:** 170–174.
7. Nagaoka I, Shimizu W, Itoh H, Yamamoto S, Sakaguchi T, Oka Y, et al. Mutation site dependent variability of cardiac events in Japanese LQT2 form of congenital long-QT syndrome. *Circ J* 2008; **72:** 694–699.
8. Makita N. Phenotypic overlap of cardiac sodium channelopathies. *Circ J* 2009; **73:** 810–817.
9. Ogawa K, Nakamura Y, Terano K, Ando T, Hishitani T, Hoshino K. Isolated non-compaction of the ventricular myocardium associated with long QT syndrome. *Circ J* 2009; **73:** 2169–2172.
10. Bazett HC. An analysis of the time relations of electrocardiograms. *Heart* 1920; **7:** 353–370.
11. Clancy CE, Rudy Y. Na^+ channel mutation that causes both Brugada and long-QT syndrome phenotypes: A simulation study of mechanism. *Circulation* 2002; **105:** 1208–1213.
12. Itoh H, Sakaguchi T, Ding WG, Watanabe E, Watanabe I, Nishio Y, et al. Latent genetic background and molecular pathogenesis of drug-induced long QT syndrome. *Circ Arrhythmia Electrophysiol* 2009; **2:** 511–523.
13. Tan HL, Bardai A, Shimizu W, Moss AJ, Schulze-Bahr E, Noda T, et al. Genotype-specific onset of arrhythmias in congenital long-QT syndrome. *Circulation* 2006; **114:** 2096–2103.
14. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 1996; **384:** 78–80.
15. Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, et al. Coassembly of KvLQT1 and minK (I_{sK}) proteins to form cardiac I_{Ks} potassium channel. *Nature* 1996; **384:** 80–83.
16. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: *HERG* encodes the I_{Kr} potassium channels. *Cell* 1995; **81:** 299–307.

17. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 1952; **117:** 500–544.
18. Newton-Cheh C, Guo CY, Larson MG, Musone SL, Surti A, Camargo AL, et al. Common genetic variant in KCNH2 is associated with QT interval duration: The Framingham Heart Study. *Circulation* 2007; **116:** 1128–1136.
19. Plant LD, Bowers PN, Liu Q, Morgan T, Zhang T, State MW, et al. A common cardiac sodium channel variant associated with sudden infant death in African Americans, SCN5A S1103Y. *J Clin Invest* 2006; **116:** 430–435.
20. Nishio Y, Makiyama T, Itoh H, Sakaguchi T, Ohno S, Gong YZ, et al. D85N, a KCNE1 polymorphism, is a disease-causing gene variant in long QT syndrome. *J Am Coll Cardiol* 2009; **54:** 812–819.
21. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PI, Yin X, Estrada K, et al. Common variants at ten loci influence QT interval duration in the QTGEN study. *Nat Genet* 2009; **41:** 399–406.
22. Pfeufer A, Sanna S, Arking DE, Müller M, Gateva V, Fuchsberger C, et al. Common variants at ten loci modulate the QT interval duration in the QTSCD study. *Nat Genet* 2009; **41:** 407–414.
23. Arking DE, Pfeufer A, Post W, Kao WH, Newton-Cheh C, Ikeda M, et al. A common genetic variant in the NOS1 regulator NOS1AP modulate cardiac repolarization. *Nat Genet* 2006; **38:** 644–651.
24. Crotti L, Monti MC, Insolia R, Peljto A, Goosen A, Brink PA, et al. NOS1AP is a genetic modifier of the long-QT syndrome. *Circulation* 2009; **120:** 1657–1663.
25. Sakaguchi T, Shimizu W, Itoh H, Noda T, Miyamoto Y, Nagaoka I, et al. Hydroxyzine, a first generation H(1)-receptor antagonist, inhibits human ether-a-go-go-related gene (HERG) current and causes syncope in a patient with the HERG mutation. *J Pharmacol Sci* 2008; **108:** 462–471.
26. Lupoglazoff JM, Cheav T, Baroudi G, Berthet M, Denjoy I, Cauchemez B, et al. Homozygous SCN5A mutation in long-QT syndrome with functional two-to-one atrioventricular block. *Circ Res* 2001; **89:** E16–E21.
27. Chang CC, Acharfi S, Wu MH, Chiang FT, Wang JK, Sung TC, et al. A novel SCN5A mutation manifests as a malignant form of long QT syndrome with perinatal onset of tachycardia/bradycardia. *Cardiovasc Res* 2004; **64:** 268–278.
28. Miura M, Yamagishi H, Morikawa Y, Matsuoka R. Congenital long QT syndrome and 2:1 atrioventricular block with a mutation of the SCN5A gene. *Pediatr Cardiol* 2003; **24:** 70–72.
29. Nakamura H, Kurokawa J, Bai CX, Asada K, Xu J, Oren RV, et al. Progesterone regulates cardiac repolarization through a nongenomic pathway: An in vitro patch-clamp and computational modeling study. *Circulation* 2007; **116:** 2913–2922.
30. Makkar RR, Fromm BS, Steinman RT, Meissner MD, Lehmann MH. Female gender as a risk factor for torsades de pointes associated with cardiovascular drugs. *JAMA* 1993; **270:** 2590–2597.
31. Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, et al. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: Findings from the International LQTS Registry. *Circulation* 1998; **97:** 2237–2244.

Journal of the American College of Cardiology
© 2011 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 57, No. 1, 2011
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2010.07.038

Risk for Life-Threatening Cardiac Events in Patients With Genotype-Confirmed Long-QT Syndrome and Normal-Range Corrected QT Intervals

Ilan Goldenberg, MD,* Samuel Horr, MA,* Arthur J. Moss, MD,* Coeli M. Lopes, PHD,† Alon Barsheshet, MD,* Scott McNitt, MS,* Wojciech Zareba, MD, PHD,* Mark L. Andrews, BBA,* Jennifer L. Robinson, MS,* Emanuela H. Locati, MD,§ Michael J. Ackerman, MD, PHD,¶ Jesaia Benhorin, MD,‖ Elizabeth S. Kaufman, MD,# Carlo Napolitano, MD,**†† Pyotr G. Platonov, MD, PHD,§§ Silvia G. Priori, MD, PHD,**†† Ming Qi, MD,‡ Peter J. Schwartz, MD,‡‡ Wataru Shimizu, MD, PHD,‖‖ Jeffrey A. Towbin, MD,¶¶ G. Michael Vincent, MD,*** Arthur A. M. Wilde, MD, PHD,## Li Zhang, MD***

Rochester and New York, New York; Milan and Pavia, Italy; Tel Aviv, Israel; Rochester, Minnesota; Cleveland, Ohio; Lund, Sweden; Suita, Japan; Houston, Texas; Amsterdam, the Netherlands; and Salt Lake City, Utah

Objectives This study was designed to assess the clinical course and to identify risk factors for life-threatening events in patients with long-QT syndrome (LQTS) with normal corrected QT (QTc) intervals.

Background Current data regarding the outcome of patients with concealed LQTS are limited.

Methods Clinical and genetic risk factors for aborted cardiac arrest (ACA) or sudden cardiac death (SCD) from birth through age 40 years were examined in 3,386 genotyped subjects from 7 multinational LQTS registries, categorized as LQTS with normal-range QTc (≤440 ms [n = 469]), LQTS with prolonged QTc interval (>440 ms [n = 1,392]), and unaffected family members (genotyped negative with ≤440 ms [n = 1,525]).

Results The cumulative probability of ACA or SCD in patients with LQTS with normal-range QTc intervals (4%) was significantly lower than in those with prolonged QTc intervals (15%) ($p < 0.001$) but higher than in unaffected family members (0.4%) ($p < 0.001$). Risk factors ACA or SCD in patients with normal-range QTc intervals included mutation characteristics (transmembrane-missense vs. nontransmembrane or nonmissense mutations: hazard ratio: 6.32; $p = 0.006$) and the LQTS genotypes (LQTS type 1:LQTS type 2, hazard ratio: 9.88; $p = 0.03$; LQTS type 3:LQTS type 2, hazard ratio: 8.04; $p = 0.07$), whereas clinical factors, including sex and QTc duration, were associated with a significant increase in the risk for ACA or SCD only in patients with prolonged QTc intervals (female age >13 years, hazard ratio: 1.90; $p = 0.002$; QTc duration, 8% risk increase per 10-ms increment; $p = 0.002$).

Conclusions Genotype-confirmed patients with concealed LQTS make up about 25% of the at-risk LQTS population. Genetic data, including information regarding mutation characteristics and the LQTS genotype, identify increased risk for ACA or SCD in this overall lower risk LQTS subgroup. (J Am Coll Cardiol 2011;57:51–9) © 2011 by the American College of Cardiology Foundation

From the *Cardiology Division of the Department of Medicine, University of Rochester Medical Center, Rochester, New York; †Cardiovascular Research Institute University of Rochester Medical Center, Rochester, New York; ‡Department of Pathology, University of Rochester Medical Center, Rochester, New York; §Cardiovascular Department De Gasperis, Niguarda Hospital, Milan, Italy; ‖Heart Institute, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ¶Departments of Medicine, Pediatrics, and Molecular Pharmacology and Experimental Therapeutics/Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic College of Medicine, Rochester, Minnesota; #The Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, Ohio; **Molecular Cardiology, Fondazione S. Maugeri, University of Pavia, Pavia, Italy; ††Leon Charney Division of Cardiology, New York University School of Medicine, New York, New York; ‡‡Department of Cardiology, Fondazione Policlinico S. Matteo IRCCS and University of Pavia, Pavia, Italy; §§Department of Cardiology, Lund University, Lund, Sweden; ‖‖Division of Cardiology, Department of Internal Medicine National Cardiovascular Center, Suita, Japan; ¶¶Department of Pediatric Cardiology, Baylor College of Medicine, Houston, Texas; ##Department of Cardiology, Academic Medical Center, Amsterdam, the Netherlands; and the ***Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah. This work was supported by research grants HL-33843 and HL-51618 from the National Institutes of Health. The authors have reported that they have no relationships to disclose.

Manuscript received May 29, 2010; revised manuscript received July 8, 2010, accepted July 12, 2010.

JACC Vol. 57, No. 1, 2011
December 28, 2010/January 4, 2011:51–9

Abbreviations and Acronyms

ACA = aborted cardiac arrest
ECG = electrocardiographic
LQTS = long-QT syndrome
LQT1 = long-QT syndrome type 1
LQT2 = long-QT syndrome type 2
LQT3 = long-QT syndrome type 3
QTc = corrected QT interval
SCD = sudden cardiac death

Congenital long-QT syndrome (LQTS) is an inherited channelopathy characterized by a prolonged corrected QT interval (QTc) at rest that is associated with an increased predisposition for polymorphic ventricular arrhythmias and sudden cardiac death (SCD) in young subjects without structural heart disease (1). To date, more than 500 mutations have been identified in 12 LQTS-susceptibility genes, with the long-QT syndrome type 1 (LQT1), long-QT syndrome type 2 (LQT2), and long-QT syndrome type 3 (LQT3) genotypes constituting more than 95% of genotype-positive LQTS and approximately 75% of all LQTS (2). Risk assessment in affected patients with LQTS relies primarily on a constellation of electrocardiographic (ECG) and clinical factors, including QTc interval and age-sex interactions (3–6). In addition, there is increasing evidence that genetic information and the molecular and cellular properties of the LQTS-causative mutation may identify subjects with increased risk for cardiac events (7–10). Despite these recent advances, however, currently there are limited data regarding the clinical course and risk factors for life-threatening events in patients with LQTS with normal resting QTc values, so-called silent mutation carriers, concealed LQTS, or normal–QT interval LQTS.

See page 60

In the present study we used combined data from 7 national LQTS registries to: 1) compare the clinical courses of patients with LQTS and normal-range QTc intervals to those of patients with prolonged QTc intervals and of genotype-negative unaffected family members; and 2) identify specific clinical and genetic risk factors for life-threatening cardiac events in patients with LQTS with normal-range QTc intervals.

Methods

Study population. The study population comprised 3,386 genotyped subjects drawn from the Rochester, New York, enrolling center (center 1) of the International LQTS Registry (n = 2,630), the Netherlands LQTS Registry (n = 391), and the Japanese LQTS Registry (n = 205), as well as from data submitted by other investigators specifically for this collaborative mutation analysis project from Denmark (n = 90), Italy (n = 28), Israel (n = 25), and Sweden (n = 17). Patients were derived from 552 proband-identified *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3) families. The proband in each family had otherwise unexplained, diagnostic QTc prolongation or experienced LQTS-related symptoms. Patients were excluded from the study if they had: 1) >1 LQTS identified mutation (n = 70); 2) Jervell and Lange-Nielsen syndrome with deafness and 2 *KCNQ1* mutations or 1 known *KCNQ1* mutation and congenital deafness (n = 2); and 3) no identified mutation on genetic testing with prolonged QTc interval (>440 ms [n = 428]).

Data collection and end point. Routine clinical and rest ECG parameters were acquired at the time of enrollment in each of the registries. Measured parameters on the first recorded electrocardiogram included QT and R-R intervals in milliseconds, with QT interval corrected for heart rate using Bazett's (11) formula. Clinical data were collected on prospectively designed forms with information on demographic characteristics, personal and family medical histories, ECG findings, therapies, and events during long-term follow-up. Data common to all LQTS registries involving genetically tested subjects were electronically merged into a common database for the present study. In addition, information regarding QT interval–prolonging medications and triggers for cardiac events was collected through a specific questionnaire for patients enrolled the U.S. portion of the registry.

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising aborted cardiac arrest (ACA; requiring external defibrillation as part of the resuscitation or internal defibrillation in patients with implantable cardioverter-defibrillators) or LQTS-related SCD (abrupt in onset without evident cause, if witnessed, or death that was not explained by any other cause if it occurred in a nonwitnessed setting such as sleep). In the multivariate models, follow-up was censored at age 41 years to avoid the influence of coronary disease on the occurrence of cardiac events. We also evaluated a secondary end point that included the occurrence of a first cardiac event of any type during follow-up (comprising syncope [defined as transient loss of consciousness that was abrupt in onset and offset], ACA, or SCD).

Phenotype characterization. For the purpose of this study, the QTc interval was categorized as normal range (≤440 ms) or prolonged (>440 ms) according to accepted criteria for the phenotypic definition of LQTS (12). Using this definition, the study population were categorized into 3 genotype and QTc subgroups: 1) LQTS with normal-range QTc interval (n = 469), comprising patients identified to have LQT1 to LQT3 mutations with QTc intervals ≤440 ms; 2) LQTS with prolonged QTc interval (n = 1,392), comprising patients with LQT1 to LQT3 mutations with QTc intervals >440 ms; and 3) unaffected family members (n = 1,525), comprising registry subjects from genotype-positive proband-identified families who were genetically tested and found to be negative for the LQTS-associated mutation, with QTc intervals ≤440 ms (i.e., genetically and phenotypically unaffected family members).

Genotype characterization. The *KCNQ1*, *KCNH2*, and *SCN5A* mutations were identified with the use of standard genetic tests performed in academic molecular genetics laboratories, including the Functional Genomics Center, University of Rochester Medical Center, Rochester, New York; Baylor College of Medicine, Houston, Texas; Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, Minnesota; Boston Children's Hospital, Boston, Massachusetts; the Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; the Department of Clinical Genetics, Academic Medical Center, Amsterdam, the Netherlands; and the Molecular Cardiology Laboratory, Policlinico S. Matteo and University of Pavia, Pavia, Italy.

Genetic alterations of the amino acid sequence were characterized by location and by the type of the specific mutation. The transmembrane region of each of the 3 LQTS channels was defined as: 1) amino acid residues from 120 through 355 in the *KCNQ1*-encoded Kv7.1 channel (S1 to S6 region); 2) amino acid residues from 398 through 657 (S1 to S6 region) in the *KCNH2*-encoded Kv11.1 channel; and 3) amino acid residues 129 through 417, 713 through 940, 1201 through 1470, and 1523 through 1740 in the *SCN5A*-encoded Nav1.5 channel (13). On the basis of prior studies that demonstrated the functional and clinical importance of missense mutations that are located in the transmembrane region of these LQTS-associated channels (9,10), mutation categories were pre-specified in the primary analysis as transmembrane-missense (mutations of the missense type in any of the 3 transmembrane regions described previously) versus nontransmembrane or nonmissense (i.e., any other identified LQT1 to LQT3 mutation that was not transmembrane-missense).

Statistical analysis. The clinical characteristics of study patients were compared by genotype and QTc categories using chi-square tests for categorical variables and *t* tests and Mann-Whitney-Wilcoxon tests for continuous variables. The Kaplan-Meier estimator was used to assess the time to a first life-threatening event and the cumulative event rates by risk groups and risk factors, and groups were compared using the log-rank test.

Cox proportional hazards regression analysis was carried out in the total study population and separately in the subset of patients with genotype-positive LQTS. Pre-specified covariates in the total population model included the 3 genotype and QTc categories, sex, and time-dependent beta-blocker therapy. The models comprising genotype-positive patients included the following pre-specified covariates: QTc category (normal range [≤440 ms] vs. prolonged [>440 ms]), the LQT1 to LQT3 genotypes, mutation location and type, sex, QTc duration (assessed both as a continuous measure [per 10-ms increase] and as a categorical covariate [dichotomized at the median value of each QTc category and assessed in separate models]), time-dependent beta-blocker therapy, and a family history of SCD in a first-degree relative. The effect of each covariate on outcome in each QTc category (i.e., in patients with LQTS with normal-range and prolonged QTc intervals) was assessed using interaction-term analysis, with interactions tested 1 at a time. Estimates of predictor hazard ratios in the separate normal and prolonged QTc categories were obtained using these interactions. To avoid violation of the proportional hazards assumption due to sex-risk crossover during adolescence, we used an age-sex interaction term in the multivariate models.

Because almost all the subjects were first-degree and second-degree relatives of probands, the effect of lack of independence between subjects was evaluated in the Cox model with grouped jackknife estimates for family membership (14). All grouped jackknife standard errors for the covariate risk factors fell within 3% of those obtained from the unadjusted Cox model, and therefore only the Cox model findings are reported. The statistical software used for the analyses was SAS version 9.20 (SAS Institute Inc., Cary, North Carolina). A 2-sided significance level of 0.05 was used for hypothesis testing.

Results

The spectrum and number of LQT1-associated, LQT2-associated, and LQT3-associated mutations by the pre-specified location and type categories are presented in Online Table 1. Totals of 100, 177, and 41 different mutations were identified in the *KCNQ1*-encoded Kv7.1, *KCNH2*-encoded Kv11.1, and *SCN5A*-encoded Nav1.5 ion channels, respectively. Study patients with identified LQTS mutations exhibited a very wide QTc interval distribution (Fig. 1), ranging from a minimum of 350 ms to a maximum of 800 ms (mean 450 ± 56 ms; median 440 ms; interquartile range: 410 to 480 ms). QTc distribution was similar among the 3 LQTS genotypes. Four hundred sixty-nine LQTS mutation–positive patients exhibited normal-range QTc intervals, constituting 25% of identified cases.

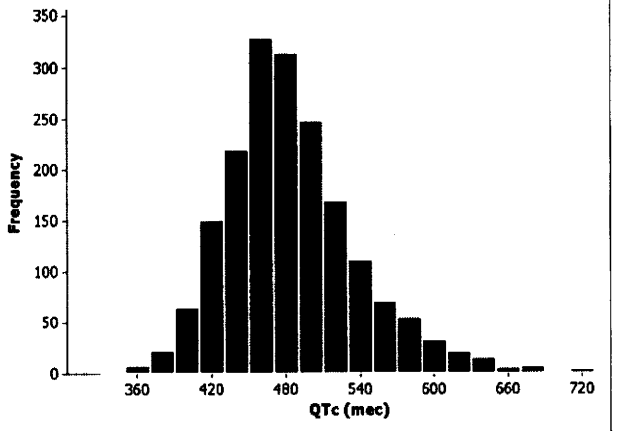

Figure 1 **Distribution of QTc Interval Duration in Genotype-Positive Patients With LQTS**

Distribution of corrected QT (QTc) interval durations in genotype-positive study patients. LQTS = long-QT syndrome.

JACC Vol. 57, No. 1, 2011
December 28, 2010/January 4, 2011:51–9

Table 1 Baseline and Follow-Up Characteristics of the Study Population by Genotype-Phenotype

Characteristic	Unaffected Family Members (n = 1,525)	Patients With LQTS With Normal-Range QTc Intervals (n = 469)	Patients With LQTS With Prolonged QTc Intervals (n = 1,392)
Female	52%	48%	61%*†
Family history of SCD	8%	12%	19%*†
QTc interval (ms)			
Mean ± SD	412 ± 22	419 ± 20	501 ± 48
Median (IQR)	420 (400–430)	420 (410–440)	490 (470–520)
Proband	8%	8%	29%*†
RR interval (ms)			
Mean ±SD	793 ± 221	888 ± 236	848 ± 214*†
Median (IQR)	800 (640–930)	900 (740–1,040)	840 (700–1,000)*†
Genotype			
LQT1	NA	40%	39%
LQT2	NA	45%	47%
LQT3	NA	16%	14%
Mutation: TM-MS			
Overall	NA	35%	43%
LQT1	NA	45%	61%
LQT2	NA	16%	29%†
LQT3	NA	64%	31%†
Therapies			
Beta-blockers	6.2%	38%	54%*†
Pacemaker	0.3%	0.6%	5%*†
LCSD	0.1%	0.2%	1.4%*†
ICD	0.6%	6%	14%*†
Events			
Syncope	10%	21%	40%*†
ACA	0.2%	1.3%	8.4%*†
SCD	0.1%	1.5%	4.4%*†
ACA/SCD‡§	0.3%	2.8%	11.3%*

*$p < 0.05$ for the comparison among the 3 genotyped categories. †$p < 0.05$ for the comparison between genotype-positive patients with QTc intervals ≤440 ms and genotype-positive patients with QTc intervals >440 ms. ‡Appropriate ICD shocks constituted 0.04% of ACAs in genotype-positive patients with QTc intervals ≤440 ms and 1.4% of ACAs in genotype-positive patients with QTc intervals >440 ms. §Only the first event for each patient was considered.

ACA = aborted cardiac arrest; ICD = implantable cardioverter-defibrillator; IQR = interquartile range; LCSD = left cardiac sympathetic denervation; LQT1 = long-QT syndrome type 1; LQT1 = long-QT syndrome type 2; LQT3 = long-QT syndrome type 3; LQTS = long-QT syndrome; MS = missense; NA = not applicable; QTc = corrected QT; SCD = sudden cardiac death; TM = transmembrane.

The clinical characteristics of the total study population by genotype and QTc subgroup are shown in Table 1. The frequency of probands (defined in the registry as the first person in a family, living or deceased, identified to have LQTS by the enrollment center) was highest in patients with prolonged QTc intervals, whereas most patients with normal-range QTc intervals (92%) were asymptomatic at the time of genetic testing. The frequency of female subjects was similar between the unaffected subjects and patients with LQTS with normal-range QTc intervals and higher in patients with prolonged QTc intervals. In mutation carriers, the frequency of the 3 main LQTS genotypes was similar between patients with and without prolonged QTc intervals. However, patients with LQT1 and LQT2 with prolonged QTc intervals had a higher frequency of transmembrane-missense mutations compared with the corresponding genotype carriers who had normal-range QTc intervals. LQTS-related therapies were administered to a significantly higher frequency of patients with prolonged QTc intervals than to subjects in the other 2 subgroups (Table 1).

Clinical course by genotype and QTc subgroup. Kaplan-Meier survival analysis (Fig. 2) demonstrated a relatively low rate of ACA or SCD in patients with LQTS with normal-range QTc intervals (4% at age 40 years and 10% at age 70 years). Event rates were significantly higher in patients with prolonged QTc intervals (15% and 24% at age 70 years; log-rank $p < 0.001$ for the comparison with the normal-range QTc subgroup) and significantly lower in unaffected family members (0.4% and 1% at age 70 years; log-rank $p < 0.001$ for the comparison with the normal-range QTc subgroup and for the overall difference among the 3 subgroups). Notably, life-threatening events in patients with normal-range QTc intervals occurred mostly after age 10 years, whereas patients with prolonged QTc intervals exhibited an earlier onset of life-threatening events (Fig. 2).

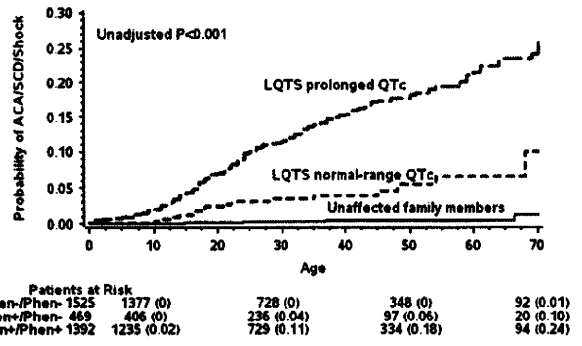

Figure 2 **Rate of ACA or SCD by Genotype and QTc Category**

Kaplan-Meier cumulative probabilities of aborted cardiac arrest (ACA) and sudden cardiac death (SCD) by genotype and corrected QT (QTc) subgroup. LQTS = long-QT syndrome.

After multivariate adjustment for sex, time-dependent beta-blocker therapy, and a family history of SCD in a first-degree relative, patients with LQTS with normal-range QTc intervals were shown to have a significant 72% ($p < 0.001$) lower risk for ACA or SCD compared with patients with prolonged QTc intervals but also exhibited a >10-fold increase in the risk for life-threatening events compared with unaffected family members (Table 2). Histories of syncope were present in 62% of patients with LQTS with normal-range QTc intervals who had life-threatening events during follow-up. Accordingly, when the composite secondary end point of a first cardiac event of any type was assessed (comprising mainly non-life-threatening syncopal episodes), patients with normal-range QTc intervals were consistently shown to be at a lower risk compared with those with prolonged QTc intervals (hazard ratio [HR]: 0.47; 95% confidence interval [CI]: 0.33 to 0.59; $p < 0.001$) and at a higher risk compared with unaffected family members (HR: 5.20; 95% CI: 4.19 to 6.44; $p < 0.001$).

Risk factors for ACA or SCD in patients with LQTS with and without prolonged QTc intervals. Interaction-term analysis demonstrated significant differences in risk factors for life-threatening events between the 2 LQTS subgroups (Table 3). In patients with normal-range QTc intervals, the LQT1 and LQT3 genotypes were associated with respective 10- and 8-fold increases in the risk for life-threatening events compared with the LQT2 genotype. In contrast, in patients with prolonged QTc intervals, the LQT1 genotype was associated with one-half the risk of the LQT2 genotype ($p = 0.002$), with a statistically significant genotype–by–QTc subgroup interaction ($p = 0.006$) (Table 3, first row), and the LQT3 genotype showed a similar risk to the LQT2 genotype, without a statistically significant genotype–by–QTc subgroup interaction (Table 3, second row).

The location and type of the LQTS mutation were shown to be significant risk factors for ACA or SCD in patients with normal-range QTc intervals. In this LQTS subset, transmembrane-missense mutations were associated with a pronounced >6-fold ($p = 0.006$) increase in the risk for ACA or SCD compared with nontransmembrane or nonmissense mutations. In contrast, in patients with prolonged QTc intervals, transmembrane-missense mutations were not independently associated with outcomes (Table 3, third row). Notably, when the secondary end point of cardiac events of any type was assessed, transmembrane-missense mutations were shown to be an independent risk factor in both LQTS subgroups (normal-range QTc interval, HR: 1.71; 95% CI: 1.16 to 2.34; prolonged QTc interval, HR: 1.39; 95% CI: 1.17 to 1.65).

Consistent results demonstrating an association between transmembrane-missense mutations and the risk for ACA or SCD in patients with normal-range QTc intervals were shown when the reference group (comprising nontransmembrane or nonmissense mutations) was further divided into 3 subcategories, including nonmissense mutations in the transmembrane region, missense mutations in the nontransmembrane region, and nonmissense mutations in the nontransmembrane region (HR >4.0 for all 3 comparisons). Accordingly, patients with normal-range QTc intervals with transmembrane-missense mutations experienced a relatively high rate of ACA or SCD during follow-up (9% at age 40 years and 21% at age 70 years), whereas patients with normal-range QTc intervals with other mutations had a very low event rate (1% at age 40 years and 5% at age 70 years; log-rank p for overall difference = 0.005) (Fig. 3A). In contrast, in patients with prolonged QTc intervals, there was no statistically significant difference in the rate of ACA or SCD between the 2 mutation categories (16% and 14% at 40 years, respectively, $p = 0.18$) (Fig. 3B).

Clinical and ECG factors, including sex and QTc duration, were shown to be associated with a significant increase in the risk for ACA or SCD only in patients with prolonged QTc intervals (Table 3, rows 4 to 6). In contrast, in patients

Table 2 **Multivariate Analysis: Risk for ACA or SCD Among the 3 Genotype and QTc Categories***

Genotype and QTc Subgroup	HR	95% CI	p Value
LQTS with prolonged QTc interval vs. unaffected family members	36.53	13.35–99.95	<0.001
LQTS with normal-range QTc interval vs. unaffected family members	10.25	3.34–31.46	<0.001
LQTS with normal-range QTc interval vs. LQTS with prolonged QTc interval	0.28	0.16–0.49	<0.001

*Model also adjusted for sex (female age >13 years) and time-dependent beta-blocker therapy.
CI = confidence interval; HR = hazard ratio; other abbreviations as in Table 1.

Table 3 Risk Factors for ACA or SCD in Patients With LQTS by QTc Interval Category*

Variable	LQTS and Normal-Range QTc Interval		LQTS and Prolonged QTc Interval		
	HR (95% CI)	p Value	HR (95% CI)	p Value	p Value for Interaction
Genotype					
LQT1 vs. LQT2	9.88 (1.26–37.63)	0.03	0.53 (0.35–0.79)	0.002	0.006
LQT3 vs. LQT2	8.04 (0.85–36.03)	0.07	1.07 (0.70–1.63)	0.77	0.08
Mutation location and type					
TM-MS vs. non-TM-MS	6.32 (1.71–23.33)	0.006	1.24 (0.88–1.76)	0.22	0.02
Sex					
Female age >13 yrs vs. male age >13 yrs	1.32 (0.42–4.17)	0.64	1.90 (1.26–2.86)	0.002	0.53
QTc interval (ms)					
Per 10-ms increase	1.20 (0.81–1.78)	0.35	1.08 (1.05–1.10)	<0.001	0.58
≥Median vs. <median†	1.03 (0.36–2.98)	0.95	2.96 (2.06–4.26)	<0.001	NA

*Cox proportional hazards regression modeling was carried out in models that included all patients with genotype-positive LQTS (n = 1,861). Covariates in the models included QTc category (≤440 ms vs. >440 ms), genotype, mutation location and type, sex, QTc interval (assessed as a continuous measure [per 10-ms increase]), time-dependent beta-blocker therapy, and a family history of SCD; the effect of each covariate in patients with normal-range (≤440 ms) and those with prolonged (>440 ms) QTc intervals was assessed by interaction-term analysis, with interactions tested 1 at a time. Estimates of predictor hazard ratios in the separate normal-range and prolonged QTc interval groups were obtained using these interactions. Virtually identical results for all pre-specified risk factors were also obtained from the models that did not include appropriate ICD shocks as part of the composite end point. †Results were obtained from separate models that assessed the risk associated with QTc values greater than or equal to the median in patients with LQTS with normal-range QTc intervals (median 420 ms) and prolonged QTc intervals (median 500 ms).

Abbreviations as in Tables 1 and 2.

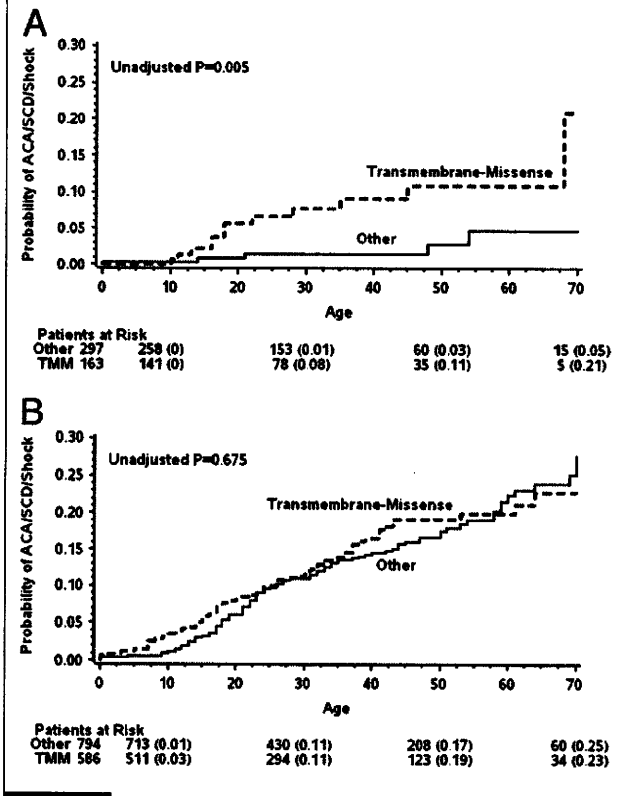

Figure 3 Rate of ACA or SCD in Patients With Normal-Range and Prolonged QTc Intervals by Mutation Location and Type

Kaplan-Meier cumulative probabilities of aborted cardiac arrest (ACA) and sudden cardiac death (SCD) by mutation location and type in patients with long-QT syndrome (LQTS) with **(A)** corrected QT (QTc) intervals ≤440 ms and **(B)** QTc intervals >440 ms.

with normal-range QTc intervals, sex was not a significant risk factor, and QTc duration was not independently associated with a significant increase in the risk for ACA or SCD when assessed as a continuous measure or when dichotomized at the median value (≥420 ms).

As suggested previously (15), the presence of a family history of SCD in any first-degree relative was not shown to be an independent predictor of ACA or SCD in patients with either normal-range QTc intervals (HR: 0.89; 95% CI: 0.63 to 1.25; p = 0.50) or prolonged QTc intervals (HR: 1.40; 95% CI: 0.32 to 6.17; p = 0.65) after adjustment for genetic and clinical factors.

Beta-blocker therapy was administered to 38% of patients who had normal-range QTc intervals compared with 54% of the patients who had prolonged QTc intervals (p < 0.001) (Table 1). Treatment with beta-blockers was associated with an overall significant 25% reduction in the risk for ACA or SCD in the total study population (95% CI: 0.70 to 0.80; p < 0.001), with similar effects in patients with normal-range QTc intervals and those with prolonged QTc intervals (p for beta-blocker–by–LQTS subset interaction = 0.45).

Characteristics of fatal or near-fatal cases with a normal-range QTc intervals. The characteristics of patients with normal-range QTc intervals who experienced ACA or SCD during follow-up are shown in Table 4. The mean age at occurrence of the lethal or near-lethal event in this population was 25.9 ± 4.5 years. Nine of the patients (53%) who experienced events were women, and 4 (24%) were treated with beta-blockers are the time of the events. In patients with normal-range QTc intervals with available data regarding therapies and triggers at the time of the events, none were reported as being treated with a QT interval–prolonging drugs at the time of ACA or SCD, and the majority of the lethal or near-lethal events were not associated with exercise or arousal triggers (Table 4).

Table 4 Characteristics of ACA and SCD Cases With Normal-Range QTc Intervals

Case	Event	Event Age (yrs)	Female	QTc Interval (ms)	BB†	LCSD†	PM†	ICD†	QT PD	Trigger*	Genotype	Mutation Location and Type
1	SCD	0.5	–	390	–	–	–	–	–	NA	LQT3	Non-TM-MS
2	ACA	10		430	–	–	–	–	–	Exercise	LQT1	TM-MS
3	ACA/shock	11	+	400	–	–	–	+	–	Non-E/A	LQT1	TM-MS
4	SCD	13	–	440	+	–	–	–	NA	NA	LQT1	TM-MS
5	ACA	14	–	410	–	–	–	–	–	Exercise	LQT1	Non-TM-MS
6	SCD	16	+	420	–	–	–	–	–	Non-E/A	LQT3	TM-MS
7	ACA	16	+	440	–	–	–	–	–	Arousal	LQT1	TM-MS
8	SCD	18	–	430	+	–	–	–	–	Non-E/A	LQT1	TM-MS
9	ACA	18	+	410	–	–	–	–	–	Exercise	LQT1	TM-MS
10	SCD	21	+	380	–	–	–	–	–	Arousal	LQT2	Non-TM-MS
11	SCD	22	–	440	–	–	–	–	NA	NA	LQT1	TM-MS
12	SCD	28	–	410	–	–	–	–	–	Exercise	LQT1	TM-MS
13	ACA	35	+	420	–	–	–	–	–	Non-E/A	LQT3	TM-MS
14	ACA	46	+	440	+	–	–	–	NA	NA	LQT2	TM-MS
15	SCD	48	–	430	+	–	–	–	–	Non-E/A	LQT2	Non-TM-MS
16	ACA	54	+	420	–	–	–	–	–	Non-E/A	LQT3	Non-TM-MS
17	SCD	69	–	380	–	–	–	–	NA	NA	LQT1	TM-MS

*Data regarding triggers for cardiac events and treatment with QT interval–prolonging medications were available for study patients who were enrolled in the U.S. portion of the International LQTS Registry. †At time of event. ‡Implanted or performed before event.

BB = beta-blocker therapy; E/A = exercise/arousal trigger for event; NA = not available; PM = pacemaker; QT PD = QT interval–prolonging drug; other abbreviations as in Tables 1 and 2.

Discussion

In this study, we assessed the clinical courses and risk factors for life-threatening events in LQTS patients with genetically-confirmed LQTS who do not exhibit the disease's phenotypic hallmark of QT interval prolongation, otherwise referred to as concealed LQTS, normal-QT interval LQTS, or genotype-positive/ECG phenotype–negative LQTS. Similar to prior studies (16), we have shown that patients with LQT1 to LQT3 exhibit a wide QTc distribution, with approximately 25% having QTc intervals well within the normal range. The rate of ACA or SCD in patients with LQTS with normal-range QTc intervals was shown to be very low (4% from birth through age 40 years, corresponding to an approximate event rate of 0.13% per year). Comparatively, however, this very low risk subset of the LQTS population still exhibited a >10-fold increase in the risk for life-threatening events compared with genetically and phenotypically unaffected family members. Importantly, predictors of life-threatening events were shown to be significantly different between LQTS patients with and without prolonged QTc intervals. In the latter LQTS subgroup, genetic data, including knowledge of genotype and mutation characteristics, were shown to identify the risk for ACA or SCD, whereas in the former LQTS subgroup, female sex in the post-adolescence period and QTc duration were identified as the predominant risk factors for life-threatening events.

The clinical courses of patients with LQTS are variable because of incomplete penetrance (17). They are influenced by age, genotype, sex, environmental factors, therapy, and possibly other modifier genes (1–10). Recent studies from the International LQTS Registry that assessed the risk for life-threatening events in patients with LQTS have consistently demonstrated that ECG and clinical risk factors, including the QTc interval and age-sex interactions, identify increased risk in the LQTS population (3–5). These studies, however, included mainly phenotype-positive patients with LQTS with QTc intervals ≥ 450 ms. Thus, the effect of genetic data on outcomes in these studies was not statistically significant after adjustment for the ECG and clinical factors. The present study population, comprising 1,861 genetically confirmed patients with the LQT1 to LQT3 genotypes, extends the data derived from prior studies and demonstrates that risk factors for life-threatening events are significantly different between patients with LQTS with and without QTc prolongation. Consistent with prior studies, we have shown that in patients with LQTS who exhibit prolonged QTc durations, ECG information and clinical factors can be used to identify the risk for life-threatening events. In contrast, in mutation-positive subjects with normal-range QTc intervals, genetic factors, including knowledge of the LQTS genotypes and the mutation location and type, identified patients who were at an increased risk for ACA or SCD after adjustment for ECG and clinical data.

Sex was not a significant risk factor for cardiac events in patients with normal-range QTc intervals. Furthermore, patients with normal-range QTc intervals displayed a similar frequency of women as unaffected family members, whereas the frequency of women was significantly higher among patients with prolonged QTc intervals. These findings are in accordance with earlier evidence of longer QTc intervals in LQTS women than in men (18), resulting in a marked female predominance in phenotypically affected patients (3–5). The biologic basis for this sex difference might be the down-regulation of expression of cardiac potassium-channel genes by female

JACC Vol. 57, No. 1, 2011
December 28, 2010/January 4, 2011:51–9

sex hormones, which have been shown to prolong the QT interval in both congenital and drug-induced LQTS (19,20). These hormonal effects may explain the present findings of a lower frequency of LQTS women with normal-range QTc intervals.

Recent genotype-phenotype studies have shown that missense mutations located in the transmembrane region, which is responsible for forming the ion conduction pathway of the channel, are associated with a significantly higher risk for cardiac events compared with mutations that are located in other regions of the LQTS channel (9,10). The present study also shows that transmembrane-missense mutations are associated with a significantly higher risk for cardiac events of any type (predominated by syncopal episodes) in patients with LQTS with both normal-range and prolonged QTc intervals. However, our findings suggest that data regarding mutation characteristics are important for the assessment of life-threatening events (comprising ACA and SCD) mainly in patients with normal-range QTc intervals, in whom information derived from ECG and clinical data is more limited. In this LQTS subset, missense mutations located in the transmembrane region were shown to be associated with a >6-fold increase in the risk for life-threatening events and with a clinically meaningful rate of ACA or SCD (9%) from birth through age 40 years.

The mechanisms relating to the occurrence of life-threatening ventricular tachyarrhythmias in phenotype-negative patients with LQTS are not clear. In the present study, none of the patients with normal-range QTc intervals who experienced ACA or SCD took QT interval–prolonging medications at the time of the events. Furthermore, most events in patients with normal-range QTc intervals were not related to exercise or arousal triggers (Table 4). An ECG tracing from a patient with the LQT1 genotype who developed arrhythmic events despite a normal-range QTc interval showed spontaneous generation of polymorphic ventricular tachycardia without preceding extrasystolic pauses or sudden sinus rate acceleration (Fig. 4), possibly explaining the occurrence of ACA or SCD in study patients with normal-range QTc intervals who were treated with beta-blockers at the time of the events.

Study limitations. Most study patients did not undergo comprehensive genetic testing for all currently known mutations that may predispose to arrhythmic risk. Thus, it is possible that the coexistence of modifier genes affected the outcomes of patients with LQTS with normal-range QTc intervals who experienced life-threatening cardiac events. In addition, to provide an estimation of event rates among unaffected family members, we included in the control group subjects who were both genotype negative and also had normal-range QTc intervals (and excluded genotype-negative subjects with prolonged QTc intervals due to possible unidentified mutations in this subset). Therefore, the overall frequency of genotype-positive subjects in the total population may not represent the true penetrance of LQTS in affected families.

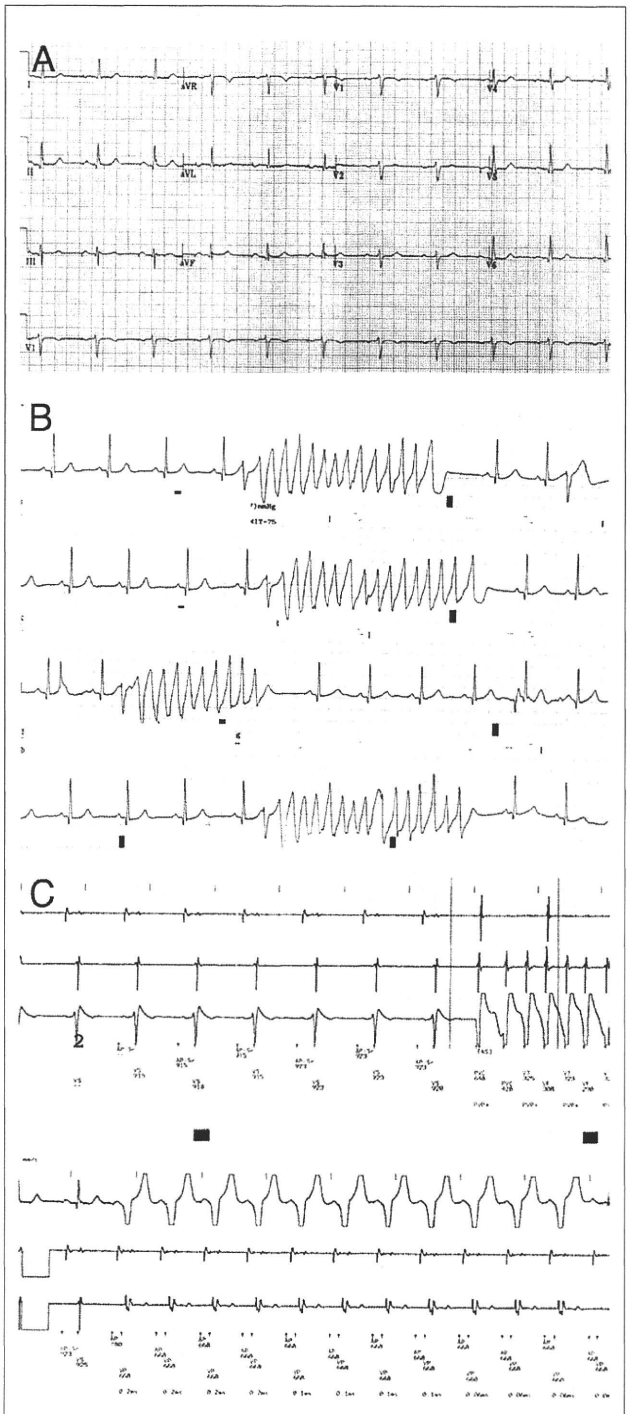

Figure 4 **Polymorphic Ventricular Tachycardia in a Patient With a Normal-Range QTc Interval**

Spontaneous generation of polymorphic ventricular tachycardia in a patient with long-QT syndrome type 1 with a normal-range corrected QT (QTc) interval. **(A)** The patient had a QTc duration of 410 ms on baseline electrocardiography. **(B)** Electrocardiographic tracing at the time of arrhythmic event demonstrates sinus rate with an RR interval of 1,000 ms without significant QT prolongation before the arrhythmia. **(C)** The patient was treated with nadolol and received an implantable cardioverter-defibrillator but continued to exhibit arrhythmic episodes that were recorded on implantable cardioverter-defibrillator interrogation.

The threshold value of 440 ms for the definition of a normal-range QTc in the present study was based on the diagnostic criteria for LQTS proposed by Schwartz et al. (12), which define a prolonged QTc interval as ≥450 ms in male patients and ≥460 ms in female patients. We chose to use a uniform approach by selecting 440 ms as the upper limit of normal rather than having separate phenotypic definitions for male and female patients. It should also be noted that 2.5% of infants and 10% to 20% of adults exceed this cutoff (21). Thus, the 440-ms value is not meant to suggest an LQTS diagnosis on its own.

Conclusions

The present study shows that patients with LQTS who exhibit normal-range QTc intervals constitute approximately 25% of the LQTS population and have a significantly lower risk for life-threatening events compared with phenotypically affected patients but also exhibit a significant increase in the risk of ACA or SCD compared with unaffected family members. Missense mutations in the transmembrane regions of the ion channels, mainly in patients with LQT1 and LQT3, were shown to identify patients with normal-range QTc intervals who have an increased risk for ACA or SCD. In contrast, increments in QTc duration were not shown to be significantly associated with increased risk for life-threatening events in this population. These findings suggest that: 1) risk assessment in phenotype-negative family members of LQTS probands should include genetic testing, because a positive genetic test result in a family member with a normal-range QTc interval implies an overall >10-fold increase in the risk for ACA or SCD compared with a negative test result in an unaffected family member; 2) genetic data may be used to identify phenotype-negative patients with LQTS who are at increased risk for fatal ventricular tachyarrhythmias independently of QTc duration; and 3) LQTS mutation–positive patients with normal-range QTc intervals who are identified as having increased risk for life-threatening events on the basis of genotype and mutation characteristics (i.e., LQT1 and LQT3 with transmembrane-missense mutations) should be carefully followed and receive a similar management strategy as phenotype-positive patients with LQTS, including avoidance of QT-prolonging medications (22), routine therapy with beta-blockers, and possibly implantable cardioverter-defibrillator therapy in those who remain symptomatic despite medical therapy. Conversely, patients with the lowest risk profile of already low risk, concealed LQTS (i.e., concealed LQT2 and non-transmembrane-missense LQT1 and LQT3) may represent the nominally near zero risk subpopulation(s) of LQTS in need of only preventative health recommendations such as QT drug avoidance.

Reprint requests and correspondence: Dr. Ilan Goldenberg, Heart Research Follow-Up Program, Box 653, University of Rochester Medical Center, Rochester, New York 14642. E-mail: ilan.goldenberg@heart.rochester.edu.

REFERENCES

1. Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome. Prospective longitudinal study of 328 families. Circulation 1991;84:1136–44.
2. Goldenberg I, Moss AJ. Long QT syndrome. J Am Coll Cardiol 2008;51:2291–300.
3. Goldenberg I, Moss AJ, Peterson DR, et al. Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. Circulation 2008;29;117:2184–91.
4. Hobbs JB, Peterson DR, Moss AJ, et al. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. JAMA 2006;296:1249–54.
5. Sauer AJ, Moss AJ, McNitt S, et al. Long QT syndrome in adults. J Am Coll Cardiol 2007;49:329–37.
6. Zareba W, Moss AJ, Locati EH, et al., International Long QT Syndrome Registry. Modulating effects of age and gender on the clinical course of long QT syndrome by genotype. J Am Coll Cardiol 2003;42:103–9.
7. Zareba W, Moss AJ, Schwartz PJ, et al., International Long-QT Syndrome Registry Research Group. Influence of genotype on the clinical course of the long-QT syndrome. N Engl J Med 1998;339:960–5.
8. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med 2003;348:1866–74.
9. Moss AJ, Shimizu W, Wilde AA, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. Circulation 2007;115:2481–9.
10. Shimizu W, Moss AJ, Wilde AA, et al. Genotype-phenotype aspects of type 2 long QT syndrome. J Am Coll Cardiol 2009;54:2052–62.
11. Bazett HC. An analysis of the time relations of electrocardiograms. Heart 1920;7:353–67.
12. Schwartz PJ, Moss AJ, Vincent GM, Crampton RS. Diagnostic criteria for the long QT syndrome: an update. Circulation 1993;88:782–4.
13. Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. J Clin Invest 2005;115:2018–24.
14. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model. New York: Springer-Verlag, 2000.
15. Kaufman ES, McNitt S, Moss AJ, et al. Risk of death in the long QT syndrome when a sibling has died. Heart Rhythm 2008;5:831–6.
16. Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. N Engl J Med 1992;327:846–52.
17. Priori SG, Napolitano C, Schwartz PJ. Low penetrance in the long-QT syndrome: clinical impact. Circulation 1999;99:529–33.
18. Stramba-Badiale M, Locati EH, Martinelli A, Courville J, Schwartz PJ. Gender and the relationship between ventricular repolarization and cardiac cycle length during 24-h Holter recordings. Eur Heart J 1997;18:1000–6.
19. Malloy KJ, Bahinski A. Cardiovascular disease and arrhythmias: unique risks in women. J Gend Specif Med 1999;2:37–44.
20. Lehmann MH, Hardy S, Archibald D, Quart B, MacNeil DJ. Sex difference in risk of torsade de pointes with d,l-sotalol. Circulation 1996;94:2535–41.
21. Johnson JN, Ackerman MJ. QTc: how long is too long? Br J Sports Med 2009;3:657–62.
22. Vincent GM, Schwartz PJ, Denjoy I, et al. High efficacy of β-blockers in long-QT syndrome type 1: contribution of noncompliance and QT-prolonging drugs to the occurrence of β-blocker treatment "failures." Circulation 2009;20:119:215–21.

Key Words: corrected QT interval ▪ long-QT syndrome ▪ sudden cardiac death.

APPENDIX

For a table about *KCNQ1*, *KCNH2*, and *SCN5A* mutations by amino acid coding, frequency, location, and type, please see the online version of this article.