

Combined assessment of sex- and mutation-specific information for risk stratification in type 1 long QT syndrome

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BACKGROUND Men and women with type 1 long QT syndrome (LQT1) exhibit time-dependent differences in the risk for cardiac events.

OBJECTIVE We hypothesized that sex-specific risk for LQT1 is related to the location and function of the disease-causing mutation in the *KCNQ1* gene.

METHODS The risk for life-threatening cardiac events (comprising aborted cardiac arrest [ACA] or sudden cardiac death [SCD]) from birth through age 40 years was assessed among 1051 individuals with LQT1 (450 men and 601 women) by the location and function of the LQT1-causing mutation (prespecified as mutations in the intracellular domains linking the membrane-spanning segments [ie, S2–S3 and S4–S5 cytoplasmic loops] involved in adrenergic channel regulation vs other mutations).

RESULTS Multivariate analysis showed that during childhood (age group: 0–13 years) men had >2-fold ($P < .003$) increased risk for ACA/SCD than did women, whereas after the onset of adolescence the risk for ACA/SCD was similar between men and women (hazard ratio = 0.89 [$P = .64$]). The presence of cytoplasmic-loop mutations was associated with a 2.7-fold ($P < .001$)

increased risk for ACA/SCD among women, but it did not affect the risk among men (hazard ratio 1.37; $P = .26$). Time-dependent syncope was associated with a more pronounced risk-increase among men than among women (hazard ratio 4.73 [$P < .001$] and 2.43 [$P = .02$], respectively), whereas a prolonged corrected QT interval (≥ 500 ms) was associated with a higher risk among women than among men.

CONCLUSION: Our findings suggest that the combined assessment of clinical and mutation location/functional data can be used to identify sex-specific risk factors for life-threatening events for patients with LQT1.

KEYWORDS: Cytoplasmic-loop mutations; Sex; Long QT syndrome; Sudden cardiac death

ABBREVIATIONS ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; HR = hazard ratio; ICD = implantable cardioverter defibrillator; LQTS = long QT syndrome; LQT1 = long QT syndrome type 1; MS = membrane spanning; QTc = corrected QT interval; SCD = sudden cardiac death (Heart Rhythm 2012;9:892–898) © 2012 Heart Rhythm Society. All rights reserved.

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ments, were established between Genaisance Pharmaceuticals (then PGxHealth and now Transgenomic) and Mayo Medical Ventures (now Mayo Clinic Health Solutions) in 2004. Dr Ackerman is also a consultant for Biotronik, Boston Scientific Corporation, Medtronic, and St Jude Medical. However, none of these entities provided financial support for this study. **Address reprint requests and correspondence:** Dr Ilan Goldenberg, MD, Heart Research Follow-up Program, Cardiology Division, University of Rochester Medical Center, Box 653, Rochester, NY 14642. E-mail address: Ilan.Goldenberg@heart.rochester.edu.

Introduction

Long QT syndrome type 1 (LQT1) is the most commonly occurring of the congenital long QT syndromes (LQTS).¹ It is caused by mutations in the *KCNQ1* gene that impair the slow-acting potassium channel that gives rise to slow delayed rectifier potassium current (I_{Ks}). The resulting prolongation of ventricular repolarization increases the potential for cardiac arrhythmogenic events that can cause syncope or sudden cardiac death (SCD). Patients with LQT1 experience the majority of their events during exercise, possibly because the phase 3 I_{Ks} repolarizing current activates during increased heart rate and is essential for QT-interval adaptation during tachycardia.^{1,2} Prior studies have shown that mutations located at the membrane-spanning (MS) region and missense vs nonmissense mutations are associated with a greater risk for cardiac events in patients with LQT1.³ The MS region includes the MS domains and the MS linkers. Mutations in the intracellular linkers that connect the MS domains of the *KCNQ1* (Kv7.1) channel subunit (defined herein as the S2–S3 and S4–S5 cytoplasmic [C]-loop mutations) were shown to affect adrenergic channel regulation by protein kinase A⁴ and may therefore predispose to increased risk for life-threatening events in this population.⁵

The phenotypic expression of LQT1 is affected by sex and age, wherein men with LQT1 experience increased risk for cardiac events, mainly during the childhood period.⁶ Prior studies, however, did not relate sex-specific risk in this population to the location and function of the disease-causing mutation in the *KCNQ1* gene. Furthermore, sex differences in the clinical course of LQT1 were related previously to a cardiac event composite end point, which comprised mostly nonfatal syncope. Accordingly, the present study was designed to evaluate whether the combined assessment of clinical and mutation location/functional data can identify sex-specific risk factors for life-threatening cardiac events in men and women with LQT1.

Methods

Study population

The study population comprised 1051 LQT1-positive subjects from 259 proband identified families. Patients were drawn from the Rochester, NY, enrolling center (center 1) of the International LQTS Registry (n = 755), the Netherlands LQTS Registry (n = 85), and the Japanese LQTS Registry (n = 83), as well as from data submitted by other investigators specifically for this collaborative mutation analysis project: Denmark (n = 43), Israel (n = 34), Sweden (n = 4), and Salt Lake City, UT (n = 47). The proband in each family had otherwise unexplained, diagnostic corrected QT-interval (QTc) prolongation or experienced LQTS-related symptoms. Patients with congenital death were excluded from the study.

Data collection and management

For each patient, information on personal history, including cardiac events, electrocardiograms, and therapies, as well as

family history was obtained at enrollment. Clinical data were then collected yearly on prospectively designed forms with information on demographic characteristics, personal and family medical history, electrocardiogram findings, medical therapies, left cardiac sympathetic denervation, implantation of a pacemaker or an implantable cardioverter defibrillator (ICD), and the occurrence of LQT1-related cardiac events. The QT interval was corrected for heart rate (QTc) by using Bazett's formula.⁷ Data common to all LQTS registries involving genetically tested individuals were merged electronically into a common database for the present study.

Genotype characterization

The *KCNQ1* mutations were identified with the use of standard genetic tests conducted in academic molecular genetic laboratories including the Functional Genomics Center, University of Rochester Medical Center, Rochester, NY; Baylor College of Medicine, Houston, TX; Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, MN; Boston Children's Hospital, Boston, MA; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands; and Molecular Cardiology Laboratory, Policlinico S. Matteo and University of Pavia, Pavia, Italy.

Mutations were defined as any nonsynonym rare variants (<1% of the healthy population) identified in a proband with a prolonged QT interval. Based on prior data regarding mutation location/function and arrhythmic risk in LQT1,^{3–5,8} mutations were categorized by their location and type in the *KCNQ1*-encoded channel subunit as follows: (1) missense mutations in the MS region: defined as amino acid residues from 120 to 355, excluding mutations within the MS linkers; (2) missense mutations in the C loops: defined as the coding sequence involving amino acid residues from 174 to 190 (S2–S3 linker) and from 242 to 259 (S4–S5 linker); (3) missense mutations in the N-terminus region, defined as amino acid residues before 120, and the C-terminus region, defined as amino acid residues after residue 355, were combined into one category labeled as the other region for this analysis (hence called the N/C terminus); and (4) other LQT1 mutations as the reference group (ie, splice sites, in-frame insertions, in-frame deletions, nonsense, and frameshift).

The specific mutations included in the present study, by location, type, and number of patients, are detailed in the Supplementary Appendix Table 1, and the distribution of the mutations in the *KCNQ1* gene by their frequency among study patients is shown in Figure 1.

End point

The primary end point of the study was the occurrence of a first life-threatening cardiac event, comprising aborted cardiac arrest (ACA) (requiring defibrillation as part of resuscitation), or LQT1-related SCD (abrupt in onset without

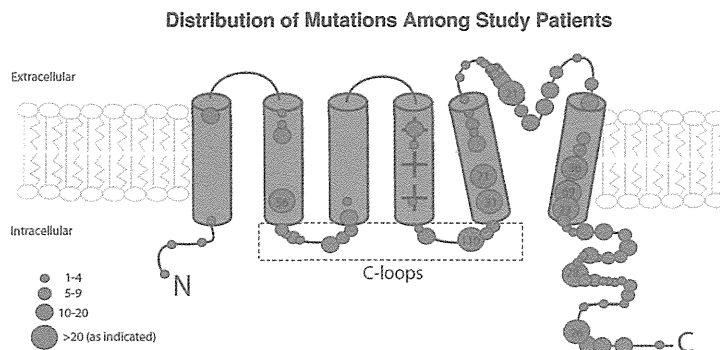


Figure 1 Distribution of mutations in the *KCNQ1* (Kv7.1) potassium channel subunit among study patients. Numbers in larger circles denote the number of patients with the mutation. C loops = cytoplasmic loops.

evident cause, if witnessed, or death that was not explained by any other cause if it occurred in a nonwitnessed setting such as sleep). To further validate the consistency of the results among patients who received an ICD during follow-up, we also assessed a secondary end point comprising the first occurrence of ACA, SCD, or appropriate ICD shock during follow-up.

Statistical analysis

The baseline and follow-up clinical characteristics of the study population were evaluated by using the chi-square test for categorical variables and the Mann-Whitney-Wilcoxon test for continuous variables. The cumulative probability of a first ACA or SCD by sex and by mutation location was assessed by using the Kaplan-Meier method, and significance was tested by using the log-rank test. Follow-up was censored at age 40 years to avoid confounding by acquired cardiovascular disease. Multivariate Cox proportional-hazards regression models were used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of ACA or SCD. Prespecified covariates in the total population model included sex, QTc duration (categorized as a 3-level covariate: >500 ms, 500–550 ms, <500 ms [reference]), mutation location and type (as defined above), the occurrence of syncope during follow-up, and medical therapy with beta-blockers. Syncope and beta-blocker therapy were assessed as time-dependent covariates in the multivariate models. To avoid violation of the proportional hazards assumption due to sex-risk crossover during adolescence, we employed an age-sex interaction term in the total population multivariate model. The effect of each covariate in men and women was assessed by interaction-term analysis (ie, by including a sex-by-risk factor interaction term in the multivariate models), with interactions tested one at a time. Patients without available baseline QTc data ($n = 151$) were included as a separate (QTc missing) covariate in the multivariate models.

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 .05 significance level was used for hypothesis testing.

Results

The clinical characteristics of the study patients by sex are shown in Table 1. Baseline QTc was similar between men and women during childhood and significantly higher among women after the onset of adolescence. During follow-up, similar numbers of men and women were treated with beta-blockers, but a higher proportion of women were treated with an ICD. There were no significant sex differences in the distribution of the mutation by location (Table 1). However, patients with missense mutations localizing to the C loops exhibited a significantly longer baseline QTc (503 ± 58 ms) than did patients with other mutations (480 ± 51 ms; $P < .001$).

Risk factors for ACA or SCD in the total LQT1 population

During follow-up, 138 study patients (13%) experienced the primary end point of a first ACA or SCD. Kaplan-Meier event rates were significantly higher among men than among women throughout follow-up ($P = .008$ for the overall difference; Figure 2). Notably, life-threatening cardiac events among men occurred predominantly during childhood, whereas among women event rates increased after this time period. Thus, by age 14 years, the cumulative probability of ACA or SCD was 10% among men as compared with only 3% among women, and by age 40 years, the respective events rates were 19% and 15% (Figure 2).

Consistent with those findings, multivariate analysis in the total study population showed that during childhood men had >2-fold ($P = .003$) increase in the risk for ACA or SCD as compared with women whereas after the onset of adolescence, there was no statistically significant difference in the risk for ACA or SCD between men and women (hazard ratio [HR] 0.89; $P = .64$; Table 2). Additional risk

Table 1 Characteristics of the study population

Characteristic	Men (n = 450)	Women (n = 601)	P
ECG parameters			
Overall QTc (ms)	473 ± 55	486 ± 55	<.001
Age ≤ 13 y	486 ± 53	485 ± 54	.21
Age > 13 y	460 ± 53	487 ± 56	<.001
QTc > 500 ms (%)	22	27	.09
RR (ms)	842 ± 220	837 ± 199	.55
Mutation location (%)			
Cytoplasmic loop (S2-S3 or S4-S5 linkers)	19	19	.92
MS	53	54	.67
N/C terminus	28	26	.58
Mutation type (%)			
Missense	83	79	.13
LQTS therapies during follow-up (%)			
Beta-blockers	45	46	.74
Pacemaker	1.6	1.5	.94
ICD	4	9	.003
LCSD	1.1	0.5	.30
Cardiac events during follow-up (%)			
Syncope	35	36	.84
ACA	3	4	.22
SCD	13	8	.007
Appropriate ICD shocks	0.2	1.7	.03
ACA or SCD*	15	11	.07

Values are mean ± SD unless otherwise indicated.

ACA = aborted cardiac arrest; ECG = electrocardiogram; ICD = implantable cardioverter defibrillator; LCSD = left cervical sympathetic denervation; LQTS = long QT syndrome; MS = membrane spanning; QTc = corrected QT interval; SCD = sudden cardiac death; SD = standard deviation.

*Only the first event for each patient was considered.

factors within the total study population included the presence of missense mutations localizing to the C loops (1.9-fold risk increase [$P = .005$]), QTc 500–550 and >550 ms (>3-fold and >4-fold risk increase, respectively [$P < .001$

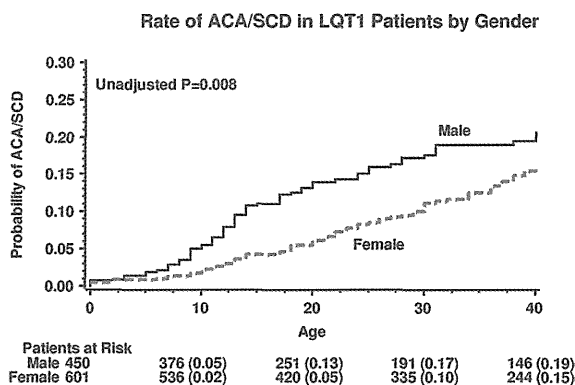


Figure 2 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in patients with LQT1 by sex. ACA = aborted cardiac arrest; LQT1 = long QT syndrome type 1; SCD = sudden cardiac death.

Table 2 Multivariate analysis: Risk factors for ACA/SCD among all patients with LQT1*

Risk factor	Relative risk		
	Hazard ratio	95% Confidence interval	P
Sex			
Men vs women ≤ 13 y	2.31	1.41–3.92	.003
Men vs women >13 y	0.92	0.61–1.51	.72
Mutation location (vs nonmissense mutations)			
Cytoplasmic loop (S2-S3/S4-S5 linkers)	1.93	1.37–2.75	.005
MS (S1, S2, S3, S4, S5, P-loop, S6)	1.02	0.71–1.85	.51
N/C terminus	0.96	0.52–1.57	.72
QTc duration (ms)			
>550 vs <500	4.18	2.06–8.46	<.001
500–550 vs <500	3.35	1.83–6.11	<.001
Time-dependent syncope			
Syncope vs no syncope	3.40	2.22–5.21	<.001

ACA = aborted cardiac arrest; LQT1 = long QT syndrome type 1; MS = membrane spanning; QTc = corrected QT interval; SCD = sudden cardiac death.

*Models were further adjusted for missing QTc values, time-dependent beta-blocker therapy.

for both]), and the occurrence of syncope during follow-up (3.4-fold risk increase [$P < .001$]; Table 2). Results were similar when the secondary end point of a first ACA, SCD, or appropriate ICD shock was assessed.

Sex-specific risk factors for life-threatening cardiac events in patients with LQT1

Kaplan-Meier survival analysis showed that women with LQT1 with missense C-loop mutations exhibited a significantly higher rate of ACA or SCD than did women whose LQT1-causative mutation localized elsewhere ($P < .001$ for the overall difference during follow-up; Figure 3A). In contrast, among men, the respective rates of ACA or SCD remained high, predominantly during the childhood period, regardless of mutation location/type ($P = .33$ for the overall difference during follow-up [Figure 3B]).

Sex-specific multivariate analysis (Table 3) showed that women with C-loop mutations exhibited nearly a 3-fold ($P = .01$) increased risk for ACA or SCD than did women with other mutation types, whereas the risk for ACA or SCD among men was not significantly different by mutation location/type (P value for mutation-location-by-sex interaction = .07). Similar results were observed in an additional analysis in which the large subset of patients with the V254M C-loop mutation was excluded from the multivariate models (risk associated with C loop vs other mutations among women: HR 2.55, 95% confidence interval [CI] 1.02–5.94; among men: HR 1.03, 95% CI 0.33–4.11).

Additional risk factors for ACA or SCD among both men and women included a prolonged QTc and the occurrence of

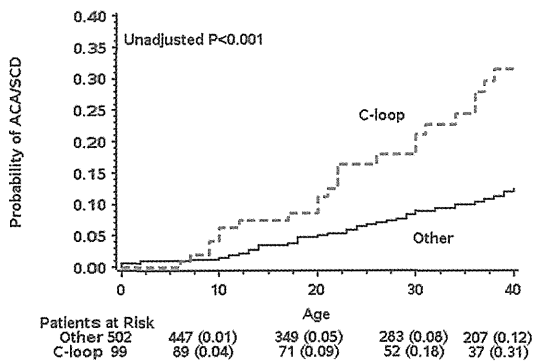
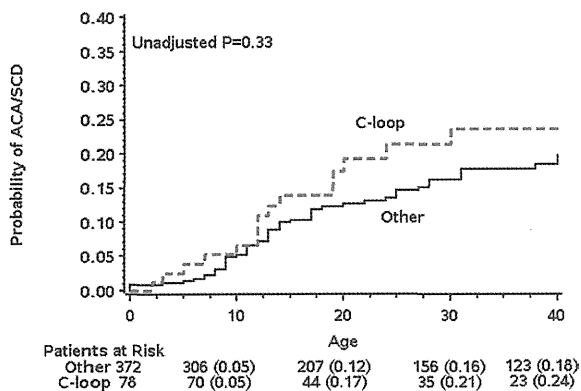
A Rate of ACA/SCD in LQT1 Females by Mutation-Location**B Rate of ACA/SCD in LQT1 Males by Mutation-Location**

Figure 3 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 mutation location. ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; LQT1 = long QT syndrome type 1; SCD = sudden cardiac death.

time-dependent syncope (Table 3). Notably, women with prolonged QTc, both in the range of 500–550 ms and >550 ms, experienced a pronounced increase (approximately 6-fold) in the risk of ACA or SCD, whereas the risk associated with a prolonged QTc in men was more modest and evident only in those with QTc >550 ms (Table 3 and Figures 4A and B, respectively). The occurrence of syncope during follow-up was associated with a 4-fold ($P < .001$) increase in the risk for subsequent ACA or SCD among men and with a 2.4-fold ($P = .002$) increase in the risk for subsequent ACA or SCD among women.

The combined assessment of clinical and genetic data identified a very low rate of life-threatening events (0.03 events per 100 patient-years) among women aged 13 years or younger without C-loop mutations, no history of prior syncope, and QTc <500 ms.

Time-dependent medical therapy with beta-blockers was associated with a significant 61% reduction in the risk for ACA or SCD in the total study population (HR 0.39; 95% CI 0.22–0.70; $P = .001$), with beta-blocker protection seen similarly between men and women (P values for beta-

blocker-by-sex interaction = .56). Notably, this analysis showed that the risk associated with C-loop mutations in women was even more pronounced among those who did not receive beta-blocker therapy (HR 4.51; 95% CI 2.57–7.23; $P < .001$).

Discussion

In the present study, we assessed for the first time sex-specific risk factors for life-threatening cardiac events in a large population of 1051 genetically confirmed patients with LQT1. Our findings show that among probands and relatives with LQT1, (1) men exhibit a significantly higher rate of life-threatening cardiac events than do women, especially prior to puberty, and (2) mutation location shows a sex-specific association with the risk for ACA or SCD. Thus, the risk for life-threatening events was shown to be increased among women with LQT1 with mutations localizing to C-loop domains (S2–S3 and S4–S5) of the *KCNQ1*-encoded protein, whereas the risk for ACA or SCD among men with LQT1 was high even among those who harbored mutations localizing to other regions of the channel that had been ascribed previously as lower-risk mutations. These findings suggest that a combined approach that incorporates clinical and genetic data can be used for improved risk assessment and management of men and women with LQT1.

A previous study from the International LQTS Registry has shown that men with LQT1 have an increase in the risk for any LQT1-related cardiac event, including syncope, during childhood, whereas after the onset of adolescence the risk for events in this population is attenuated without a significant sex difference.⁶ Because of a limited sample of 243 patients with LQT1, the study did not assess sex-related differences in the risk for only life-threatening cardiac events (ACA or SCD) in this population. Thus, our findings of the present study extend prior observations and show that men with LQT1 display a higher rate of ACA or SCD than do women from birth through age 40 years, with a predominant risk increase during the childhood period.

Patients with LQT1 experience ventricular tachyarrhythmias more frequently during physical effort,² possibly due to the lack of adaptive QT shortening with decreasing RR intervals during tachycardia.¹⁰ Thus, the early predominance of life-threatening cardiac events among men may be related to sex differences in the level of physical activity during childhood among registry patients. After the onset of adolescence, an increase in the levels of testosterone, which was shown to shorten action potential duration and ventricular repolarization,^{11–13} may result in a reduction in the risk for arrhythmic events in men. This mechanism is supported by the fact that the risk for life-threatening events during childhood was higher among men despite the fact that the average QTc was similar between men and women during this time period, whereas after the onset of adolescence the QTc was significantly reduced in men and remained virtually unchanged in women (Table 1).

Table 3 Multivariate analysis: Risk factors for ACA/SCD among patients with LQT1 by sex*†

	Men with LQT1		Women with LQT1	
	HR (95% CI)	P	HR (95% CI)	P
Mutation location (vs nonmissense mutations)				
Cytoplasmic loop (S2–S3/S4–S5 linkers)	1.21 (0.72–2.04)	0.48	2.62 (1.59–4.26)	<.001
MS	1.02 (0.63–1.97)	0.54	1.01 (0.62–1.89)	.56
N/C terminus	0.89 (0.52–1.91)	0.87	1.14 (0.51–2.37)	.43
QTc duration (ms)				
500–550 vs <500	1.70 (0.63–4.57)	0.29	6.85 (2.74–17.10)	<.001
>550 vs < 500	3.11 (1.19–8.15)	0.02	5.93 (1.89–18.62)	.002
Time-dependent syncope				
Syncope vs no syncope	4.06 (2.22–7.41)	<0.001	2.43 (1.43–4.85)	.002

ACA = aborted cardiac arrest; CI = confidence interval; HR = hazard ratio; LQT1 = long QT syndrome type 1; QTc = corrected QT interval; SCD = sudden cardiac death; MS = membrane spanning.

*Findings were further adjusted for missing QTc values, time-dependent beta-blocker therapy.

†Models were carried out in the total population by using interaction-term analysis, with interactions tested one at a time; cytoplasmic loop-by-sex interaction = .07; all other interaction P values were >.10.

Mutations located in the MS region, including the MS domains and the C loops, of the *KCNQ1* protein have been associated with greater prolongation in the QTc during exercise¹⁴ and an increase in the risk for cardiac events in patients with LQT1.³ Importantly, the C loops were shown to modify the function of *KCNQ1* channel subunit, including functional interaction with the auxiliary beta subunits encoded by *KCNE1* and modulation of the channel's protein kinase A (PKA)-dependent, adrenergic regulation.⁴ Thus, patients who harbor C-loop mutations may be sensitive to even mild degrees of adrenergic stimulation, resulting in arrhythmic events that may occur during less intense physical activity. This mechanism may explain the sex-specific association of mutation location to arrhythmic risk shown in the present study, as women who carry mutations in the adrenergic-sensitive C loops may have an increased risk for life-threatening events even during milder degrees of physical activity. In contrast, participation in more intense physical activity among men with LQT1, especially during childhood, may predispose them to arrhythmic events even among those who carry non-C-loop mutations (which are less sensitive to sympathetic activation). It is also possible that sex differences in the regulation of the ion channel contribute to the differential effect of mutation location/function on arrhythmic risk between men and women.

Similar to prior studies,^{15–17} we have also shown that QTc is a major risk factor for cardiac events in patients with LQTS. However, our data suggest that in LQT1 the risk associated with QTc is more pronounced among women (who exhibited >6-fold risk with QTc exceeding 500 ms, whereas a significant risk increase among men was evident only among those with QTc >550 ms).

It was suggested recently that patients with LQT1 experience a very low rate of cardiac events during beta-blocker therapy.¹⁸ In the present study, medical therapy with beta-blockers was associated with a pronounced reduction in the risk for ACA or SCD in the total LQT1 population, without a statistically significant difference between men and

women. However, our findings suggest that sex-specific risk factors should be taken into account in the management of patients with LQT1. These clinical and mutation-related risk factors are shown in Figure 1 of the Supplementary Appendix and include (1) the preadolescence period in men, especially among those who experience syncope during childhood and those with QTc >550 ms, and (2) following the onset of adolescence in women, especially among those with C-loop mutations, QTc ≥500 ms, and/or history of syncope.

Study limitations

Although we have shown recently that the S2–S3 and S4–S5 C-loop linkers have an important functional role in adrenergic channel regulation through PKA,^{4,5} further studies are needed to relate the functional expression of discrete *KCNQ1* mutations to sex-specific risk in LQT1, and their interaction with possible hormonal modulation of cardiac risk in this population.

The present study shows that beta-blocker therapy is associated with a significant reduction in the risk for life-threatening events in both men and women with LQT1. However, because of sample size limitations, we did not evaluate sex-specific differences in response to beta-blocker therapy between patients with LQT1 with higher-risk (and adrenergic-sensitive) C-loop mutations and those with mutations localizing elsewhere. In addition, we did not carry out comprehensive analysis of the relationship between all functional regions of the *KCNQ1*-encoded protein (including functional areas within the C-terminus or N-terminus domains) and sex-specific risk.

We excluded patients with congenital deafness from the study. However, 12 patients (1%) had 2 different mutations in the *KCNQ1* gene. To validate the consistency of the results for patients with single mutations, all multivariate models were repeated after excluding the 12 patients with >1 mutation. This confirmatory analysis yielded virtually identical results regarding the risk associated with clinical factors and mutation location as in the primary analysis.

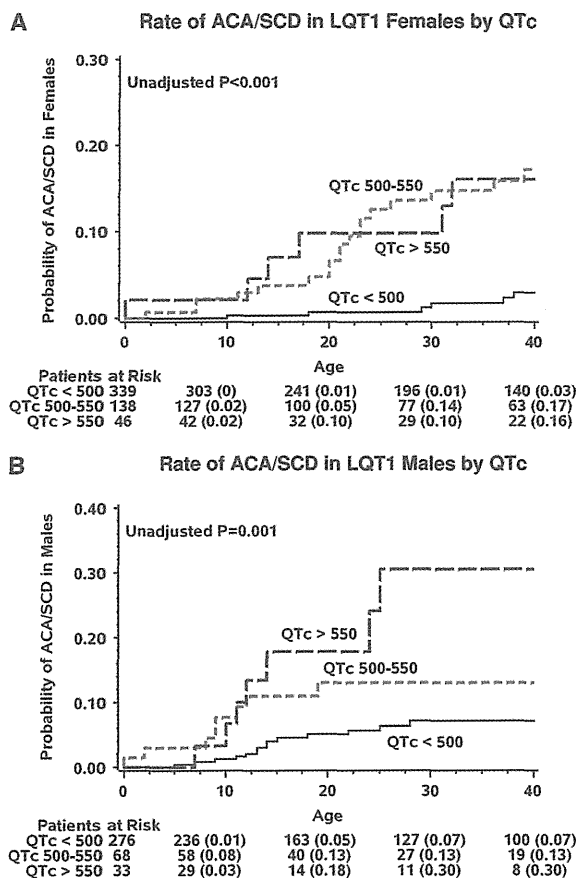


Figure 4 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in (A) women with LQT1 and (B) men with LQT1, by QTc duration. ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; LQT1 = long QT syndrome type 1; QTc = corrected QT 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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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 $\approx 35\%$ 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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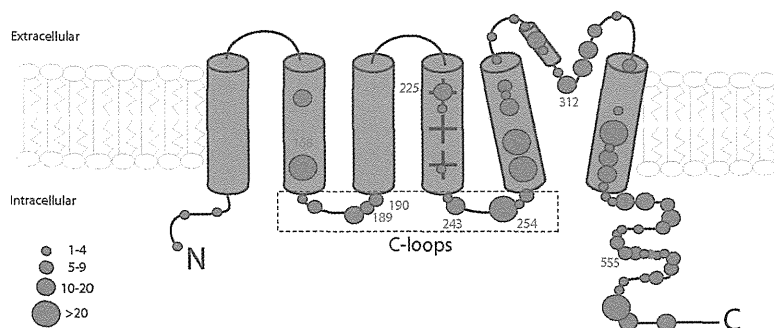


Figure 1. Frequency and location of mutations in the KCNQ1 potassium channel. Diagrammatic location of 99 different mutations in the KCNQ1 potassium channel involving 860 subjects. The α -subunit involves the N-terminus (N), 6 membrane-spanning segments, 2 cytoplasmic loops (S2-S3 and S4-S5), and the 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 β 1-adrenergic receptors is the major signaling pathway contributing to the increase in heart rate and cardiac output during exercise. β 1-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 (≈ 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 mutations⁸ 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 Multi-center 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 subunits¹¹ (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 μ mol/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 M Ω . 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			
	C/N Terminus	Membrane Spanning	C Loops	Nonmissense
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

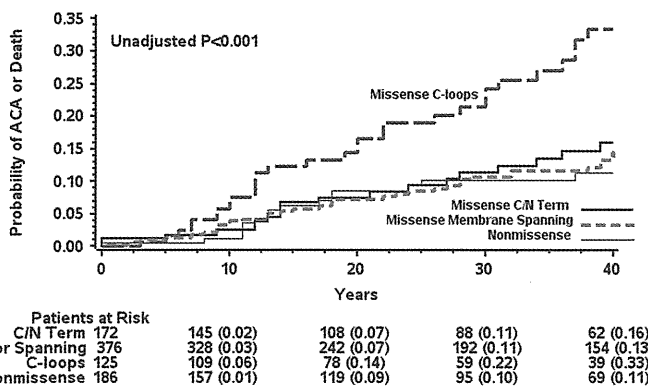


Figure 2. Kaplan-Meier estimates of cumulative probability of life-threatening cardiac events by mutation location and type. The numbers in parentheses reflect the cumulative event rate at that point in time. ACA indicates aborted cardiac arrest; LQTS, long-QT syndrome.

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 $P=0.028$.

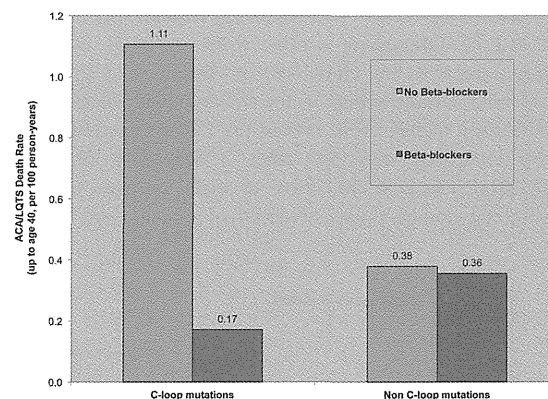


Figure 3. Risk for life-threatening cardiac events by mutation location and β -blocker treatment. Sixty-three of the 125 subjects (50%) with C-loop missense mutations were treated with β -blockers during a mean follow-up of 26.2 years; 305 of the 735 subjects (42%) with non-C-loop missense mutations 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.

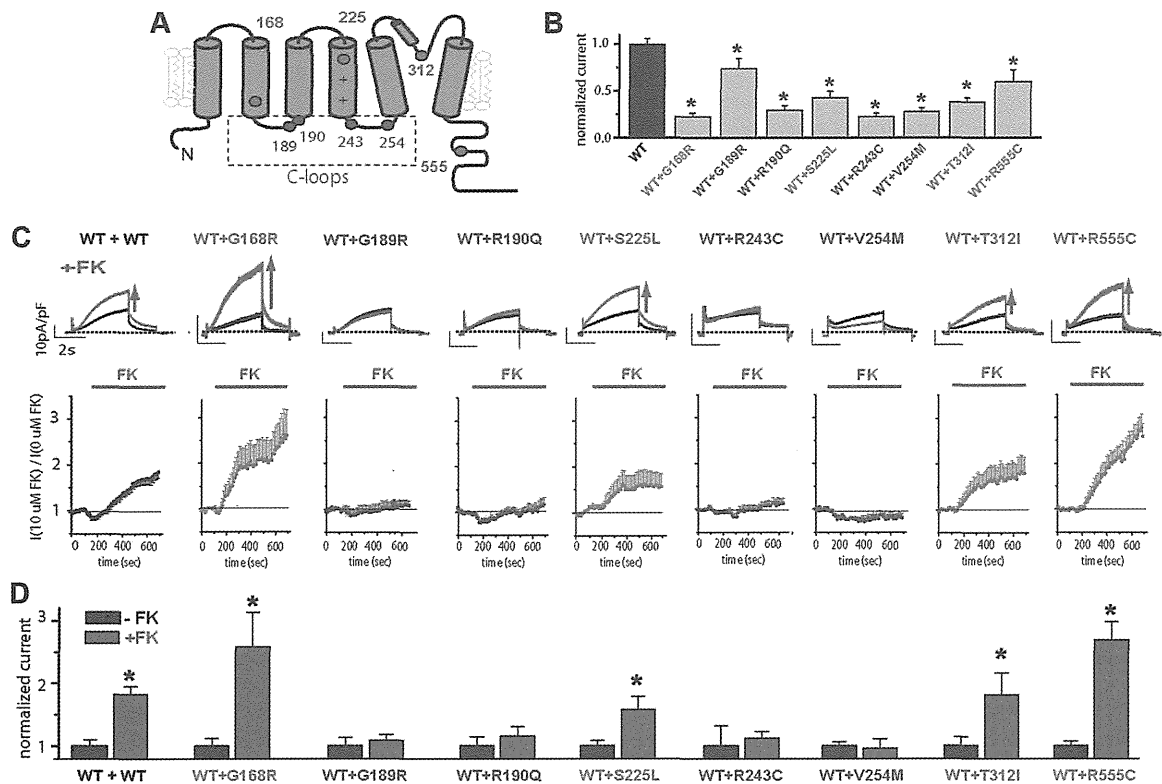


Figure 4. Regulation of LQT1 mutant channels by protein kinase A. **A**, Schematic representation of location of the mutations used in the study. **B**, Effect of each of the mutations studied in basal nonstimulated cell currents. Average current measured for cells expressing wild-type (WT) and mutant 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 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 $\mu\text{mol/L}$) for 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 KCNE1 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 impor-

tant implications regarding risk assessment and management in this study sample. First, patients with missense mutations located in the C loop 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 *KCNQ1*(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 guidelines²⁷ 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 greater 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_{K_s} 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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Disclosures

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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 β 1-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 in Patients With Long QT Syndrome

Type 1: Clinical Predictability of Cardiac Models

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Objectives	The study was designed to assess the ability of computer-simulated electrocardiography parameters to predict clinical outcomes and to risk-stratify patients with long QT syndrome type 1 (LQT1).
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	A total of 633 LQT1-genotyped subjects with 34 mutations from multinational long QT syndrome registries were studied. Cellular electrophysiology function was determined for the mutations and introduced in a 1-dimensional transmural electrocardiography computer model. The mutation effect on transmural repolarization was determined for each mutation and related to the risk for cardiac events (syncope, aborted cardiac arrest, and sudden cardiac death) among patients.
Results	Multivariate analysis showed that mutation-specific transmural repolarization prolongation (TRP) was associated with an increased risk for cardiac events (35% per 10-ms increment [$p < 0.0001$]; \geq upper quartile hazard ratio: 2.80 [$p < 0.0001$]) and life-threatening events (aborted cardiac arrest/sudden cardiac death: 27% per 10-ms increment [$p = 0.03$]; \geq upper quartile hazard ratio: 2.24 [$p = 0.002$]) independently of patients' individual QT interval corrected for heart rate (QTc). Subgroup analysis showed that among patients with mild to moderate QTc duration (<500 ms), the risk associated with TRP was maintained (36% per 10 ms [$p < 0.0001$]), whereas the patient's individual QTc was not associated with a significant risk increase after adjustment for TRP.
Conclusions	These findings suggest that simulated repolarization can be used to predict clinical outcomes and to improve risk stratification in patients with LQT1, with a more pronounced effect among patients with a lower-range QTc, in whom a patient's individual QTc may provide less incremental prognostic information. (J Am Coll Cardiol 2012;60:2182-91) © 2012 by the American College of Cardiology Foundation

Long QT syndrome (LQTS) may cause 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

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caused by loss-of-function mutations in the *KCNQ1* gene, which encodes the alpha subunit of the cardiac ion channel

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involved in the slow delayed rectifier potassium current (IKs) (1). To date, >300 different mutations have been identified in this gene (2).

The occurrence of cardiac events in patients with LQT1 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 patients with LQT1. QT interval corrected for heart rate (QTc) is one of the most effective risk stratifiers in LQTS, with previous studies showing a 4.2-fold risk increase in aborted cardiac arrest (ACA) or SCD 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 in patients with genetically confirmed LQTS 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. The authors hypothesized that: 1) mutation-specific transmural repolarization prolongation (TRP), obtained by simulating transmural electrocardiograms (ECGs) using a 1-dimensional (1D) cable model, will be an independent predictor of cardiac events among patients with LQT1; and 2) data regarding mutation-specific simulated TRP will identify increased risk for cardiac events among patients with LQT1 with mild to moderate QTc prolongation, in whom a patient's individual QTc provides less incremental prognostic information.

Methods

Simulation of pseudo-transmural ECGs with a 1D cable model. The 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 was adapted from the Flaim-Giles-McCulloch (FGM) reconstruction of the canine cardiac cell (14). The FGM model reconstructs 3 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 the cable model (70% to 100% wall distance in Online Fig. 1). The FGM endocardial cells correspond to

the leftmost edge of the cable (0%), while the midmyocardial cell is mapped 10% wall depth in the subendocardium. For the present model, the profiles of conductance of late sodium current (I_{NaL}) and IKs were linearly interpolated between the different stereotyped cell types (Online Fig. 1). The model conduction velocity and action potential duration distributions are similar to experimental data (Online Fig. 2). 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 models in some published studies that report only a single electrode voltage located near the epicardium (15,16), the present 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 by 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 (Online Fig. 3). 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 parameters for the mutation were provided without a cross-reference to the mutation). For additional details on the in silico methods, see Online Appendix.

Study population data collection and clinical endpoints. The study population comprised 633 subjects derived from 103 LQT1 proband-identified families with genetically confirmed *KCNQ1* mutations for which mutant channel properties are known, permitting simulation with the method described here (see Online Table 1 for details of mutations included [8]). Patients were drawn from 3 LQTS registries—the U.S. part of the Rochester-based LQTS Registry (n = 488), the Japan-based LQTS Registry (n = 56), and the Netherlands-based LQTS Registry (n = 23)—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 (n = 9).

Abbreviations and Acronyms

ACA	= aborted cardiac arrest
AP	= action potential
ECG	= electrocardiography
FGM	= Flaim-Giles-McCulloch
IKs	= rectifier potassium current
LQT1	= long QT syndrome type 1
LQTS	= long QT syndrome
Q4	= upper quartile
QTc	= QT interval corrected by heart rate
SCD	= sudden cardiac death
TRP	= transmural repolarization prolongation
WT	= wild-type

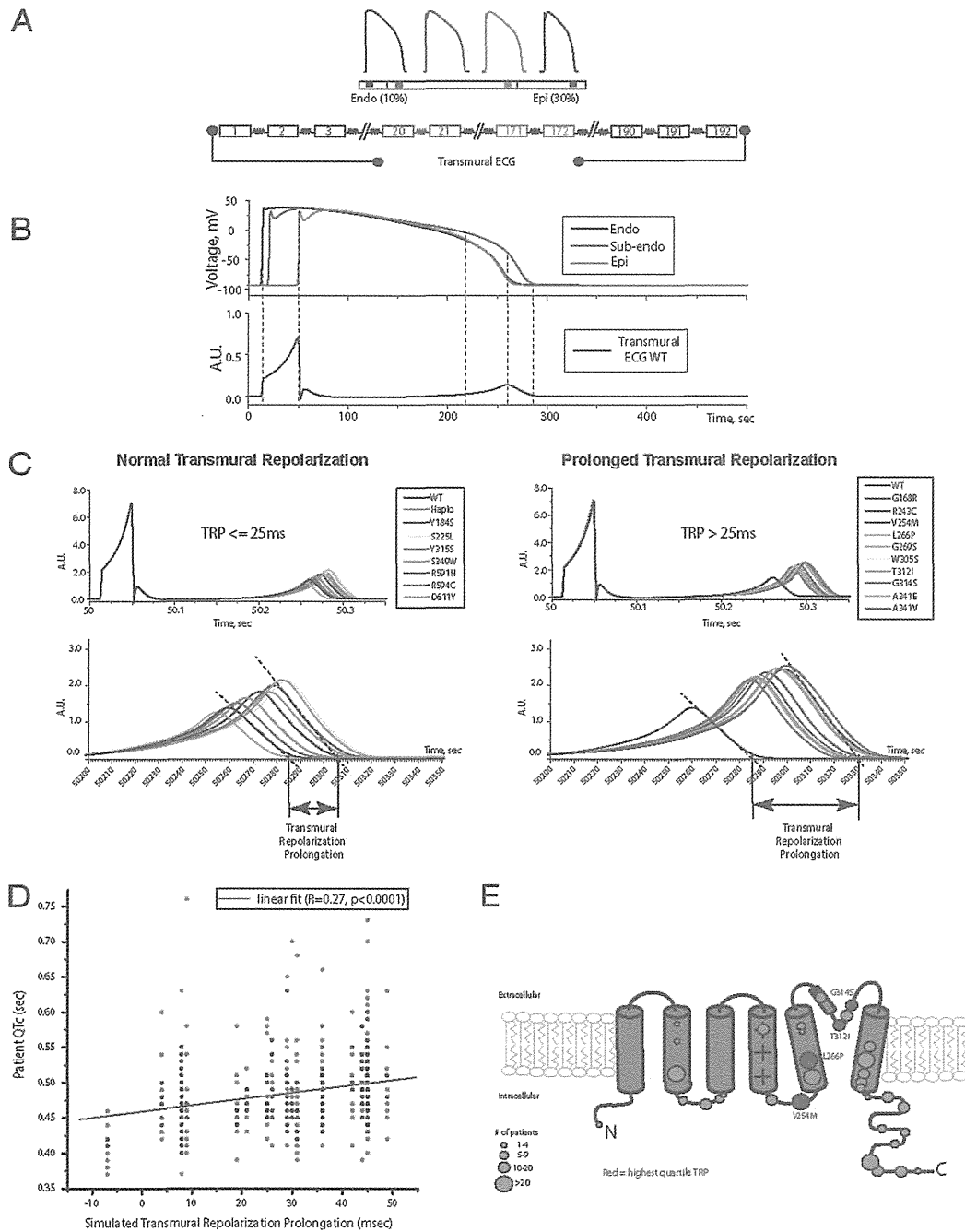


Figure 1 Determination of TRP for Ion Channels Associated With LQT1

(A) The 192-cell array used in the computational simulation of transmural repolarization prolongation (TRP) in patients with long QT syndrome type 1 (LQT1). Action potential determination for wild-type channels at the different cell types is shown. (B) Determination of pseudo-transmural electrocardiography (ECG) based on the wild-type channel action potential duration across the array of cells. (C) Pseudo-transmural ECG simulated for mutant channels in the study with lower (left) and higher (right) prolongation observed. A unique TRP parameter was determined for each mutant ion channel as indicated. (D) Correlation of TRP determined from simulation with patient QT interval corrected for heart rate (QTc) for all patients genotype positive for each mutation. (E) Location of mutations in the study. Mutations associated with the top quartile TRP are marked in red. Circle size indicates number of patients with the mutation.

Upon enrollment in the registry, clinical history was obtained; thus follow-up data in the current study comprised historical data from birth to enrollment and prospective information collected at yearly intervals after enrollment. The mean age at enrollment in the registry among the patients from the Rochester registry (77% of the patients studied) was 26 years, and follow-up for the survival analysis was from birth through age 40 years. On average, 69% of the follow-up data were obtained retrospectively at enrollment. Only patients for whom a complete medical history and prospective information were available were included in the present study. Clinical history data were collected on forms and included 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, ACA requiring defibrillation, and SCD without a known cause. Each subject had a single individual QTc value assigned, obtained at the time of entry in the LQT registry.

Genotyping. The *KCNQ1* mutations were identified with the use genetic tests performed in academic molecular-genetic laboratories, including the Functional Genomics Center, University of Rochester Medical Center, Rochester, New York; Baylor College of Medicine, Houston, Texas; Mayo Clinic College of Medicine, Rochester, Minnesota; Boston Children's Hospital, Boston, Massachusetts; Laboratory of Molecular Genetics, National Cardiovascular Center, Suita, Japan; Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands; and Statens Serum Institut, Copenhagen, Denmark. For the proband in each family, the 5 most common LQT loci (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) were fully sequenced to identify the mutation by comparing the sequence with those of healthy individuals without LQTS. Once a mutation was identified in other family members, and the *KCNQ1* gene was sequenced to confirm the presence or absence of the mutation.

Specifically, after informed consent was obtained, blood samples from patients were sent for detailed mutational analysis involving either 12 LQTS genes (*KCNQ1*, *KCNH2*, *SCN5A*, *ANK2*, *KCNE1*, *KCNE2*, *CACNA1C*, *KCNJ2*, *CAV3*, *SCN4B*, *AKAP9*, and *SNTA1*) or 5 LQTS genes (*KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) and were screened for multiple LQTS mutations/polymorphisms. Genetic tests included next-generation DNA sequencing of all of the coding exons. For that, exons and adjacent splice sites of each of the genes were sequenced using a solid-state sequencing-by-synthesis process (Illumina, Inc., San Diego, California). There are over 230 exons to be sequenced in LQTS; 12 gene panels and both alleles of each exon (maternal and paternal) were sequenced simultaneously. Polymerase chain reaction primers flanking each exon of the regions of interest were

designed to generate amplicons ranging in size from ~200 to 600 bp, avoiding any nonunique sequences, as well as positions of known polymorphisms and mutations. The products of all multiplex reactions for a specific patient were pooled. Sequencing itself first required cluster generation, which is the process of attaching individual fragments from a patient's library to a chambered glass slide and replicating them in situ to produce distinct islands of homogeneous fragments. Using the Genome Analyzer II (Illumina, Inc.), sequencing-by-synthesis chemistry was performed in the slide chambers to which the clusters were attached. Each base (A, C, G, or T) added to a growing DNA strand liberates 1 of 4 associated fluorochromes in a local reaction that was captured by photoimaging cycle for each cluster. Photoimaged data from each cluster of molecules were converted to base calls and aligned to a reference sequence for each gene of interest using Illumina-provided software. Various annotation databases (e.g., dbSNP, HGMD, and the rapidly growing local compilations) were used, when possible, to classify nucleotide differences into various categories, ranging from polymorphisms to pathogenic mutations.

Electrophysiology. The electrophysiologic properties of mutant *KCNQ1* channels were obtained by overexpression in *Xenopus laevis* oocytes as previously described (8,17,18). Experiments were performed in cells expressing WT human *KCNQ1* (NP_000209.2) and WT human *KCNE1* (NP_001121142). Mutations were generated by polymerase chain reaction-based site direct mutagenesis of the WT *KCNQ1* construct, using PFU ultra DNA polymerase and construct sequences confirmed by DNA sequencing. The average measurement of at least 18 cells expressing mutant channels were used to determine changes in ion channel parameters compared to WT channels. Thus, the same *KCNQ1* source was used for all of the mutant ion channels, with the only difference being the mutation specified in the study. All mutations analyzed for TRP were present among study subjects.

In brief, WT 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 these 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 had 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