associated with each type of LQT syndrome. Although gene-specific differences might be associated with a discrepancy in the occurrence of cardiac events,[39] further investigations are required.

Clinical Implications

The present results indicate a need for more specific medical therapy, although further assessments are required. For example, amounts of medication should be increased in summer and taken in the morning by patients with LQT1, increased over summer to autumn and taken before falling asleep by patients with LQT2, and increased before falling asleep for patients with LQT3.

Study Limitations

First, the timing and number of events might have been underestimated because they were based on patients' recall and medical records. Not all cardiac events were memorized like those recorded by an implantable cardioverter defibrillator. However, more extreme symptoms such as syncope and cardiac arrest or death were usually memorable and a history was taken from not only the patients but also their families. Thus, underestimation of these more disastrous events was considered to be low. In contrast, the frequency of events such as presyncope could be overestimated because they could arise as a result of causes other than ventricular tachyarrhythmias. However, we defined the symptoms of presyncope as sudden dizziness, palpitations, and chest pain persisting for over 30 s without a complete loss of consciousness that were confirmed by ECG recordings as being associated with ventricular tachyarrhythmias at least once, and we attempted to minimize false-positive cases. Second, the influence of drug therapy was not considered in this study, so the precise effect of time- or season-dependent exposure to β-blockers on the distribution of events was not analyzed. However, patients usually take medications in the morning and we did not change the medication according to the season. Third, some patients who had experienced a large number of events might have distorted the results. However, the tendencies of the seasonal and circadian distribution of cardiac events were similar, even when patients with a large number of cardiac events ($\geq$10) were excluded. In addition, the tendency remained similar regardless of the severity of cardiac events (presyncope, syncope, cardiac arrest, and death). Finally, this was a retrospective study, and the population size and the number of events was small, especially among patients with LQT3. In addition, unavoidable bias was conferred by excluding patients with LQTS whose first manifestation of illness was sudden death. Therefore, further studies of a large number of patients (with an implantable cardioverter defibrillator if possible) are required to validate the present findings and to define the underlying mechanisms.

Acknowledgments

We gratefully acknowledge the expert statistical assistance of Akiko Kada. Drs Shimizu and Horie were supported in part by a Research Grant for Cardiovascular Diseases (21C-8, 22-4-7, 23-4-7, 24-033) from the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid for Scientific Research on Innovative Areas (22136011).

Disclosures

This manuscript represents original work that has not been published and is not being considered for publication elsewhere in whole or in part in any language except as an abstract. All co-authors have read and approved the submission of the manuscript. There are no financial or other relationships that could lead to a conflict of interest (Conflict of Interest: none declared).

References

1. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, et al. Spectrum of mutations in long-QT syndrome genes: KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000; **102:** 1178–1185.
2. Anand K, Aryana A, Cloutier D, Hee T, Esterbrooks D, Mooss AN, et al. Circadian, daily, and seasonal distributions of ventricular tachyarrhythmias in patients with implantable cardioverter-defibrillators. *Am J Cardiol* 2007; **100:** 1134–1138.
3. Arntz HR, Willich SN, Schreiber C, Bruggemann T, Stern R, Schultheiss HP. Diurnal, weekly and seasonal variation of sudden death: Population-based analysis of 24,061 consecutive cases. *Eur Heart J* 2000; **21:** 315–320.
4. Muller JE, Ludmer PL, Willich SN, Tofler GH, Aylmer G, Klangos I, et al. Circadian variation in the frequency of sudden cardiac death. *Circulation* 1987; **75:** 131–138.
5. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. *Am J Cardiol* 1987; **60:** 801–806.
6. Spencer FA, Goldberg RJ, Becker RC, Gore JM. Seasonal distribution of acute myocardial infarction in the second National Registry of Myocardial Infarction. *J Am Coll Cardiol* 1998; **31:** 1226–1233.
7. Englund A, Behrens S, Wegscheider K, Rowland E. Circadian variation of malignant ventricular arrhythmias in patients with ischemic and nonischemic heart disease after cardioverter defibrillator implantation: European 7219 Jewel Investigators. *J Am Coll Cardiol* 1999; **34:** 1560–1568.
8. Muller D, Lampe F, Wegscheider K, Schultheiss HP, Behrens S. Annual distribution of ventricular tachycardias and ventricular fibrillation. *Am Heart J* 2003; **146:** 1061–1065.
9. Lampert R, Rosenfeld L, Batsford W, Lee F, McPherson C. Circadian variation of sustained ventricular tachycardia in patients with coronary artery disease and implantable cardioverter-defibrillators. *Circulation* 1994; **90:** 241–247.
10. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. Genotype-phenotype correlation in the long-QT syndrome: Gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001; **103:** 89–95.
11. Maron BJ, Semsarian C, Shen WK, Link MS, Epstein AE, Estes NA 3rd, et al. Circadian patterns in the occurrence of malignant ventricular tachyarrhythmias triggering defibrillator interventions in patients with hypertrophic cardiomyopathy. *Heart Rhythm* 2009; **6:** 599–602.
12. Takigawa M, Noda T, Shimizu W, Miyamoto K, Okamura H, Satomi K, et al. Seasonal and circadian distributions of ventricular fibrillation in patients with Brugada syndrome. *Heart Rhythm* 2008; **5:** 1523–1527.
13. Wilde AA, Jongbloed RJ, Doevendans PA, Duren DR, Hauer RN, van Langen IM, et al. Auditory stimuli as a trigger for arrhythmic events differentiate HERG-related (LQTS2) patients from KVLQT1-related patients (LQTS1). *J Am Coll Cardiol* 1999; **33:** 327–332.
14. Moss AJ, Robinson JL, Gessman L, Gillespie R, Zareba W, Schwartz PJ, et al. Comparison of clinical and genetic variables of cardiac events associated with loud noise versus swimming among subjects with the long QT syndrome. *Am J Cardiol* 1999; **84:** 876–879.
15. Ackerman MJ, Tester DJ, Porter CJ. Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. *Mayo Clin Proc* 1999; **74:** 1088–1094.
16. Ali RH, Zareba W, Moss AJ, Schwartz PJ, Benhorin J, Vincent GM, et al. Clinical and genetic variables associated with acute arousal and nonarousal-related cardiac events among subjects with long QT syndrome. *Am J Cardiol* 2000; **85:** 457–461.
17. Kloner RA, Poole WK, Perritt RL. When throughout the year is coronary death most likely to occur? A 12-year population-based analysis of more than 220 000 cases. *Circulation* 1999; **100:** 1630–1634.
18. Page RL, Zipes DP, Powell JL, Luceri RM, Gold MR, Peters R, et al. Seasonal variation of mortality in the Antiarrhythmics Versus Implantable Defibrillators (AVID) study registry. *Heart Rhythm* 2004; **1:** 435–440.
19. Noda T, Takaki H, Kurita T, Suyama K, Nagaya N, Taguchi A, et al. Gene-specific response of dynamic ventricular repolarization to sympathetic stimulation in LQT1, LQT2 and LQT3 forms of congenital long QT syndrome. *Eur Heart J* 2002; **23:** 975–983.
20. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, et al. Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long-QT syndrome. *J Am Coll Cardiol* 2003; **41:** 633–

642.
21. Shimizu W, Noda T, Takaki H, Nagaya N, Satomi K, Kurita T, et al. Diagnostic value of epinephrine test for genotyping LQT1, LQT2, and LQT3 forms of congenital long QT syndrome. *Heart Rhythm* 2004; **1:** 276–283.
22. Shimizu W. Clinical impact of genetic studies in lethal inherited cardiac arrhythmias. *Circ J* 2008; **72:** 1926–1936.
23. Sakaguchi T, Shimizu W, Itoh H, Noda T, Miyamoto Y, Nagaoka I, et al. Age- and genotype-specific triggers for life-threatening arrhythmia in the genotyped long QT syndrome. *J Cardiovasc Electrophysiol* 2008; **19:** 794–799.
24. Sriboonlue P, Tungsanga K, Tosukhowong P, Sitprija V. Seasonal changes in serum and erythrocyte potassium among renal stone formers from northeastern Thailand. *Southeast Asian J Trop Med Public Health* 1993; **24:** 287–292.
25. Guo J, Massaeli H, Xu J, Jia Z, Wigle JT, Mesaeli N, et al. Extracellular K+ concentration controls cell surface density of IKr in rabbit hearts and of the HERG channel in human cell lines. *J Clin Invest* 2009; **119:** 2745–2757.
26. Compton SJ, Lux RL, Ramsey MR, Strelich KR, Sanguinetti MC, Green LS, et al. Genetically defined therapy of inherited long-QT syndrome: Correction of abnormal repolarization by potassium. *Circulation* 1996; **94:** 1018–1022.
27. Etheridge SP, Compton SJ, Tristani-Firouzi M, Mason JW. A new oral therapy for long QT syndrome: Long-term oral potassium improves repolarization in patients with HERG mutations. *J Am Coll Cardiol* 2003; **42:** 1777–1782.
28. Hayashi H, Sumiyoshi M, Yasuda M, Komatsu K, Sekita G, Kawano Y, et al. Prevalence of the Brugada-type electrocardiogram and incidence of Brugada syndrome in patients with sick sinus syndrome. *Circ J* 2010; **74:** 271–277.
29. JCS Joint Working Group. Guidelines for risks and prevention of sudden cardiac death (JCS 2010): Digest version. *Circ J* 2012; **76:** 489–507.
30. Khan IA, Nair CK. Brugada and long QT-3 syndromes: Two phenotypes of the sodium channel disease. *Ann Noninvasive Electrocardiol* 2004; **9:** 280–289.
31. Makita N. Phenotypic overlap of cardiac sodium channelopathies: Individual-specific or mutation-specific? *Circ J* 2009; **73:** 810–817.
32. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, et al. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest* 2008; **118:** 2219–2229.
33. Bonnemeier H, Richardt G, Potratz J, Wiegand UK, Brandes A, Kluge N, et al. Circadian profile of cardiac autonomic nervous modulation in healthy subjects: Differing effects of aging and gender on heart rate variability. *J Cardiovasc Electrophysiol* 2003; **14:** 791–799.
34. Trinder J, Kleiman J, Carrington M, Smith S, Breen S, Tan N, et al. Autonomic activity during human sleep as a function of time and sleep stage. *J Sleep Res* 2001; **10:** 253–264.
35. Shinar Z, Akselrod S, Dagan Y, Baharav A. Autonomic changes during wake-sleep transition: A heart rate variability based approach. *Auton Neurosci* 2006; **130:** 17–27.
36. Rasch B, Dodt C, Molle M, Born J. Sleep-stage-specific regulation of plasma catecholamine concentration. *Psychoneuroendocrinology* 2007; **32:** 884–891.
37. Tsukada T, Ikeda T, Ishiguro H, Abe A, Miyakoshi M, Miwa Y, et al. Circadian variation in out-of-hospital cardiac arrests due to cardiac cause in a Japanese patient population. *Circ J* 2010; **74:** 1880–1887.
38. Jeyaraj D, Haldar SM, Wan X, McCauley MD, Ripperger JA, Hu K, et al. Circadian rhythms govern cardiac repolarization and arrhythmogenesis. *Nature* 2012; **483:** 96–99.
39. Tan HL, Bardai A, Shimizu W, Moss AJ, Schulze-Bahr E, Noda T, et al. Genotype-specific onset of arrhythmias in congenital long-QT syndrome: Possible therapy implications. *Circulation* 2006; **114:** 2096–2103.

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

Jason Costa, MA,* Coeli M. Lopes, PhD,† Alon Barsheshet, MD,* Arthur J. Moss, MD,* Dmitriy Migdalovich, BS,* Gregory Ouellet, MA,* Scott McNitt, MS,* Slava Polonsky, MS,* Jennifer L. Robinson, MS,* Wojciech Zareba, MD, PhD,* Michael J. Ackerman, MD, PhD,‡ Jesaia Benhorin, MD,§ Elizabeth S. Kaufman, MD,‖ Pyotr G. Platonov, MD,¶ Wataru Shimizu, MD, PhD,# Jeffrey A. Towbin, MD,** G. Michael Vincent, MD,†† Arthur A.M. Wilde, MD, PhD,‡‡ Ilan Goldenberg, MD*

*From the *Cardiology Division, University of Rochester Medical Center, Rochester, New York; †Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York; ‡Departments of Medicine, Pediatrics, and Molecular Pharmacology and Experimental Therapeutics, Divisions of Cardiovascular Diseases and Pediatric Cardiology, Mayo Clinic, Rochester, Minnesota; §Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ‖Heart and Vascular Research Center, MetroHealth Campus of Case Western Reserve University, Cleveland, Ohio; ¶Department of Cardiology, Lund University, Lund, Sweden; #Department of Internal Medicine, Division of Cardiology, National Cardiovascular Center, Suita, Japan; **Department of Pediatrics, University of Cincinnati Children's Hospital, Cincinnati, Ohio; ††LDS Hospital, Salt Lake City, Utah; ‡‡Department of Cardiology, Academic Medical Center, Amsterdam, The Netherlands.*

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)

Jason Costa, Coeli M. Lopes, Alon Barsheshet, and Ilan Goldenberg contributed equally to this article. This work was supported by research grants HL-33843 and HL-51618 from the National Institutes of Health, Bethesda, MD, and by a research grant from GeneDx to the Heart Research Follow-Up Program in support of the LQTS Registry. Dr Ackerman is a consultant for Transgenomic (approved by Mayo Clinic's Medical-Industry Relations Office and Conflict of Interests Review Board). In addition, "cardiac channel gene screen" and "know-how relating to long QT genetic testing" license agreements, resulting in consideration and royalty payments, were established between Genaissance 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.

1547-5271/$ -see front matter doi:10.1016/j.hrthm.2012.01.020

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 A[4] 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 deafness 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

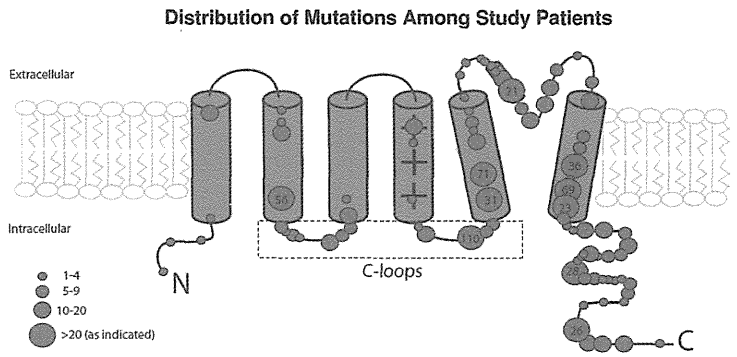

Figure 1 Distribution of mutations in the *KCNQ1* (Kv7.1) potassium channel subunit among study patients. Numbers in larger circles denote the number of patients with the mutation. C loops = cytoplasmic loops.

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 [$P < .001$ 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.

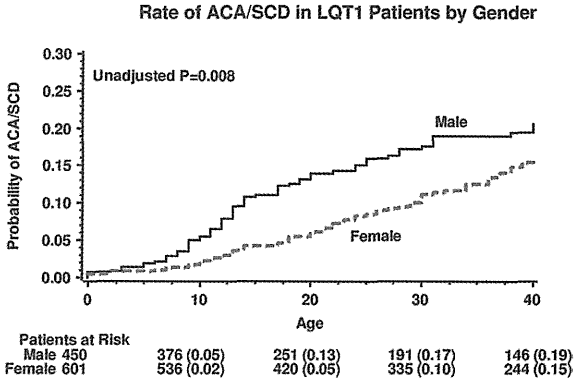

Figure 2 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in patients with LQT1 by sex. ACA = aborted cardiac arrest; LQT1 = long QT syndrome type 1; SCD = sudden cardiac death.

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.

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

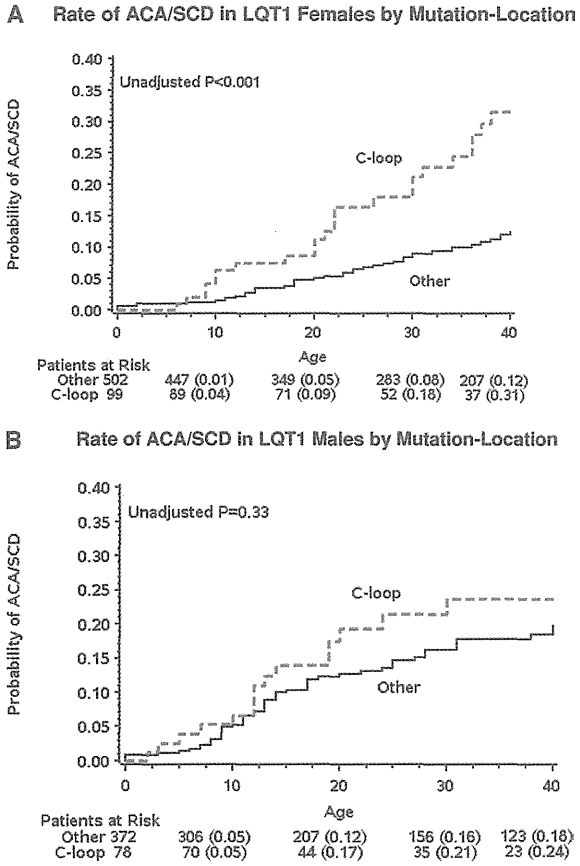

Figure 3 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in (**A**) women with LQT1 and (**B**) men with LQT1, by mutation location. ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; LQT1 = long QT syndrome type 1; SCD = 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 exercise[14] 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.

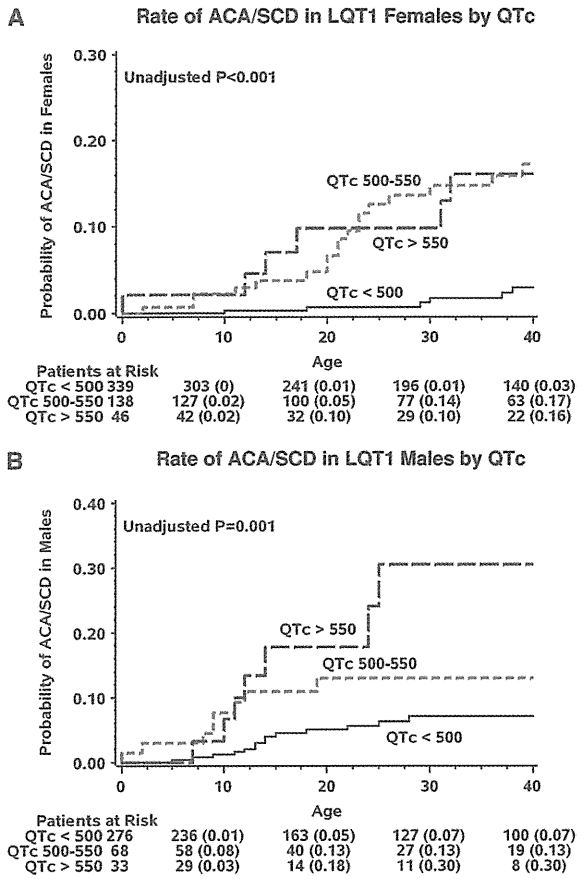

Figure 4 Kaplan-Meier estimates of the cumulative probability of aborted cardiac arrest or sudden cardiac death in (**A**) women with LQT1 and (**B**) men with LQT1, by QTc duration. ACA = aborted cardiac arrest; C-loop mutations = cytoplasmic-loop mutations; LQT1 = long QT syndrome type 1; QTc = corrected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

References

1. Goldenberg I, Moss AJ. Long QT syndrome. J Am Coll Cardiol 2008;51:2291–2300.
2. Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. Circulation 2001;103:89–95.
3. Moss AJ, Shimizu W, Wilde AAM, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the *KCNQ1* gene. Circulation 2007;115:2481–2489.
4. Matavel A, Medei E, Lopes CMB. PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions. Channels (Austin) 2010;4:3–11.
5. Barsheshet A, Goldenberg I, O-Uchi J, et al. Mutation Specific Risk and Response to Therapy in Type 1 Long QT Syndrome. American Heart Association Scientific Sessions, Chicago, IL, 2010.
6. Zareba W, Moss AJ, Locati EH, et al; International Long QT Syndrome Registry. Modulating effects of age and gender on the clinical course of long QT syndrome by genotype. J Am Coll Cardiol 2003;42:103–109.
7. Bazett H. An analysis of the time relations of electrocardiograms. Heart 1920;7:353–367.
8. Splawski I, Shen J, Timothy KW, et al. Spectrum of mutations in long-QT syndrome genes: *KVLQT1*, *HERG*, *SCN5A*, *KCNE1*, and *KCNE2*. Circulation 2000;102:1178–1185.
9. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model. New York, NY: Springer-Verlag; 2000.
10. Swan H, Viitasalo M, Piippo K, Laitinen P, Kontula K, Toivonen L. Sinus node function and ventricular repolarization during exercise stress test in long QT syndrome patients with KvLQT1 and HERG potassium channel defects. J Am Coll Cardiol 1999;34:823–829.
11. Hara M, Danilo P Jr, Rosen MR. Effects of gonadal steroids on ventricular repolarization and on the response to E4031. J Pharmacol Exp Ther 1998;285:1068–1072.
12. Brouillette J, Trépanier-Boulay C, Fiset C. Effect of androgen deficiency on mouse ventricular repolarization. J Physiol 2003;546:403–413.
13. Ridley JM, Shuba YM, James AF, Hancox JC. Modulation by testosterone of an endogenous hERG potassium channel current. J Physiol Pharmacol 2008;59:395–407.
14. Shimizu W, Horie M, Ohno S, et al. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: multicenter study in Japan. J Am Coll Cardiol 2004;44:117–125.
15. Moss AJ, Schwartz PJ, Crampton RS, et al. The long QT syndrome: prospective longitudinal study of 328 families. Circulation 1991;84:1136–1144.
16. Priori SG, Napolitano C, Schwartz PJ, et al. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. JAMA 2004;292:1341–1344.
17. Priori SG, Schwartz PJ, Napolitano C, et al. Risk stratification in the long-QT syndrome. N Engl J Med 2003;348:1866–1874.
18. Vincent GM, Schwartz PJ, Denjoy I, et al. High efficacy of β-blockers in long-QT syndrome type 1: contribution of noncompliance and QT-prolonging drugs to the occurrence of β-blocker treatment "failures". Circulation 2009;119:215–221.

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

Alon Barsheshet, MD*; Ilan Goldenberg, MD*; Jin O-Uchi, MD, PhD*; Arthur J. Moss, MD; Christian Jons, MD; Wataru Shimizu, MD; Arthur A. Wilde, MD, PhD; Scott McNitt, MS; Derick R. Peterson, PhD; Wojciech Zareba, MD, PhD; Jennifer L. Robinson, MS; Michael J. Ackerman, MD; Michael Cypress, BS; Daniel A. Gray, MD, PhD; Nynke Hofman, MS; Jorgen K. Kanters, MD; Elizabeth S. Kaufman, MD; Pyotr G. Platonov, MD, PhD; Ming Qi, PhD; Jeffrey A. Towbin, MD; G. Michael Vincent, MD; Coeli M. Lopes, PhD

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

Received February 24, 2011; accepted February 27, 2012.

From the Cardiology Division (A.B., I.G., A.J.M., S.M., W.Z., J.L.R.), Department of Biostatistics and Computational Biology (D.R.P.), and Nephrology Division (M.C.), University of Rochester Medical Center, Rochester, NY; Cardiovascular Research Institute (J.O-U., C.M.L.) and Department of Pathology (M.Q.), University of Rochester School of Medicine and Dentistry, Rochester, NY; Gentofte University Hospital, Copenhagen, Denmark (C.J., J.K.K.); National Cardiovascular Center, Suita, Japan (W.S.); Department of Cardiology (A.A.W.) and Department of Clinical Genetics (N.H.), Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; Department of Pediatrics, Division of Pediatric Cardiology, Mayo Clinic, Rochester, MN (M.J.A.); Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, OH (E.S.K.); Department of Cardiology, Lund University, Lund, Sweden (P.G.P.); The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH (J.A.T.); and Department of Medicine, University of Utah School of Medicine, Salt Lake City (G.M.V.).

*Drs Barsheshet, Goldenberg, and O-Uchi contributed equally to this article.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.111.048041/-/DC1.

Correspondence to Coeli M. Lopes, PhD, Department of Medicine, Box CVRI, Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, Rochester, NY 14642 (E-mail Coeli_Lopes@URMC.Rochester.edu); or Ilan Goldenberg, MD, Heart Research Follow-Up Program, University of Rochester Medical Center, Box 653, Rochester, NY 14642 (E-mail Ilan.Goldenberg@heart.rochester.edu).

Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.111.048041**

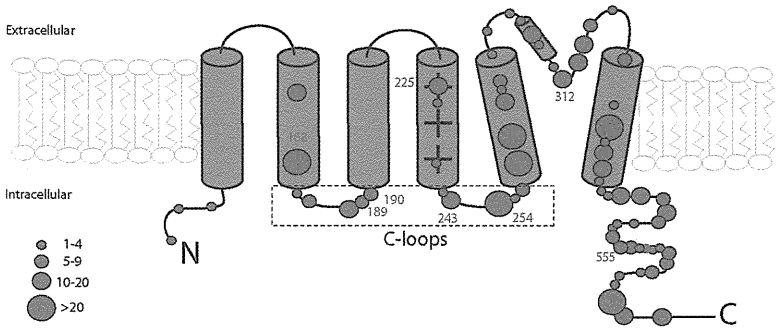

Figure 1. Frequency and location of mutations in the KCNQ1 potassium channel. Diagrammatic location of 99 different mutations in the *KCNQ1* potassium channel involving 860 subjects. The α-subunit involves the N-terminus (N), 6 membrane-spanning segments, 2 cytoplasmic loops (S2–S3 and S4-S5), and the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

Editorial see p 1961
Clinical Perspective on p 1996

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

	Missense			
Parameter	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

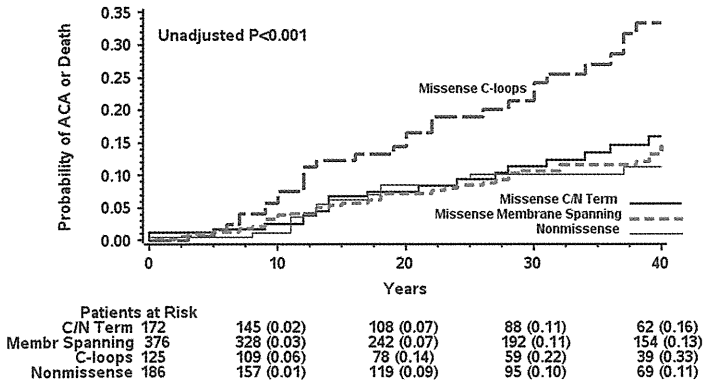

Figure 2. Kaplan-Meier estimates of cumulative probability of life-threatening cardiac events by mutation location and type. The numbers in parentheses reflect the cumulative event rate at that point in time. ACA indicates aborted cardiac arrest; LQTS, long-QT syndrome.

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 $P=0.028$.

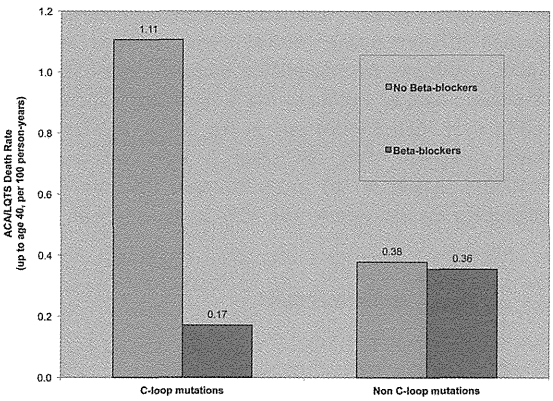

Figure 3. Risk for life-threatening cardiac events by mutation location and β-blocker treatment. Sixty-three of the 125 subjects (50%) with C-loop missense mutations were treated with β-blockers during a mean follow-up of 26.2 years; 305 of the 735 subjects (42%) with non–C-loop missense mutations were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

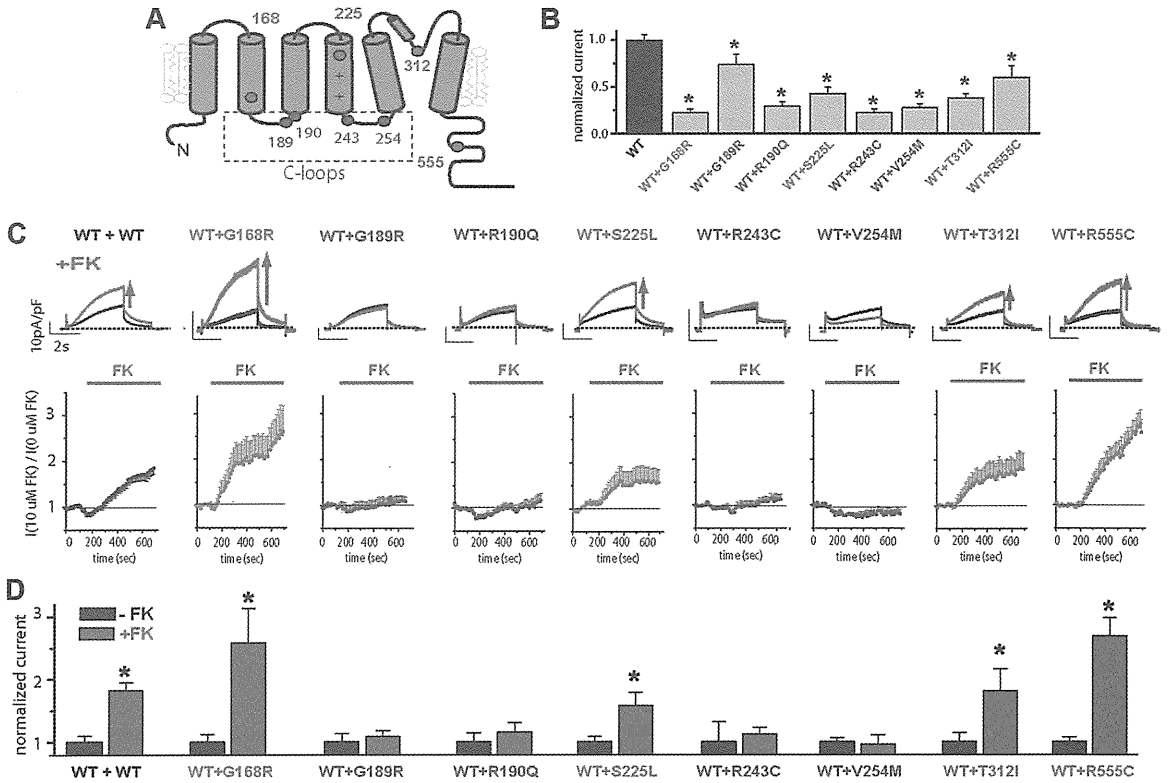

Figure 4. Regulation of LQT1 mutant channels by protein kinase A. **A**, Schematic representation of location of the mutations used in the study. **B**, Effect of each of the mutations studied in basal nonstimulated cell currents. Average current measured for cells expressing wild-type (WT) and mutant 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 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.

Acknowledgments

This research was carried out while Dr Barsheshet was a Mirowski-Moss Career Development Awardee at the University of Rochester Medical Center, Rochester, NY. We thank Jaime Sorenson, Nobiru Suzuki, and Mehreen Butt for their technical assistance.

Sources of Funding

This work was supported by research grants HL-33843 and HL-51618 from the National Institutes of Health, Bethesda, MD, and by a research grant from BioReference Labs to the Heart Research Follow-Up Program in support of the LQTS Registry.

Disclosures

Dr Ackerman has a consulting relationship and license agreement/royalty arrangement with Transgenomic. Dr Kaufman receives research support from Cambridge Heart. Dr O-Uchi is the recipient of an American Heart Association Postdoctoral Fellowship Grant (09POST2310079), a Foreign Study Grant Award of the Kanae Foundation (Tokyo, Japan), and an Irisawa Memorial Promotion Award for Young Physiologists (Physiological Society of Japan). Dr Kanters is a recipient of the Danish Council for Strategic Research grant. Dr Shimizu is a recipient of research grant from the Ministry of Health, Labour and Welfare, Japan. Dr Moss is a recipient of National Institutes of Health grants and receives research support from BioReference Labs. Dr Wilde has a consulting relationship with Transgenomic. The other authors report no conflicts.

References

1. Goldenberg I, Moss AJ. Long QT syndrome. *J Am Coll Cardiol.* 2008; 51:2291–2300.
2. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, Denjoy I, Guicheney P, Breithardt G, Keating MT, Towbin JA, Beggs AH, Brink P, Wilde AA, Toivonen L, Zareba W, Robinson JL, Timothy KW, Corfield V, Wattanasirichaigoon D, Corbett C, Haverkamp W, Schulze-Bahr E, Lehmann MH, Schwartz K, Coumel P, Bloise R. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation.* 2001;103:89–95.
3. Walsh KB, Kass RS. Regulation of a heart potassium channel by protein kinase A and C. *Science.* 1988;242:67–69.
4. Marx SO, Kurokawa J, Reiken S, Motoike H, D'Armiento J, Marks AR, Kass RS. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science.* 2002;295:496–499.
5. Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. *J Am Coll Cardiol.* 2000;35:778–786.
6. Goldenberg I, Bradley J, Moss A, McNitt S, Polonsky S, Robinson JL, Andrews M, Zareba W. Beta-blocker efficacy in high-risk patients with the congenital long-QT syndrome types 1 and 2: Implications for patient management. *J Cardiovasc Electrophysiol.* 2010;21:893–901.
7. Jespersen T, Grunnet M, Olesen SP. The KCNQ1 potassium channel: from gene to physiological function. *Physiology.* 2005;20:408–416.
8. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, Hofman N, Seth R, Kamakura S, Miyamoto Y, Goldenberg I, Andrews ML, McNitt S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation.* 2007;115:2481–2489.
9. Matavel A, Medei E, Lopes CM. PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions. *Channels.* 2010; 4:3–11.
10. Jons C, J OU, Moss AJ, Reumann M, Rice JJ, Goldenberg I, Zareba W, Wilde AA, Shimizu W, Kanters JK, McNitt S, Hofman N, Robinson JL, Lopes CM. Use of mutant-specific ion channel characteristics for risk stratification of long QT syndrome patients. *Sci Transl Med.* 2011;3: 76ra28.
11. Jhun BS, J OU, Wang W, Ha CH, Zhao J, Kim JY, Wong C, Dirksen RT, Lopes CM, Jin ZG. Adrenergic signaling controls RGK-dependent trafficking of cardiac voltage-gated L-type Ca^{2+} channels through PKD1. *Circ Res.* 2012;110:59–70.
12. Hobbs JB, Peterson DR, Moss AJ, McNitt S, Zareba W, Goldenberg I, Qi M, Robinson JL, Sauer AJ, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Towbin JA, Vincent GM, Zhang L. Risk of aborted cardiac arrest or sudden cardiac death during adolescence in the long-QT syndrome. *JAMA.* 2006;296:1249–1254.
13. Goldenberg I, Moss AJ, Peterson DR, McNitt S, Zareba W, Andrews ML, Robinson JL, Locati EH, Ackerman MJ, Benhorin J, Kaufman ES, Napolitano C, Priori SG, Qi M, Schwartz PJ, Towbin JA, Vincent GM, Zhang L. Risk factors for aborted cardiac arrest and sudden cardiac death in children with the congenital long-QT syndrome. *Circulation.* 2008; 117:2184–2191.
14. Locati EH, Zareba W, Moss AJ, Schwartz PJ, Vincent GM, Lehmann MH, Towbin JA, Priori SG, Napolitano C, Robinson JL, Andrews M, Timothy K, Hall WJ. Age- and sex-related differences in clinical manifestations in patients with congenital long-QT syndrome: findings from the international LQTS registry. *Circulation.* 1998;97:2237–2244.
15. Zareba W, Moss AJ, Sheu G, Kaufman ES, Priori S, Vincent GM, Towbin JA, Benhorin J, Schwartz PJ, Napolitano C, Hall WJ, Keating MT, Qi M, Robinson JL, Andrews ML. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J Cardiovasc Electrophysiol.* 2003;14:1149–1153.
16. Sauer AJ, Moss AJ, McNitt S, Peterson DR, Zareba W, Robinson JL, Qi M, Goldenberg I, Hobbs JB, Ackerman MJ, Benhorin J, Hall WJ, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L. Long QT syndrome in adults. *J Am Coll Cardiol.* 2007;49:329–337.
17. Lin DY, Wei LJ. The robust inference for the proportional hazards model. *J Am Stat Assoc.* 1989;84:1074–1078.
18. Therneau TM, Grambsch PM. *Modeling Survival Data: Extending the Cox Model.* New York, NY: Springer-Verlag; 2000.
19. Terrenoire C, Clancy CE, Cormier JW, Sampson KJ, Kass RS. Autonomic control of cardiac action potentials: role of potassium channel kinetics in response to sympathetic stimulation. *Circ Res.* 2005;96: e25–e34.
20. Potet F, Scott JD, Mohammad-Panah R, Escande D, Baro I. Akap proteins anchor CAMP-dependent protein kinase to KVLQT1/ISK channel complex. *Am J Physiol Heart Circ Physiol.* 2001;280:H2038–H2045.
21. Shimizu W, Horie M, Ohno S, Takenaka K, Yamaguchi M, Shimizu M, Washizuka T, Aizawa Y, Nakamura K, Ohe T, Aiba T, Miyamoto Y, Yoshimasa Y, Towbin JA, Priori SG, Kamakura S. Mutation site-specific differences in arrhythmic risk and sensitivity to sympathetic stimulation in the LQT1 form of congenital long QT syndrome: multicenter study in Japan. *J Am Coll Cardiol.* 2004;44:117–125.
22. Isacoff EY, Jan YN, Jan LY. Putative receptor for the cytoplasmic inactivation gate in the shaker K^+ channel. *Nature.* 1991;353:86–90.
23. Franqueza L, Lin M, Shen J, Splawski I, Keating MT, Sanguinetti MC. Long QT syndrome-associated mutations in the S4-S5 linker of KVLQT1 potassium channels modify gating and interaction with mink subunits. *J Biol Chem.* 1999;274:21063–21070.
24. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, Dorn T, Goedel A, Hohnke C, Hofmann F, Seyfarth M, Sinnecker D, Schomig A, Laugwitz KL. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med.* 2010;363:1397–1409.
25. Heijman J, Spatjens RL, Seyen SR, Lentink V, Kuijpers HJ, Boulet IR, de Windt LJ, David M, Volders PG. Dominant-negative control of cAMP-dependent IKs upregulation in human long-QT syndrome type 1. *Circ Res.* 2012;110:211–219.
26. Choveau FS, Rodriguez N, Ali FA, Labro AJ, Rose T, Dahimene S, Boudin H, Le Henaff C, Escande D, Snyders DJ, Charpentier F, Merot J, Baro I, Loussouarn G. KCNQ1 channels voltage dependence through a voltage-dependent binding of the S4-S5 linker to the pore domain. *J Biol Chem.* 2011;286:707–716.

27. Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, Gregoratos G, Klein G, Moss AJ, Myerburg RJ, Priori SG, Quinones MA, Roden DM, Silka MJ, Tracy C, Smith SC Jr, Jacobs AK, Adams CD, Antman EM, Anderson JL, Hunt SA, Halperin JL, Nishimura R, Ornato JP, Page RL, Riegel B, Blanc JJ, Budaj A, Dean V, Deckers JW, Despres C, Dickstein K, Lekakis J, McGregor K, Metra M, Morais J, Osterspey A, Tamargo JL, Zamorano JL. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death): developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation.* 2006;114:e385–e484.
28. Huffaker R, Lamp ST, Weiss JN, Kogan B. Intracellular calcium cycling, early afterdepolarizations, and reentry in simulated long QT syndrome. *Heart Rhythm.* 2004;1:441–448.

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.

Journal of the American College of Cardiology
© 2012 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 60, No. 21, 2012
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2012.07.053

In Silico Cardiac Risk Assessment in Patients With Long QT Syndrome

Type 1: Clinical Predictability of Cardiac Models

Ryan Hoefen, MD, PhD,* Matthias Reumann, PhD,† Ilan Goldenberg, MD,* Arthur J. Moss, MD,* Jin O-Uchi, MD, PhD,‡ Yiping Gu, PhD,§ Scott McNitt, MS,* Wojciech Zareba, MD, PhD,* Christian Jons, MD,* Jorgen K. Kanters, MD,‖ Pyotr G. Platonov, MD, PhD,¶ Wataru Shimizu, MD,# Arthur A. M. Wilde, MD, PhD,** John Jeremy Rice, PhD,§ Coeli M. Lopes, PhD‡

Rochester, New York; Melbourne, Australia; Yorktown Heights, New York; Copenhagen, Denmark; Lund, Sweden; Suita, Japan; and Amsterdam, the Netherlands

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 [$p < 0.0001$]; ≥upper quartile hazard ratio: 2.80 [$p < 0.0001$]) and life-threatening events (aborted cardiac arrest/sudden cardiac death: 27% per 10-ms increment [$p = 0.03$]; ≥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

From the *Division of Cardiology, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York; †IBM Research Collaboratory for Life Sciences, Melbourne, Australia; ‡Cardiovascular Research Institute, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York; §IBM Research Functional Genomics and Systems Biology, IBM T. J. Watson Research Center, Yorktown Heights, New York; ‖Gentofte University Hospital & University of Copenhagen, Copenhagen, Denmark; ¶Department of Cardiology, Lund University, Lund, Sweden; #National Cardiovascular Center, Suita, Japan; and the **Department of Clinical Genetics (NH), Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands. Dr. Wilde is a member of the advisory board of Sorin and PGxHealth. Dr. Goldenberg has been a paid consultant for Boston Scientific. Dr. Jons reports receiving speaker's fees from Boehringer Ingelheim. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Hoefen and Reumann are joint first authors; they contributed equally to this work. Drs. Lopes and Goldenberg are first authors for this paper.

Manuscript received January 7, 2012; revised manuscript received July 19, 2012, accepted July 23, 2012.

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

See page 2192

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.

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

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).