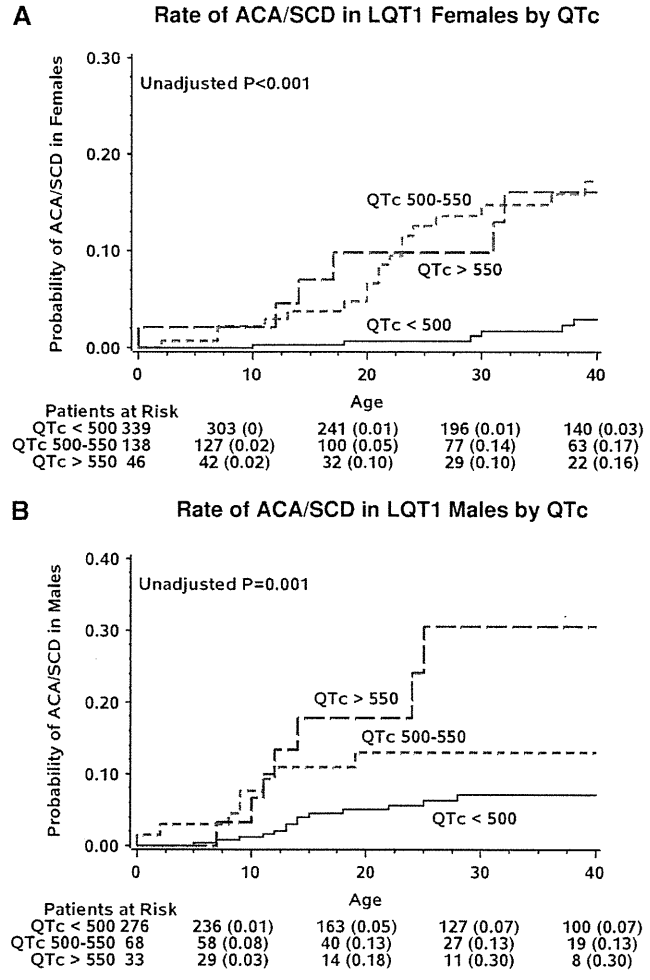

Figure 4 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in (**A**) women with LQT1 and (**B**) men with LQT1, by QTc duration. ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; LQT1 = long QT syndrome type 1; QTc = corrected QT interval; SCD = sudden cardiac death.

However, because of a small sample of patients with >1 mutation, the current results should be interpreted with caution in the risk assessment of this subset.

Conclusions and clinical implications

Our data extend prior knowledge regarding genotype-specific risk assessment in LQTS.[16,17] The present results suggest that the functional effects of mutations in the *KCNQ1*-encoded channel subunit may explain differences in the risk for life-threatening cardiac events between men and women with LQT1. Here, both men and women with LQT1-causative mutations localizing to the C loops (S2–S3 and S4–S5 linkers), the intracellular domains that connect the MS domains of the *KCNQ1*-encoded protein, have increased risk for not only LQT1-triggered syncope but also LQT1-triggered life-threatening cardiac events of ACA and SCD, possibly due to the increased sensitivity of these functional domains to adrenergic stimulation. In contrast, men with LQT1 were shown to have an increased risk for ACA or SCD even in the presence of mutations localizing elsewhere predicted at the molecular/cellular level to be associated with lower risk. These findings suggest that a genotype-specific approach, incorporating clinical and mutation location/functional data, might further improve the risk assessment and management of patients with the most common genetic subtype of LQTS.

Appendix

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hrthm.2012.01.020.

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CASE REPORT

A left ventricular noncompaction in a patient with long QT syndrome caused by a *KCNQ1* mutation: a case report

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Abstract A 5-year-old girl developed cardiopulmonary arrest after crying. From the electrocardiogram and echocardiography, a left ventricular noncompaction (LVNC) with long QT syndrome (LQT) was suspected as the cause of the cardiopulmonary arrest, and treatment with a β-blocker and a calcium antagonist was then begun. A genetic screening of LQT-related genes revealed a previously reported heterozygous *KCNQ1* mutation. The association of LVNC and LQT is an extremely rare condition, and long-term treatment based on the characteristics of both disorders is required. Also, the association of cardiomyopathy and LQT could become a new clinical entity in the future.

Keywords Long QT syndrome · Left ventricular noncompaction · Epilepsy · Cardiopulmonary arrest · *KCNQ1* mutation

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Background

Long QT syndrome (LQT) is a group of ion-channel disorders of the myocardium that may prolong the repolarization of the cardiac cycle [1]. According to the genotype investigation, 12 subtypes (LQT1–12) have been reported [2]; each subtype has its own clinical characteristics, and the treatment strategy differs for each subtype. Long QT syndrome is known as the most important cause of sudden cardiac death in the young [3], and may mostly result from the occurrence of ventricular fibrillation (VF) or torsade de pointes (TdP).

Here we report the case of a girl with left ventricular noncompaction (LVNC) and LQT, which were confirmed after resuscitation from cardiopulmonary arrest.

Case report

A 5-year-old girl had syncope after intense crying at her kindergarten. Her mother noticed cyanosis around her lips and then she developed cardiopulmonary arrest. Bystander cardiopulmonary resuscitation (CPR) was started immediately by a kindergarten teacher, who called for an ambulance. An automated external defibrillator (AED) revealed pulseless electrical activity, and there was no indication for defibrillation. She was transported to our hospital under continuous CPR.

She had been followed up for a diagnosis of epilepsy after two episodes of afebrile convulsions when she was 3 years old. She had a syncopal attack during the follow-up period, and multifocal spike waves were noted on the electroencephalogram. She had been administered carbamazepine since then, after which the spike waves disappeared during the follow-up period. Except for an

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episode of afebrile convulsions at age 4 years, she did not experience any further episodes of convulsions or syncope. Magnetic resonance imaging revealed no brain abnormalities.

On arrival at our hospital, sinus rhythm had resumed; however, she required intubation and respiratory support for her respiratory failure and cardiac dysfunction. Her cardiac function then improved gradually, but about 6 h after arrival, TdP and VT emerged in the intensive care unit. While we were preparing to defibrillate her, performing cardiac compressions for about 1 min, sinus rhythm resumed spontaneously (Fig. 1) and her cardiac function improved.

The electrocardiogram obtained after the CPR exhibited a prolonged QTc interval (Fig. 2, QTc = 0.6 s), and the patient was suspected as having LQT. There were no electrolyte imbalances at the time of hospitalization. Her cardiac function improved gradually after CPR. An echocardiogram revealed a spongy dysplastic left ventricular myocardium with prominent trabeculations and deep recesses, indicating LVNC (Fig. 3). We therefore started the patient on propranolol and verapamil to control her VT. Her respiratory support was discontinued 3 days after hospitalization. After administration of propranolol and verapamil, TdT and VT no longer emerged.

Electrocardiographic examinations and a genetic screening of LQT-related genes were performed on the patient, her sister and brother, her parents, her paternal and maternal grandfathers and grandmothers, and a maternal uncle (Fig. 4). She, her brother, her father, and her paternal grandmother were found to have a previously reported heterozygous *KCNQ1* mutation c.1831 G > T in exon 15 (p. D611T). No prolongation of QT intervals or echocardiographic abnormalities were found in family members (her brother, her father, and her paternal grandmother) who had a *KCNQ1* mutation. Although her development had been normal until this event, the patient manifested mild mental retardation because of ischemic brain damage. She underwent rehabilitation and attended a school for handicapped children with a restriction on swimming.

Discussion

Left ventricular noncompaction is a congenital cardiomyopathy with a spongy morphological appearance and deep intertrabecular sinusoids in communication with the ventricular cavity [4]. The diagnosis is mainly made by two-dimensional echocardiography, cardiac magnetic resonance imaging, or left ventricular angiography.

The echocardiogram reveals that prominent trabeculations and deep recesses are noted in the ventricular myocardium [5]. However, so far there has been no distinct definition of LVNC [6]. Koh et al. [7] reported that a left ventricular myocardial deformation is reduced in the longitudinal and circumferential dimensions and manifests with tight systolic–diastolic coupling in children with LVNC.

Genetic mutations were first reported in the *G4.5* gene in patients with an isolated LVNC [8]. Z-line and mitochondrial mutations and X-linked inheritance resulting from mutations in the *G4.5* gene encoding tafazzin could be a pathogenesis for the disease. In this report, the gene defect differed among the families, and thus there did not appear

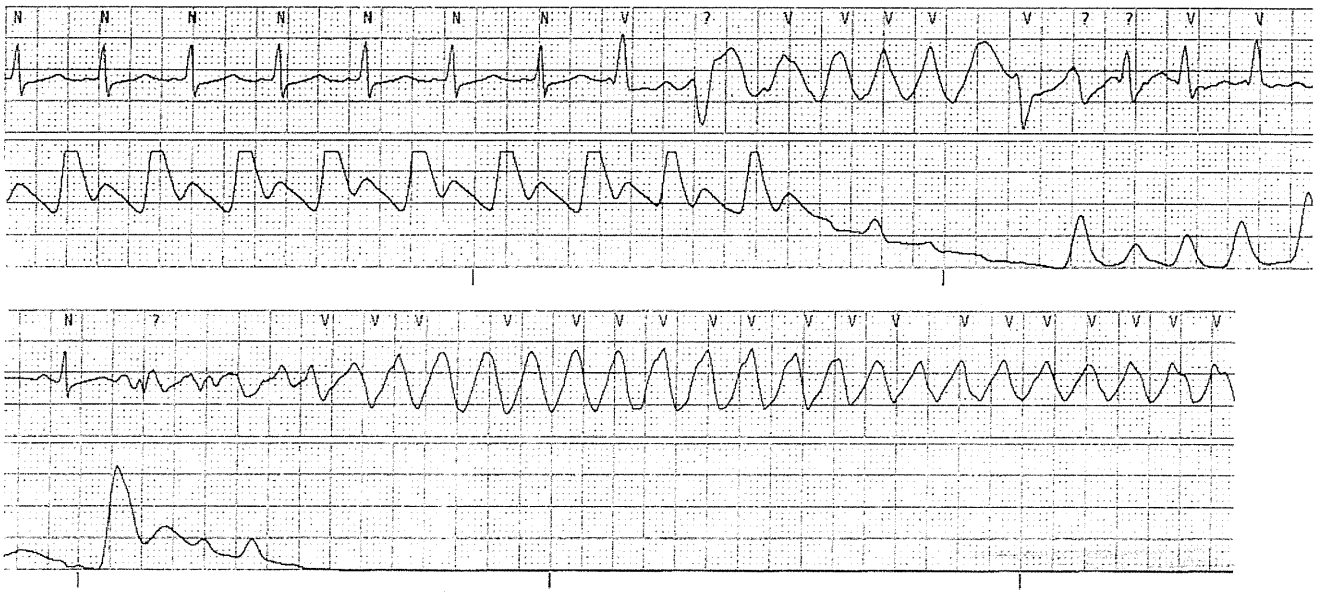

Fig. 1 Monitor recording obtained while the patient was in the intensive care unit. *Upper panel* occurrence of torsade de pointes (TdP), which terminated within several beats. *Lower panel* occurrence of long-lasting TdP or ventricular fibrillation

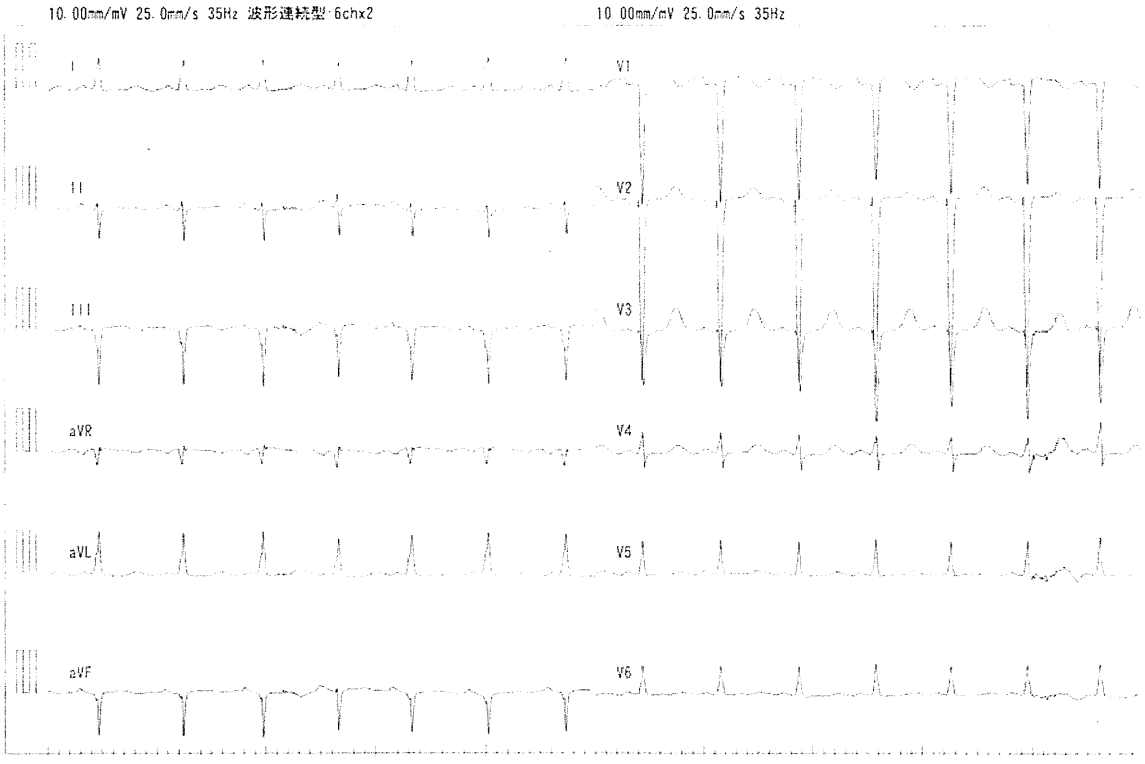

Fig. 2 Electrocardiogram recorded after the resuscitation. The electrocardiogram after the resuscitation showed normal sinus rhythm with left QRS axis deviation (−15°). The QTc interval was prolonged to 0.6 s. There were also flattened T waves in the left precordial leads (V5 and V6)

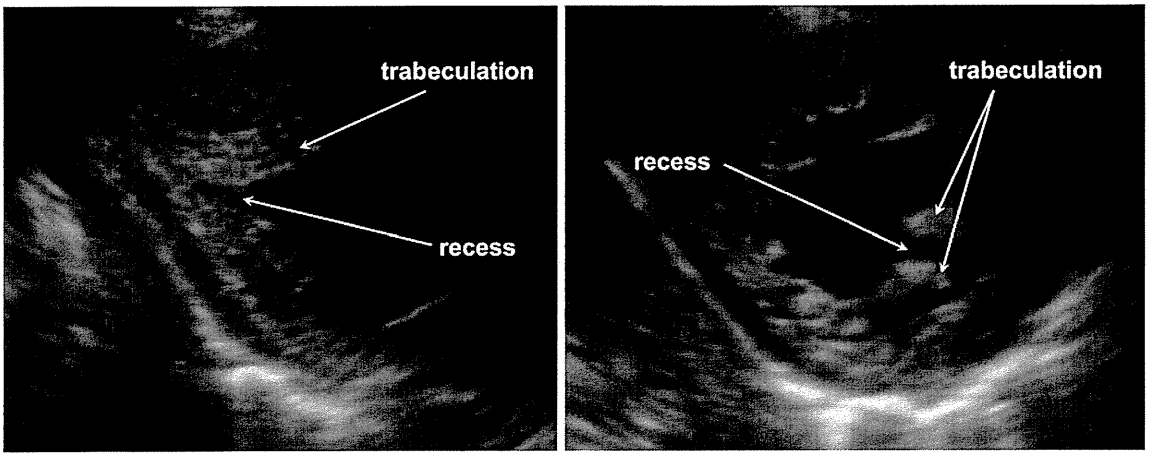

Fig. 3 Echocardiogram. Prominent trabeculations and a deep recess were detected

to be any obvious genotype–phenotype correlation that would allow for the differentiation of the clinical course to be predicted. In addition, the cardiac phenotypes that occur as a result of *G4.5* mutations may vary significantly. On the other hand, in patients with LVNC associated with congenital heart disease, a so-called nonisolated LVNC, mutations in the α-dystrobrevin gene have been reported [9]. In this report, the α-dystrobrevin mutation resulted in a phenotype of a dilated hypertrophic cardiomyopathy with deep trabeculations associated with congenital heart disease, consistent with the criteria for LVNC. However, the phenotype in this family had considerable variability. Consequently, the details of the relation between an ion-channel dysfunction and the maldevelopment of the ventricular myocardium are not well described. Further genetic studies are needed to discover whether a combined mutation with *G4.5* or α-dystrobrevin and *KCNQ1* could have contributed to this clinical manifestation in this patient.

The association of LVNC with LQT is extremely rare. *SCN5A* mutations are frequently associated with LVNC.

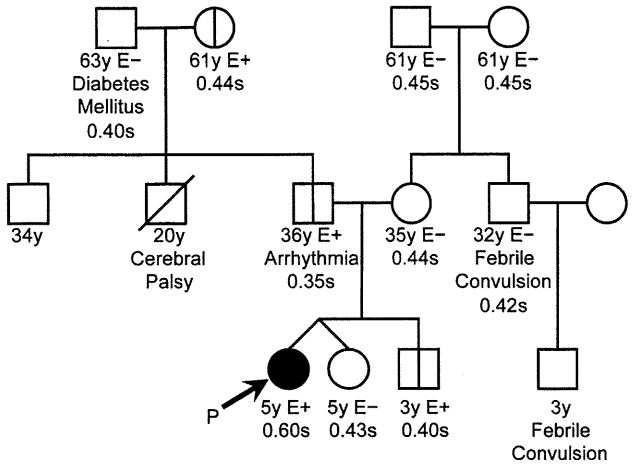

Fig. 4 Family tree of our patient. The *arrow* shows the proband. *KCNQ1* mutations were detected in the proband, and her brother, father, and grandmother

However, only 2 of 62 patients were found to have LQT in *SCN5A*-positive LVNC [10]. *SCN5A* mutations are well known as a cause of LQT3 syndrome and Brugada syndrome. Ogawa et al. [11] reported a *KCNH2* mutation in two patients with LQT and LVNC. *KCNH2* mutations have been known to be the cause of LQT2 syndrome.

KCNQ1 mutations are known as a cause of LQT [12]. However, to the best of our knowledge there have been no previous reports on the association of a *KCNQ1* mutation and LVNC, so this is the first report suggesting an association between LQT1 and LVNC.

The association of cardiomyopathy and LQT could become a new clinical entity in the future. In 2006, the American Heart Association scientific statement on the classification of cardiomyopathies formally classified LVNC as its own disease entity, as a primary cardiomyopathy with a genetic origin, in the same category as ion-channel disorders [13]. Long-term follow-up will be required to reveal further associations between both disorders.

Conclusion

The association of LQT with LVNC is extremely rare. There have been only two patients with *SCN5A* mutations and two patients with *KCNH2* mutations reported to date. This is the first report of a *KCNQ1* mutation with LQT and LVNC. A genetic screening of LQT-related genes is recommended for patients with a long QT interval and LVNC.

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Mutations in Cytoplasmic Loops of the KCNQ1 Channel and the Risk of Life-Threatening Events

Implications for Mutation-Specific Response to β-Blocker Therapy in Type 1 Long-QT Syndrome

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Background—β-Adrenergic stimulation is the main trigger for cardiac events in type 1 long-QT syndrome (LQT1). We evaluated a possible association between ion channel response to β-adrenergic stimulation and clinical response to β-blocker therapy according to mutation location.

Methods and Results—The study sample comprised 860 patients with genetically confirmed mutations in the *KCNQ1* channel. Patients were categorized into carriers of missense mutations located in the cytoplasmic loops (C loops), membrane-spanning domain, C/N terminus, and nonmissense mutations. There were 27 aborted cardiac arrest and 78 sudden cardiac death events from birth through 40 years of age. After multivariable adjustment for clinical factors, the presence of C-loop mutations was associated with the highest risk for aborted cardiac arrest or sudden cardiac death (hazard ratio versus nonmissense mutations=2.75; 95% confidence interval, 1.29–5.86; P=0.009). β-Blocker therapy was associated with a significantly greater reduction in the risk of aborted cardiac arrest or sudden cardiac death among patients with C-loop mutations than among all other patients (hazard ratio=0.12; 95% confidence interval, 0.02–0.73; P=0.02; and hazard ratio=0.82; 95% confidence interval, 0.31–2.13; P=0.68, respectively; P for interaction=0.04). Cellular expression studies showed that membrane spanning and C-loop mutations produced a similar decrease in current, but only C-loop mutations showed a pronounced reduction in channel activation in response to β-adrenergic stimulation.

Conclusions—Patients with C-loop missense mutations in the *KCNQ1* channel exhibit a high risk for life-threatening events and derive a pronounced benefit from treatment with β-blockers. Reduced channel activation after sympathetic activation can explain the increased clinical risk and response to therapy in patients with C-loop mutations. **(*Circulation.* 2012;125:1988-1996.)**

Key Words: adrenergic beta-antagonists ■ ion channels ■ long QT syndrome ■ mutation

Long-QT syndrome type 1 (LQT1) is the most common type of inherited long-QT syndrome (LQTS), accounting for ≈35% of all patients and >50% of genotyped patients.[1] LQT1 arises from a decrease in repolarizing potassium current resulting from mutations in the *KCNQ1* gene. Four *KCNQ1*-derived α-subunits assemble to form the I_{Ks} channel along with obligatory auxiliary subunits derived from *KCNE1*. Exercise is the main trigger for cardiac arrhythmic

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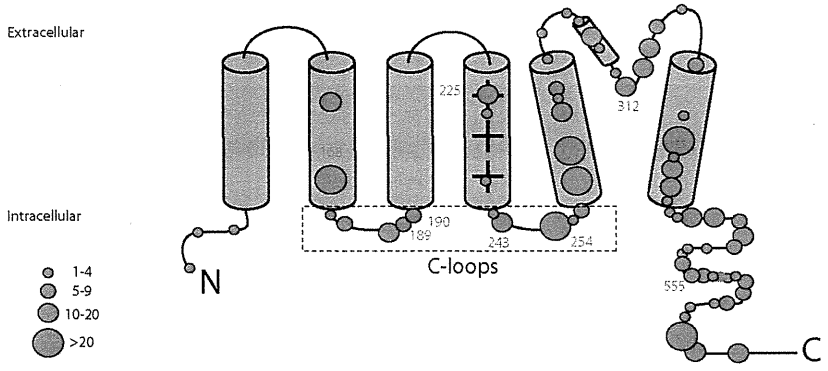

Figure 1. Frequency and location of mutations in the KCNQ1 potassium channel. Diagrammatic location of 99 different mutations in the *KCNQ1* potassium channel involving 860 subjects. The α-subunit involves the N-terminus (N), 6 membrane-spanning segments, 2 cytoplasmic loops (S2–S3 and S4-S5), and the C-terminus portion (C). The size of the circles reflects the number of subjects with mutations at the respective locations.

events in patients with LQT1.[2] Activation of β1-adrenergic receptors is the major signaling pathway contributing to the increase in heart rate and cardiac output during exercise. β1-Adrenergic receptor activation leads to activation of protein kinase A (PKA), which directly phosphorylates the *KCNQ1* subunit, increasing I_{Ks} function.[3,4] The increase in I_{Ks} is thought to suppress the premature beats and afterdepolarization induced by increased L-type Ca^{2+} currents during β-adrenergic stimulation.[5] Accordingly, β-blockers have been considered the first-line therapy in LQT1 patients without a history of aborted cardiac arrest (ACA). Data from several prior LQTS studies[1,6] demonstrate that despite the reduction in the risk of cardiac events with β-blocker therapy among LQT1 patients, there is a considerable cardiac residual event rate among patients who are being treated with this mode of medical therapy ($\approx$10 cardiac events per 100 person-years),[6] suggesting that β-blockers may be less effective in certain subgroups of LQT1 patients.

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The *KCNQ1* protein consists of 676 amino acid residues with an intracellular N-terminus region, 6 membrane-spanning segments with 2 connecting cytoplasmic loops (C loops), and an intracellular C-terminus region.[7] Prior genotype-phenotype studies have provided important information on the effect of location and coding type of the channel mutations on the phenotypic manifestations and clinical course of LQT1 patients. These studies have shown that missense mutations and mutations located at the transmembrane region (including the C loops) were associated with greater risk for cardiac events.[8] However, the mechanism related to the increased risk associated with transmembrane mutations has not been studied. C loops, part of the transmembrane region, were suggested to affect adrenergic channel regulation by PKA.[9] We therefore hypothesized that the previously reported finding about the risk associated with transmembrane mutations[8] is related to the effect of C-loop mutations within this region. Accordingly, the present study was carried out in a large cohort of subjects having a spectrum of *KCNQ1* mutations from the International LQTS Registry and was designed to investigate the clinical outcomes among *KCNQ1* mutation carriers by further dividing the transmembrane region into membrane-spanning and C-loop domains, to determine a possible differential response to β-blocker therapy depending on mutation location and function related to PKA regulation, and to relate the clinical data to functional studies of changes in I_{Ks} function and β-adrenergic receptor regulation in mammalian cells.

Methods

Study Sample

The study comprised 860 patients with genetically confirmed *KCNQ1* mutations derived from 170 proband-identified families. The proband in each family had QTc prolongation not resulting from a known secondary cause. The subjects were drawn from the Rochester (n=637), the Netherlands (n=94), the Japanese (n=82), the Danish (n=43), and the Swedish (n=4) portions of the Multicenter Mutation Registry. All subjects or their guardians provided informed consent for the genetic and clinical studies. Patients with congenital deafness and patients with multiple LQTS-associated mutations were excluded from the study.

Phenotype Characterization

On enrollment, routine clinical and ECG information was obtained from birth to the participants' enrolled age, and ongoing clinical information was obtained at yearly intervals thereafter. For each patient, data on personal and family histories, cardiac events, and therapy were systematically recorded at enrollment and at each visit or medical contact. Clinical data, recorded on prospectively designed forms, included patient and family histories and demographic, ECG, therapeutic, and cardiac event information. Data on β-blocker therapy included the starting date and discontinuation date if appropriate. Information on the end point of ACA or sudden cardiac death (SCD) was also verified through requested medical records. Every effort was made to confirm an underlying life-threatening arrhythmia when observed or documented by medical staff.

Genotype Characterization

The *KCNQ1* mutations were identified with the use of standard genetic tests performed in academic molecular genetics laboratories. Genetic alterations of the amino acid sequence were characterized by location and by the specific type of mutation (missense, splice site, in-frame insertions/deletions, nonsense, stop codon, and frameshift).

We evaluated the risk associated with 4 main prespecified subgroups: C- or N-terminus missense, membrane-spanning missense, C-loop missense, and nonmissense (ie, splice sites, in-frame insertions, in-frame deletions, stop codons, and frameshift). The membrane-spanning region of the KCNQ1-encoded channel was defined as the coding sequence involving amino acid residues between 124 and 170 (S1-S2), 196 and 241 (S3-S4), and 263 and 355 (S5-S6), with the C-loop region between residues 171 to 195 (S2-S3) and 242 to 262 (S4-S5; Figure 1). The N-terminus region was defined before residue 124 and the C-terminus region after residue 355.

To minimize survival bias, we included patients who died before they were genotyped (n=64). They were assumed to have the mutation that their first-degree relatives had. All other patients were confirmed through genotyping.

Cellular Expression Studies

To study the mechanism underlying the risk for cardiac events in patients with missense C-loop mutations, we measured channel function and regulation for channels formed with wild-type (WT) subunits coexpressed with 4 mutant subunits present in C loops (G189R, R190Q, R243C, and V254M) and 4 mutant subunits present in the non–C-loop domains: 3 in the membrane-spanning domain (T312I, G168R, and S225L) and 1 in the C terminus (R555C). The mutations chosen included the most common mutations in the LQT1 registry. WT and mutant KCNQ1 subunit cDNA and KCNE1 subunit cDNA were transfected into HEK293T cells.[10] Mutant KCNQ1 cDNA was transfected in combination with WT-KCNQ1 to mimic the heterozygous nature of the disease (WT-KCNQ1:mutant KCNQ1:KCNE1=0.5:0.5:1). Fluorescence-conjugated and -tagged constructs were used to evaluate the efficiency of the cotransfection of WT and mutant subunits[11] (see the online-only Data Supplement). Of the HEK293T cells cotransfected with both WT and mutant subunits, 85% to 90% showed fluorescence of at least 1 subunit transfected, and 85% to 95% of transfected cells expressed all the subunits transfected (Figure I in the online-only Data Supplement). All electrophysiology determinations were performed with the untagged subunit. Expression of WT and mutant subunits was confirmed by Western blot (Figure II in the online-only Data Supplement). Expression levels were not significantly decreased for the mutant subunits compared with WT. We measured ion channel currents after channel depolarization to 20 mV for 4 seconds from −80-mV holding potential before and after application of forskolin, a PKA activator (10 μmol/mL), with standard electrophysiological techniques and physiological solutions. Current was normalized for all voltages to cell capacitance, and further normalization was performed between WT and mutant. The normalization to WT currents was accomplished by use of WT cell currents transfected and measured on the same day as the currents measured from mutant channel.[10] Pipettes used had resistances ranging from 2 to 6 MOhm. Series resistance compensation of >70% was used to compensate for voltage drops in the pipette. All experiments were performed at room temperature. Details of the molecular biology and electrophysiological methods are given in the Materials and Methods section of the online-only Data Supplement.

End Point

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising ACA requiring external defibrillation as part of the resuscitation 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 an unwitnessed setting) from birth through 40 years of age. Follow-up after 40 years of age was not included to minimize the influence of coronary disease on cardiac events. The consistency of the results among patients who received an implantable cardioverter-defibrillator during follow-up was evaluated in a secondary analysis that included the occurrence of a first appropriate implantable cardioverter-defibrillator shock in the composite ACA or SCD end point.

Statistical Analysis

Characteristics of the 4 subgroups of patients categorized by mutation location and type were compared by use of a 1-way ANOVA test or χ^2 and Fisher exact tests as appropriate. The probability of a first life-threatening cardiac event by the mutation location and type subgroup was graphically displayed according to the Kaplan-Meier method, with comparison of instantaneous risk by the log-rank test. The Cox proportional-hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of a life-threatening cardiac event from birth through 40 years of age. The Cox regression models, stratified by decade of birth year and allowing for time-dependent covariates, were fit to estimate the adjusted hazard ratio function of age. Therefore, to fulfill the assumption of proportional hazards for sex over the entire age range, a time-dependent covariate for sex (via an interaction with time) was incorporated, allowing for different hazard ratios by sex before and after 13 years of age. This was justified by the known higher risk of cardiac events or life-threatening cardiac events among male subjects before adolescence and a similar or higher female risk after the onset of adolescence.[12–16] Patients who did not have an ECG for QTc measurement (n=127) were identified in the Cox models as QTc missing, and all Cox models were adjusted for this QTc-missing parameter. The influence of time-dependent β-blocker therapy (the age at which β-blocker therapy was initiated) on outcome in the subgroups of patients with and without C-loop missense mutations was determined by adding a time-dependent β-blocker–by–mutation category interaction term to the multivariable Cox model. We have adjusted for the effect of potential lack of independence between subjects using the robust sandwich estimator for family membership.[17,18] This robust sandwich covariance estimator is used with correlated data. Correlations among data points in the Cox model lead to underestimation of the SE used in significance testing, whereas the robust estimator uses an inflated variance estimate, taking family membership or other clustering connection into account. All significant predictors of life-threatening event risk remained significant with or without the use of this robust measure of variance. It should be noted that there is seldom more than a single observed outcome (ACA/SCD) per family (only 7% of families had >1 event); thus, the standard model-based SEs, confidence intervals, P values, and likelihood ratio tests are valid.[16]

We have carried out the following additional secondary analyses: (1) including the biophysical function of the mutations (categorized as dominant negative, haploinsufficiency, and unknown) as a covariate in the model, (2) excluding the large subgroup of patients with V254M mutations, and (3) including appropriate implantable cardioverter-defibrillator shocks in the composite end point. In addition, to assess whether fuller adjustment for family membership was important, regression models that included shared frailty terms (ie, random effects) for family were fit.

The statistical software used for the analyses was SAS version 9.20 (SAS Institute Inc, Cary, NC). For the fitting of models with frailty terms, the software used was Splus 7.0.0 for Sun SPARC. For electrophysiology and biochemistry experiments, 1-way ANOVA followed by the Tukey post hoc test was applied to assess statistical significance for multiple-group comparisons by use of SPSS Statistics (IBM). An unpaired Student t test was used for 2-group comparisons. A 2-sided significance level of 0.05 was used for hypothesis testing.

Results

Study Sample

The spectrum of mutations as categorized by location and type and their respective number of carriers are presented in Table I-A in the online-only Data Supplement. The location and frequency of missense mutations are presented diagrammatically in Figure 1. Of the 99 total different KCNQ1 mutations identified, 77 were missense mutations and 22 were nonmissense mutations. Missense mutations were further categorized according to their location: 28 different mutations in C-terminus or N-terminus regions (26 in C terminus), 34 mutations in membrane-spanning regions, and 15 mutations in the C-loop regions (8 in S2-S3 loop and 7 in S4-S5 loop). The clinical characteristics of patients in the 4 mutation location/type subgroups are presented in Table 1. Of the 860 study subjects, 20% had C/N terminal missense mutations, 44% had membrane-spanning missense mutations, 15% had C-loop missense mutations, and 22% had nonmissense mutations. Patients with C-loop missense mutations exhibited the longest QTc interval at enrollment, were treated with β-blockers more frequently during follow-up, and had a higher frequency of cardiac events of any type, including

Table 1. Demographic and Clinical Characteristics

Parameter	Missense			Nonmissense
	C/N Terminus	Membrane Spanning	C Loops	
Patients, n (%)	172 (20.0)	376 (43.7)	125 (14.5)	187 (21.7)
Female, n (%)	94 (54.7)	221 (58.8)	70 (56.0)	119 (63.6)
Age at enrollment, median (interquartile range), y	21 (9–41)	25 (11–41)	19 (5–35)	21 (11–39)
QTc at enrollment, mean±SD, ms*	467±63	480±51	503±58	470±41
QTc at enrollment ≥500 ms, n (%)	39/154 (25.3)	94/312 (30.1)	47/102 (46.1)	41/165 (24.8)
Therapy during follow-up, n (%)				
β-blockers	64 (37.0)	167 (44.4)	63 (50.4)	74 (39.8)
Pacemaker	2 (1.2)	7 (1.9)	1 (0.8)	4 (2.2)
Defibrillator	6 (3.5)	25 (6.6)	10 (8.0)	13 (7.0)
Sympathectomy	0 (0.0)	1 (0.3)	1 (0.8)	1 (0.5)
Cardiac event during follow-up, n (%)				
Syncope	52 (30.1)	122 (32.4)	69 (55.2)	46 (24.7)
Aborted cardiac arrest	6 (3.5)	10 (2.7)	8 (6.4)	3 (1.6)
Sudden cardiac death	18 (10.4)	29 (7.7)	24 (19.2)	13 (7.0)
Any cardiac event	63 (36.4)	144 (38.3)	84 (67.2)	55 (29.6)

C loop indicates cytoplasmic loop.

*Of the 127 long-QT syndrome type 1 mutation carriers who did not have an ECG for QTc measurement, 58 (46%) died suddenly at a young age without a documented ECG.

syncope, ACA, and LQTS death, compared with the other mutation subgroups. The clinical characteristics of probands only are presented in Table I-B in the online-only Data Supplement.

Clinical Outcome of Patients According to Mutation Location and Type

There were 105 first life-threatening cardiac events (27 first ACA events and 78 first LQTS-related SCD events) among the 860 study patients. Patients were enrolled in the registry between 1978 and 2007 with follow-up through 2008; the last reported life-threatening cardiac event occurred in 2005. Figure 2 presents the cumulative probabilities of first life-threatening cardiac events in the 4 subgroups. There was a significantly higher event rate in the C-loop missense subgroup compared with the other 3 subgroups (log-rank $P<0.001$). Thus, at 40 years of age, the rate of life-threatening cardiac events was 33% in patients with C-loop missense mutations compared with ≤16% in patients with other mutations.

The findings from the multivariable analysis for the end point of a first life-threatening cardiac event are shown in Table 2. Notably, the adjusted hazard ratio for C-loop missense versus nonmissense mutation was 2.75 ($P=0.009$), and there was no statistically significant difference in the risk among the other mutation location/type subgroups.

Secondary confirmatory analyses (Table II in the online-only Data Supplement) showed that patients with C-loop missense mutations had an adjusted hazard ratio of 2.74 (95% confidence interval, 1.68–4.46; $P<0.001$) for life-threatening events compared with patients with other mutations. The results were consistent when the biophysical function of the mutations was added as a covariate to the multivariable model. To show that our results do not depend on the C-loop V254M mutation, which is the most common mutation in the C-loop subgroup (Table I in the online-only Data Supplement), accounting for 50% of C-loop patients, we

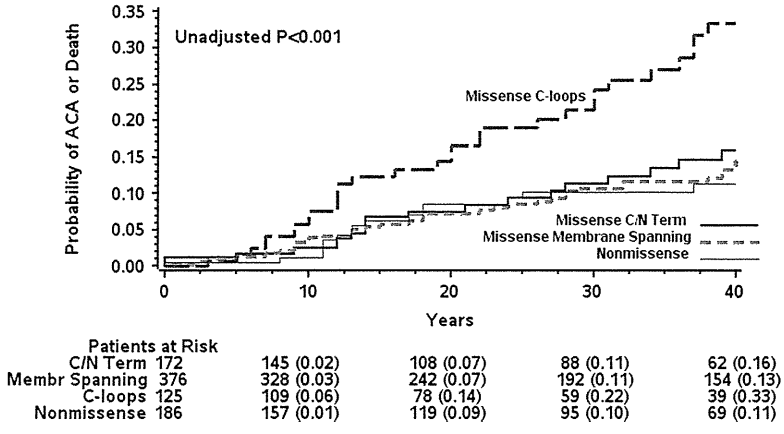

Figure 2. Kaplan-Meier estimates of cumulative probability of life-threatening cardiac events by mutation location and type. The numbers in parentheses reflect the cumulative event rate at that point in time. ACA indicates aborted cardiac arrest; LQTS, long-QT syndrome.

Table 2. Multivariable Analysis: Risk Factors for Aborted Cardiac Arrest or Sudden Cardiac Death

	Hazard Ratio	95% Confidence Interval	P
Sex/age			
Male (vs female) age <13 y	1.93	1.08–3.45	0.03
Male (vs female) age 13 to 40 y	1.13	0.67–1.91	0.65
QTc ≥500 ms (vs QTc <500 ms)	3.55	1.83–6.89	<0.001
Mutation type and location			
Cytoplasmic loops (missense) vs nonmissense	2.75	1.29–5.86	0.009
C/N terminus (missense) vs nonmissense	1.47	0.64–3.39	0.37
Membrane spanning (missense) vs nonmissense	0.85	0.41–1.78	0.67

The models are adjusted for sex by age and corrected QT category (including missing QT), mutation type and location category, and time-dependent β-blocker treatment. Of the 127 long-QT syndrome type 1 mutation carriers without available ECG data, 58 (46%) died suddenly at a young age without a documented ECG. The hazard ratio for missing QTc versus available was 10.49 (95% confidence interval, 6.61–16.66; $P<0.001$).

have carried out an additional separate analysis excluding patients who carried this mutation. Results were consistent, with patients with C-loop mutations having a greater risk for life-threatening events, demonstrating that our findings were independent of this mutation. The results were also consistent after inclusion of appropriate implantable cardioverter-defibrillator shocks in the composite end point (adjusted hazard ratio for C-loop missense mutations versus nonmissense mutations. 2.64; 95% confidence interval, 1.64–4.23; $P<0.001$) and after stratifying patients by enrolling center. To assess whether fuller adjustment for family membership was important, regression models that included frailty terms (ie, random effects) for family were fit in the multivariable Cox models. Models with gamma and gaussian frailty terms were fit, and the C-loop term had a consistent effect size with the original models while remaining statistically significant. Furthermore, in both of these models, the frailty terms were nonsignificant. The consistency of the results provides further support for the higher risk associated with C-loop mutations.

β-Blocker Therapy

In the present study, the effect of β-blocker therapy on the risk of life-threatening events among the different mutation subgroups was assessed as a time-dependent covariate (ie, β-blockers were given to patients at different time points during follow-up, and this information was taken into account in the multivariable models). Multivariable analysis showed a significant differential effect of β-blocker therapy on the outcome of patients with C-loop missense mutations compared with those who had other mutations (Table 3). β-Blocker therapy was associated with a significant 88% reduction ($P=0.02$) in the risk of life-threatening events among patients with C-loop missense mutations, whereas the benefit of β-blocker therapy was significantly attenuated among patients with other mutations in the *KCNQ1* channel

Table 3. Multivariable Analysis: Response to β-Blocker Therapy

β-Blocker vs No β-Blocker Therapy	Hazard Ratio	95% CI	P
All LQT1 patients	0.49	0.19–1.23	0.13
LQT1 patients with C-loop missense mutations*	0.12	0.02–0.73	0.02
LQT1 patients with other mutations (non–C-loop missense mutations)*	0.82	0.31–2.13	0.68

CI indicates confidence interval; LQT1, long-QT syndrome type 1; and C-loop, cytoplasmic loop. The models are adjusted for sex by age and corrected QT category (including missing QT), mutation type and location category, and time-dependent β-blocker treatment.

*P for interaction for mutation location-by–β-blocker treatment=0.04.

(adjusted hazard ratio, 0.82; $P=0.68$; P for treatment-by–mutation location/type interaction=0.04).

Consistent with those findings, the rate of ACA or SCD (Figure 3) was lowest among patients with C-loop missense mutations who were treated with β-blockers and highest among patients with C-loop missense mutations who were not treated with β-blockers (0.17 versus 1.11 per 100 patient-years, respectively), whereas patients with other mutations in the KCNQ1 channel exhibited intermediate and similar rates of life-threatening events with and without β-blocker therapy (0.36 and 0.38 per 100 patient-years, respectively; Figure 3).

In addition, we have repeated analysis for only patients who were treated by β-blockers (at any point in time) and consistently found that there is differential response to β-blocker therapy depending on mutation location (β-blocker versus no β-blocker therapy in patients with C-loop mutations: hazard ratio, 0.15; 95% confidence interval, 0.01–1.73; $P=0.13$; β-blocker versus no β-blocker therapy in patients with non–C-loop mutations: hazard ratio, 1.89; 95% confidence interval, 0.54–6.63; $P=0.32$) with a value for treatment-by–mutation location/type interaction of $P=0.028$.

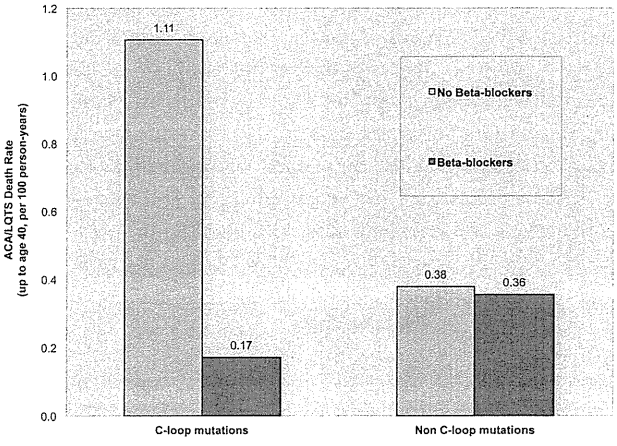

Figure 3. Risk for life-threatening cardiac events by mutation location and β-blocker treatment. Sixty-three of the 125 subjects (50%) with C-loop missense mutations were treated with β-blockers during a mean follow-up of 26.2 years; 305 of the 735 subjects (42%) with non–C-loop missense mutations were treated by β-blockers during a mean follow-up of 27.5 years. Event rates per 100 person-years were calculated by dividing the number of events during the period of β-blocker therapy or the absence of β-blocker therapy by person-years and multiplying the results by 100. ACA indicates aborted cardiac death; LQTS, long-QT syndrome.

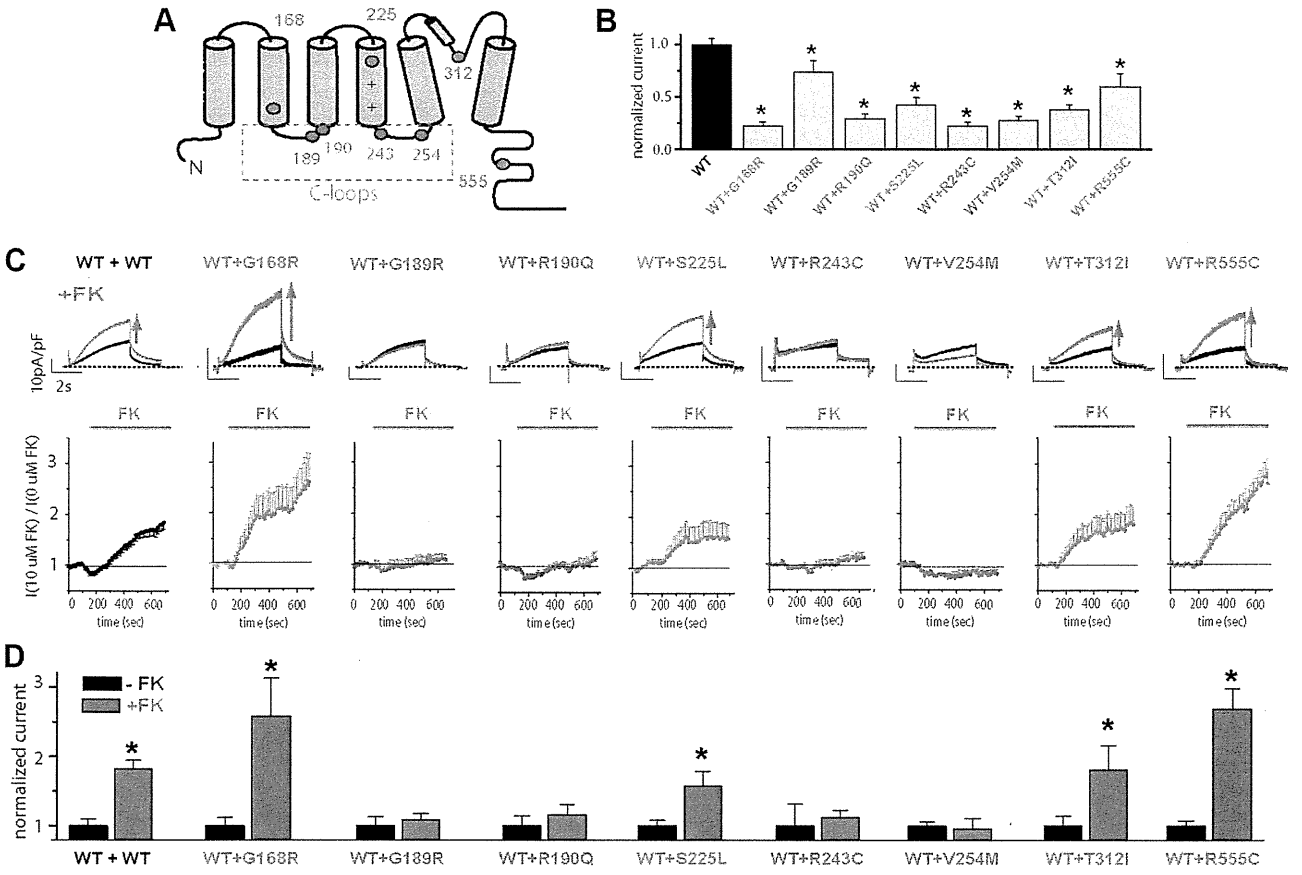

Figure 4. Regulation of LQT1 mutant channels by protein kinase A. **A**, Schematic representation of location of the mutations used in the study. **B**, Effect of each of the mutations studied in basal nonstimulated cell currents. Average current measured for cells expressing wild-type (WT) and mutant subunits measured at 40 mV after 3 seconds of depolarization. KCNQ1 and KCNE1 subunit were expressed at a ratio of 0.5 WT-KCNQ1:0.5 KCNQ1 mutant:1 KCNE1 or 0.5 WT-KCNQ1:0.5 vector:1 KCNE1 for T haploinsufficient channels. *$P<0.05$ vs WT. **C**, Top, Typical ion channel current measured before and after 10 minutes of application of the protein kinase A activator forskolin (FK; 10 μmol/L) for WT and WT and mutant subunits coexpressed. Scale bars in each panel are 10 pA/pF and 2 seconds. Scale bars are the same for all constructs. Bottom, Time course of current regulation by forskolin measured at 20 mV after 3 seconds of depolarization for channels formed by either WT or mutant coexpressed with WT subunits as indicated. Current was normalized to current in the absence of forskolin application. KCNQ1 and KCNE1 subunits were expressed at a ratio of 0.5 WT-KCNQ1:0.5 KCNQ1 mutant:1 KCNE1 or 1 WT-KCNQ1:1 KCNE1 for WT channels. Currents were activated by 4-second depolarizing steps to 20 mV from a −80-mV holding potential. These were followed by a step to −20 mV. **D**, Summary data for experiments done as in **C**. *$P<0.05$ vs the current before stimulation (black bar) in each group.

Cellular Expression Studies

To understand the mechanism underlying the increase in risk associated with C-loop mutations, we measured channel basal function and regulation in 8 mutant channels associated with LQT1: 3 in the membrane-spanning domains (T312I, G168R, and S225L), 4 located in C loops (G189R, R190Q, R243C, and V254M), and 1 in the C terminus (R555C; Figure 4A). WT and mutant subunits were coexpressed for all experiments. Basal channel current was decreased for all mutations studied compared with WT subunits (Figure 4B). In addition, because activation by PKA is thought to be particularly important for I_{Ks} function and to underlie arrhythmogenesis in LQT1,[4,19,20] we measured the effect of the PKA activator forskolin. All C-loop mutations tested showed dramatically impaired response to forskolin, whereas the other mutations showed a strong activation by forskolin, as did the WT KCNQ1 channel (Figure 4C and 4D).

Discussion

The present analysis of 860 LQT1 patients with a wide range of mutations in the *KCNQ1* channel provides several important implications regarding risk assessment and management in this study sample. First, patients with missense mutations located in the C loops exhibit the highest risk for life-threatening cardiac events independently of clinical and ECG variables. Second, β-blocker therapy is associated with a pronounced reduction in the risk of ACA or SCD among carriers of missense mutations in the C loop, whereas the benefit of this mode of medical therapy is significantly attenuated in LQT1 patients with other mutations. Third, expression studies of C-loop mutations suggest that an impaired regulation by PKA is the mechanism underlying the increased risk for cardiac events independently of patient QTc and may explain the pronounced response to medical therapy with β-blockers among patients with C-loop mutation carriers.

We have recently shown that patients with mutations located in the transmembrane region have a significantly higher rate of cardiac events than those with mutations located in the C terminus.[8] In addition, mutations in the transmembrane domain were suggested to be associated with

a greater prolongation of the QTc during exercise.[21] The present findings confirm previous work indicating that mutations in the transmembrane region are associated with higher risk but suggest that within the transmembrane region there are distinct functional domains, with the C loops (but not in the membrane-spanning domain) being associated with increased risk for life-threatening cardiac events compared with other mutations in the KCNQ1 channel. The S2-S3 and S4-S5 C loops have previously been suggested to have an important functional role in modifying the function of voltage-gated potassium channels.[22] In particular for I_{Ks}, the S4-S5 loop has been suggested to mediate a functional interaction with the auxiliary *KCNE1* subunits.[23] Most recently, LQT1 mutations in C loops, when expressed in the absence of WT subunits, were suggested to affect adrenergic channel regulation.[9] Our results showed that even when expressed in the presence of WT subunits, C-loop mutations can dramatically affect channel regulation. Consistent with our results, induced pluripotent stem cells differentiated into cardiomyocytes from a patient carrying R190Q were recently shown to lack adrenergic regulation of their I_{Ks} current.[24] Also consistent with our results, for haploinsufficient mutations, not tested here, a simple lack of mutant subunit expression is expected to maintain normal adrenergic regulation, contributing to the milder phenotype of these mutations.[8] It is conceivable that a decrease in channel regulation, as observed for the C-loop mutations, will lead to an increase in the burden of the mutation during adrenergic stimulus. The increase in cardiac risk associated with C-loop mutations is independent of traditional clinical variables; this can be explained by a blunted PKA-mediated activation because QTc is generally measured at rest. Thus, our results suggest that exercise may exacerbate the QTc prolongation for C-loop mutants. It has recently been suggested that the mutation KNCQ1(A341V) also caused an impairment in β-adrenergic activation.[25] This mutation is located at the end of the S6 domain, a region suggested to interact with the S4-S5 loop.[26] It is possible that other mutations causing functional impairment similar to that of the C-loop mutations may also carry the increased cardiac risk and β-blocker efficacy.

Current guidelines recommend empirical therapy with β-blockers in all LQTS patients.[27] The present study shows, for the first time, a mutation-specific response to β-blocker therapy in LQT1, demonstrating that β-blockers were associated with a significantly greater reduction in the risk of life-threatening cardiac events among patients with mutations located in the C loops compared with all other mutations. It is conceivable that during β-adrenergic stimulation, patients with mutations located in the C loops have an unopposed increase in inward Ca^{2+} currents and prolongation of repolarization caused by blunted PKA-mediated activation of I_{Ks}.[5] β-Blockers may decrease these unopposed inward Ca^{2+} currents, shorten repolarization, and reduce the risk of ventricular arrhythmias,[28] whereas patients with other mutations do not exhibit such an effect.

Study Limitations

Clinical history was obtained on enrollment in the registry, so follow-up data in the current study comprised historical data from birth to enrollment and prospective information collected at yearly intervals after enrollment.

The International LQTS Registry records therapies that are prescribed at the discretion of the treating physicians to enrolled subjects; therefore, β-blocker administration was not randomized. However, because the patient's physician would have been blinded to whether the patient had a C-loop mutation, the interaction of this with β-blocker therapy is still compelling. Prior studies from the International LQTS Registry have shown that β-blocker therapy is associated with a significant reduction in the risk of cardiac events in LQTS patients. However, the present study is the first to assess the benefit of β-blocker therapy for the reduction in the risk of ACA or SCD among LQT1 patients. We have shown that β-blocker therapy is associated with a significant 88% ($P=0.02$) reduction in the risk of life-threatening cardiac events among LQT1 carriers of the higher-risk C-loop mutations. Risk reduction associated with β-blocker therapy in the total study sample and among carriers of the low-risk non–C-loop mutations did not reach statistical significance. The lack of a significant β-blocker effect may be due to sample size limitation and a more limited number of events among carriers of lower risk mutations. Thus, lower-risk patients should still be treated with β-blocker therapy according to guidelines[27] because the cumulative probability of ACA or SCD from birth through 40 years of age among patients with non–C-loop missense mutations was still considerable (between 11% and 16%). These limitations also suggest that further studies in independent populations are needed before the results can be extrapolated to clinical practice.

The present results, derived from LQTS families enrolled in the registry, may be confounded by familial factors such as ethnicity. To minimize bias, we adjusted for family membership in the multivariable models and carried out a secondary analysis in which additional adjustment was made for proband status. These analyses yielded similar results, further supporting the consistency of our findings. Of the 127 LQT1 mutation carriers without available ECG data, 58 (46%) died suddenly at a young age without a documented ECG. To minimize this bias related to exclusion of higher-risk patients, all multivariable analyses included adjustment for a QTc-missing category in addition to the category of QTc >500 milliseconds.

Channel current and response to forskolin were analyzed for 8 mutations of 99 mutation types observed in this study, but the robust findings in these expression studies strongly support our suggested mechanism. Experimental data were performed at room temperature; results may be different at physiological temperature.

Conclusions

We used a combination of clinical analysis and cellular electrophysiology experiments to investigate the molecular determinants and mechanisms underlying the clinical outcomes of a large cohort of subjects having a spectrum of *KCNQ1* mutations categorized by their code type and location. Patients with *KCNQ1* missense mutations located in the cytoplasmic loops had a significantly greater risk for life-threatening cardiac events and gained grater benefit when

treated with β-blockers compared with patients having other *KCNQ1* missense or nonmissense mutations independently of clinical risk factors. We suggest that a combination of a decrease in basal function and altered adrenergic regulation of the I_{Ks} channel underlies the increased cardiac risk in this subgroup of patients. Our results highlight the importance of understanding the molecular determinants and mechanisms underlying arrhythmogenesis to identify cardiac risk factors for LQT1 patients.

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CLINICAL PERSPECTIVE

Long-QT syndrome type 1 (LQT1) arises from a decrease in repolarizing potassium current resulting from mutations in the *KCNQ1* gene. The main trigger for cardiac arrhythmic events in patients with LQT1 is activation of β1-adrenergic receptors during exercise. Despite the observed reduction in the risk of cardiac events with β-blocker therapy among LQT1 patients, there is still a considerable cardiac residual event rate, suggesting that subgroups of LQT1 patients have differential response to β-blockers. The present study of 860 patients from the International LQTS Registry shows that the presence of missense mutations in distinct functional domains of the KCNQ1 protein, the S2-S3 and S4-S5 cytoplasmic loops (C loops), is associated with a significantly increased risk for life-threatening cardiac events compared with other mutations. Furthermore, patients with missense C-loop mutations gained greater benefit when treated with β-blockers compared with patients having other *KCNQ1* mutations independently of clinical risk factors, demonstrating that LQT1 patients have differential response to β-blocker therapy depending on mutation location. Both a decrease in basal function and altered adrenergic regulation of the I_{Ks} channel underlie the increased cardiac risk and response to β-blockers in this subgroup of patients. Patients with missense C-loop mutations should be considered a high-risk group of patients but with a pronounced response to β-blockers.

In Silico Cardiac Risk Assessment of Long QT patients: clinical predictability of cardiac models

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ABSTRACT

Background: Although attempts have been made to correlate mutation-specific ion channel dysfunction with patient phenotype in Long QT syndrome, these have been largely unsuccessful. Systems level computational models can be used to predict consequences of complex changes in channel function to the overall heart rhythm.

Methods: 633 LQT1 genotyped subjects with 34 mutations from multinational LQTS registries were studied. Cellular electrophysiology function was determined for the mutations and introduced in a 1D transmural ECG computer model. The mutation effect on transmural repolarization was determined for each mutant and related to the risk of cardiac events (syncope, aborted cardiac arrest (ACA) and sudden cardiac death (SCD)) among patients.

Results: Multivariate analysis showed that mutation-specific transmural repolarization prologation (TRP) was associated with increased risk of cardiac events (35% per 10ms increment [$p<0.0001$], ≥upper quartile [Q_4] HR=2.80 [$p<0.0001$]) and life threatening events (SCD/ACA: 27% per 10ms increment [$p=0.03$], ≥Q_4:HR=2.24 [$p=0.002$]) independently of the patient's individual QTc. Subgroup analysis showed that among patients with mild to moderate QTc duration (<500 msec) the risk associated with TRP was maintained (36% per 10ms [$p<0.0001$]) whereas the patient's individual QTc was not associated with a significant risk increase after adjustment for TRP.

Conclusions: Our findings suggest that simulated repolarization can be used to predict clinical outcomes and to improve risk stratification in LQT1 patients, with a more pronounced effect among patients with a lower-range QTc, in whom the patient's individual QTc may provide less incremental prognostic information.

INTRODUCTION

Long QT syndrome (LQTS) causes torsade de pointes arrhythmia, ventricular fibrillation, and sudden cardiac death (SCD). The disease can either be inherited as a congenital ion channel mutation or acquired as a result of drugs that block these cardiac ion currents. Type 1 long QT syndrome (LQT1), the most common form of LQTS, is caused by loss-of-function mutations in the KCNQ1 gene, which encodes the alpha subunit of the cardiac ion channel responsible for the slow delayed rectifier potassium current (IKs) [1]. To date, more than three hundred different mutations have been identified in this gene [2].

The occurrence of cardiac events in LQT1 patients is variable, with proper risk stratification needed to optimize patient treatment.[3-6] Several phenotype variables have been associated with a more severe clinical course in LQT1 patients. QT interval corrected for heart rate (QTc) is one of the most effective risk stratifiers in LQT syndrome with previous studies showing a 4.2-fold risk increase in aborted cardiac arrest or sudden cardiac death among patients with a QTc ≥500 ms [4]. However, QTc can vary temporally and among individuals with the same mutation [7]. Mutation characteristics have recently been shown to determine cardiac risk on patients with genetic confirmed LQT but normal range QTc intervals [8-10]. This suggests a strong genetic component to cardiac risk that is not currently understood.

Although several attempts have been made to correlate decrease in IKs function associated with specific mutations with patient phenotype, these attempts have been largely unsuccessful [8, 11-13]. Systems level computational models are highly developed in the field of cardiac physiology and can be used to predict consequences of complex changes in channel function to the overall heart rhythm. We hypothesized that: 1) mutation-specific prolongation of transmural repolarization, obtained by simulating transmural ECGs using a one-dimensional (1D) cable model will be an independent predictor of cardiac events among LQT1 patients; and 2) data regarding mutation-specific simulated transmural prolongation will identify increased risk for cardiac events among LQT1 patients with mild to moderate QTc prolongation, in whom the patient's individual QTc provides less incremental prognostic information.

METHODS

Simulation of Pseudo Transmural-ECGs with a 1D Cable Model

The one-dimensional (1D) cable model of 192 cells was constructed and parameterized to represent the transmural heterogeneity across the ventricular wall. As shown in Figure 1A, each of the 192 cells was assigned varying properties based on its position within the ventricular wall. The cell model is adapted from the Flaim-Giles-McCulloch (FGM) reconstruction of the canine cardiac cell [14]. The FGM model reconstructs three stereotyped cell types: epicardial (Epi), endocardial (Endo) and midmyocardial (M) cell responses. The FGM epicardial cell corresponds to the rightmost 30% of the wall in our cable model (70-100% wall distance in Fig. S2). The FGM endocardial cells correspond to the leftmost edge of the cable (0%) while the midmyocardial cell is mapped 10% wall depth in the sub-endocardium. For our model, the profiles of conductance of I_{NaL} and IKs were linearly interpolated between the different stereotyped cell types (see Fig. S2). The model conduction velocity and APD distributions are similar to experimental data (see Fig. S1). The pseudo-transmural-ECG was computed based on the transmural voltage gradient from the epicardial to the endocardial sides of the heart. In contrast to most published studies that report only a single electrode voltage located near the epicardium [15, 16], our model generates T-waves sensitive to the whole transmural repolarization profile.

Wild type (WT) IKs current parameters in the model were modified to mimic human IKs currents and currents measured for channels containing LQT1 mutant subunits previously reported in Jons et al.[8] The first 5 cells are stimulated with applied current, and the action potential (AP) propagates to the end of the array. The AP propagates along an array of 288 cells, with the central 192 cells used in the analysis to circumvent edge artifacts (Fig. S3). The pacing protocol consists of 60 beats at a 1000 ms interval to bring the model to steady state. During the simulation studies, the modeling team was blinded to as which mutant corresponded to a given simulation (i.e., the modified parameter for the mutant were provided without a cross-reference to the mutant). For additional details on the *in silico* methods see supplementary data.

Study Population Data Collection and Clinical Endpoints

The study population comprised 633 subjects derived from proband identified families (n=103) with genetically confirmed *KCNQ1* mutations for which mutant channel properties are known, permitting simulation with the method described here (see Table S1 for details of mutations included [8]). Patients were drawn from four LQTS registries: the U.S. part of the Rochester-based LQTS Registry (n=488), the Netherlands LQTS Registry (n=23), the Japanese LQTS Registry (n=56), as well as from data submitted by other investigators specifically for this collaborative mutation analysis project: Denmark (n =12) and Sweden (n =4). The proband in each family had diagnostic QTc prolongation and may or may not have experienced LQTS-related symptoms. Patients were excluded from the study if they had > 1 LQTS-causing mutation.

Follow up data for the study comprised prospective information collected at yearly intervals after enrollment and retrospective data from birth to enrollment. Only patients for whom complete medical history and prospective information was available are included in the present study. Clinical history data were collected on forms with information on demographic characteristics, personal and family medical history, ECG findings, medical therapies, left cardiac sympathetic denervation, implantation of a pacemaker or an implantable cardioverter defibrillator (ICD), and the occurrence of LQTS-related cardiac events.

LQTS-related cardiac events included syncope, defined as transient loss of consciousness abrupt in onset and offset, aborted cardiac arrest (ACA) requiring defibrillation, and sudden cardiac death without a known cause (SCD).

Electrophysiology

The electrophysiological properties of mutant KCNQ1 channels were obtained by overexpression in *Xenopus laevis* oocytes as previously described [8, 17, 18]. In brief, wild-type and mutant KCNQ1 cRNA were injected into oocytes in a 1:1 ratio, together with the KCNE1 subunit (0.5:0.5:1 ratio WTKCNQ1:MutKCNQ1:KCNE1). The IKs tail current at -40 mV was measured after depolarization to a series of voltage steps from -50 to +80 mV every 10 mV and a Boltzmann fit ($G = g_{max}/(1 + \exp[-(V - V_{1/2})/k]$) of this data was used to determine the steepness or slope factor (k), the voltage that elicits half of the maximal activation ($V_{1/2}$) of activation and the maximal conductance (g_{max}). Nonsense mutation effects were assumed to have a

haploinsufficient phenotype, and effects were evaluated by measuring currents with decreased WT expression (0.5:1 ratio WTKCNQ1:KCNE1).

Statistics

Linear regression was used to test for correlation between simulated repolarization time using the model described above and QTc measured from patients. Kaplan-Meier survival analysis was used to determine the cumulative probability of cardiac events by simulated repolarization times and by measured QTc interval, and significance was tested by the log-rank test. Multivariate Cox proportional hazards regression modeling was used to evaluate the independent contribution of simulated repolarization times to the occurrence of cardiac events from birth through age 40 years. Additional prespecified covariates in the multivariate models included gender, the patient's individual QTc, and time-dependent β-blocker therapy (i.e. by taking into account in the multivariate models information regarding administration of β-blockers given to patients at different time-points during follow-up). Patients who did not have an ECG for QTc measurement (n=92) were identified in the Cox models as "QTc missing", and all Cox models were adjusted for this QTc missing parameter. Data from the International LQTS Registry demonstrate that an age-interaction exists regarding the effect of gender and genotype on the occurrence of cardiac events, with a crossover effect for both genotype and gender after the onset of adolescence [19,20]. Therefore, to avoid a violation of the proportional-hazards assumption, models were carried out separately within prespecified younger- (0 through 14 years) and older- (15 through 40 years) age-groups.

Because almost all the subjects were first- 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.[9] 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, NC). A 2-sided 0.05 significance level was used for hypothesis testing.

RESULTS