Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing

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OBJECTIVES The purpose of this study was to determine the spectrum and prevalence of cardiac channel mutations among a large cohort of consecutive, unrelated patients referred for long QT syndrome (LQTS) genetic testing.

BACKGROUND Congenital LQTS is a primary cardiac channelopathy. More than 300 mutations have been identified in five genes encoding key ion channel subunits. Until the recent release of the commercial clinical genetic test, LQTS genetic testing had been performed in research laboratories during the past decade.

METHODS A cardiac channel gene screen for LQTS-causing mutations in *KCNQ1* (LQT1), *KCNH2* (LQT2), *SCN5A* (LQT3), *KCNE1* (LQT5), and *KCNE2* (LQT6) was performed for 541 consecutive, unrelated patients (358 females, average age at diagnosis 24 ± 16 years, average QTc 482 ± 57 ms) referred to Mayo Clinic's Sudden Death Genomics Laboratory for LQTS genetic testing between August 1997 and July 2004. A comprehensive open reading frame and splice site analysis of the 60 protein-encoding exons was conducted using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing.

RESULTS Overall, 211 putative pathogenic mutations in *KCNQ1* (88), *KCNH2* (89), *SCN5A* (32), *KCNE1* (1), and *KCNE2* (1) were found in 272 unrelated patients (50%). Among the genotype positive patients (N = 272), 243 had single pathogenic mutations (LQT1: n = 120 patients; LQT2: n = 93; LQT3: n = 26; LQT5: n = 3; LQT6: n = 1), and 29 patients (10% of genotype-positive patients and 5% overall) had two LQTS-causing mutations. The majority of mutations were missense mutations (154/210 [73%]), singletons (identified in only a single unrelated patient: 165/210 [79%]), and novel (125/211 [59%]). None of the mutations identified were seen in more than 1,500 reference alleles. Those patients harboring multiple mutations were younger at diagnosis (15 ± 11 years vs 24 ± 16 years, P =.003).

CONCLUSIONS In this comprehensive cardiac channel gene screen of the largest cohort of consecutive, unrelated patients referred for LQTS genetic testing, half of the patients had an identifiable mutation. The majority of mutations continue to represent novel singletons that expand the published compendium of LQTS-causing mutations by 35%. These observations should facilitate diagnostic interpretation of the clinical genetic test for LQTS.

KEYWORDS Long QT syndrome; Genetic testing; Potassium channels; Sodium channels

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Introduction

Lethal ventricular arrhythmias claim the lives of more than 300,000 individuals in the United States each year. The majority of these sudden deaths involve middle-aged and elderly persons with coronary artery disease. However, a tragic minority involves previously healthy infants, children, adolescents, and young adults. After excluding structural heart diseases such as hypertrophic cardiomyopathy and anomalous coronary arteries, the most common pathogenic mechanism underlying these unexplained sudden deaths are heritable arrhythmia syndromes or cardiac channelopathies such as congenital long QT syndrome (LQTS).

LQTS affects one in 5,000 individuals. It is characterized by (1) a heterogeneous clinical natural history ranging from asymptomatic longevity to sudden death in infancy, (2) a heterogeneous ECG phenotype ranging from a completely normal resting ECG to extreme QT prolongation with manifest T-wave alternans, and (3) heterogeneous genetic underpinnings.[1] Since the initial identification of the first LQTS genetic locus on chromosome 11 in 1991[2] to the first identification of LQTS-causing mutations involving the *KCNH2*-encoded potassium channel[3] and the *SCN5A*-encoded cardiac sodium channel[4] in 1995, LQTS is understood predominantly as a cardiac channelopathy.[5,6] To date, more than 300 mutations involving five genes that encode critical cardiac channel subunits have been reported in LQTS.[7,8] More recently, mutations in ankyrin B have been established as the pathogenic basis for the previously elusive and rare type 4 LQTS (LQT4).[9]

Over the past decade, LQTS genetic testing has been conducted in select few research laboratories as part of Institutional Review Board (IRB)-approved genotype-phenotype research studies resulting in numerous novel revelations, including the identification of relatively gene-specific ECG profiles, gene-specific responses to epinephrine QT stress testing, gene-specific arrhythmogenic triggers and arrhythmogenic temporal states such as swimming, alarm clocks, and the postpartum period, gene-specific responsiveness to beta-blocker therapy, gene-directed treatment strategies, and gene-specific risk stratification.[10–20] However, with genetic testing taking anywhere from 6 to 24 months to complete and the requirement to disseminate the genetic test information to the study subject rather than to the referring physician, the patients and families afflicted with this potential sudden death syndrome and the physicians evaluating and managing such families have not similarly benefited from the genomic breakthroughs in LQTS.

Recently, however, LQTS genetic testing has become a commercially available, clinical diagnostic test, thus representing one of the first comprehensive genetic tests for a cardiac condition akin to *BRCA1* and *BRCA2* breast cancer genetic testing.[21] LQTS genetic testing now joins cystic fibrosis genetic testing as the only ion channel genetic tests currently available clinically. Accordingly, we report the results from our 7-year experience of LQTS genetic testing conducted for 541 consecutive, unrelated patients with a referral diagnosis of LQTS. These observations provide key insights to guide the proper utilization and interpretation of this newly available clinical test.

Methods

Comprehensive mutational analysis of unrelated LQTS cases

Informed written consent was obtained in accordance with study protocols approved by the Mayo Foundation IRB. Between August 1997 and July 2004, 541 consecutive, unrelated patients with a suspected clinical diagnosis of congenital LQTS were referred for LQTS molecular genetic testing at Mayo Clinic's Sudden Death Genomics Laboratory. Regardless of the clinical diagnostic score for LQTS ("Schwartz score")[22], a sample was accepted for genetic testing if the referring physician had made a tentative clinical diagnosis of LQTS. Clinical data, including 12-lead ECG, personal history of syncope, seizures, or aborted cardiac arrest, temporally related triggers, and family history, were extracted and maintained in a custom database, blinded to patient genotype. Sufficient data to derive a clinical Schwartz score were available in the majority of cases (n = 417 [77%]). A Schwartz score ≥4 indicates high clinical probability/strong clinical evidence in support of a congenital LQTS diagnosis.[22] Differences between continuous variables were assessed using unpaired Student's t-tests. Nominal variables were analyzed using Chi-square analysis. $P < .05$ was considered significant.

Patient genomic DNA was analyzed for mutations in all protein-coding exons (N = 60), including splice site regions of the *KCNQ1*/*KVLQT1*-encoded I_{Ks} potassium channel α-subunit (LQT1), the *KCNH2*/*HERG*-encoded I_{Kr} potassium channel α-subunit (LQT2), the *SCN5A*-encoded cardiac sodium channel NaV1.5 (LQT3), the *KCNE1*/*minK*-encoded I_{Ks} β-subunit (LQT5), and the *KCNE2*/*MiRP1*-encoded I_{Kr} β-subunit (LQT6) using polymerase chain reaction, denaturing high-performance liquid chromatography, and automated DNA sequencing.[17,18,23,24]

All putative LQTS-associated variants were denoted using known and accepted nomenclature.[25] For example, the single letter amino acid code was used to designate nonsynonymous, missense variants using the P73T format. Here, at amino acid position 73, the "wild-type" amino acid (P = proline) is replaced by a threonine (T) on one of the alleles. To be annotated as a putative LQTS-associated variant, the variant must have involved a conserved residue or splice site that altered the primary amino acid structure of the encoded protein. Hence, synonymous single nucleotide polymorphisms were excluded from consideration. In addition, to be considered as a probable pathogenic LQTS-causing variant, the nonsynonymous variant must have been absent in all published databases of channel polymorphisms and our previous comprehensive analysis of 1,488 reference

Table 1 Demographics of 541 consecutive, unrelated patients referred for long QT syndrome genetic testing

	Total cohort	LQT1	LQT2	LQT3	Multiples	Genotype negative
No. of unrelated patients	541	120	93	26	29	269
Age at diagnosis	24 ± 16	24 ± 17	22 ± 15	24 ± 17	15 ± 11	25 ± 16
Range (yr)	(0–78)	(0–69)	(0–65)	(0–57)	(0–50)	(0–78)
Sex (male/female)	183/358	44/76	26/67	8/18	15/14	89/180
Ethnicity (% white)	93	92	92	88	84	96
Average QTc (ms)	482 ± 57	483 ± 40	507 ± 56	494 ± 61	506 ± 57	470 ± 60
Range	(365–759)	(410–650)	(402–648)	(413–700)	(433–637)	(365–759)
Percent with QTc >480 ms	46	47	66	50	75	35
Percent with syncope	42	50	43	31	57	38
Percent with cardiac arrest	12	14	15	4	14	12
Percent with positive family history	42	47	48	42	43	38
Percent with "Schwartz" score ≥4	29	40	39	24	61	17

QTc = corrected QT interval.

alleles from four ethnic groups for the potassium channel genes[26] and 1,658 reference alleles for *SCN5A*.[27] As such, the sole or concomitant presence of a common polymorphism such as P448R-KCNQ1, K897T-KCNH2, H558R-SCN5A, or D85N-KCNE1 would not, by definition, warrant the annotation of LQT1, LQT2, LQT3, or LQT5, respectively, and would not be counted toward the assignment of compound or multiple mutation status to an individual.

Results

Table 1 summarizes the demographics for this cohort of 541 consecutive, unrelated cases (358 females) having a suspected clinical diagnosis of LQTS. The majority of this cohort was white (93%), with 19 Hispanic patients (4%), 11 blacks (2%), 3 Asians (0.5%), and 1 Native-American. Ethnicity was not available for 24 participants (4%). The average age at diagnosis was 24 ± 16 years (range 1 day to 78 years). The average QTc was 482 ± 57 ms (range 365–759 ms). Approximately 46% of the subjects had a QTc >480 ms, 42% had fainted, 12% had survived sudden cardiac death, and 29% had an overall clinical diagnostic Schwartz score ≥4 indicating high clinical probability of LQTS. Eighty-nine of these 123 subjects (72%) with a Schwartz score ≥4 had a putative LQTS-causing mutation (data not shown). A positive family history was identified in approximately half the cases. None of the subjects displayed the autosomal recessive phenotype of Jervell and Lange-Nielsen syndrome with deafness.

Confirming and extending our previous observations with respect to particular arrhythmogenic triggers, Figure 1 summarizes the spectrum and distribution of LQTS genotypes associated with specific arrhythmogenic triggers that were documented among the index cases. In this cohort of consecutive, unrelated patients, 124 patients (23%) had documentation of either exertional syncope (N = 101) and/or aborted cardiac arrest during exertion (N = 23). Although 56 of 69 patients (81%) with an identifiable mutation were LQT1 genotype, many patients remained genotype negative (N = 55) following analysis of the five LQTS-associated genes despite a clinical presentation involving exertional syncope/aborted cardiac arrest (Figure 1).

There were 71 unrelated patients with either a personal or family history of a drowning or near-drowning. Among the patients with this swimming phenotype, 46 of 71 (65%) were LQT1, 5 (7%) LQT2, 1 LQT3, and 18 (25%) genotype negative (Figure 1). Previously, half of these genotype negative patients harboring a swimming phenotype were shown to host catecholaminergic polymorphic ventricular tachycardia (CPVT)-associated mutations involving the *RyR2*-

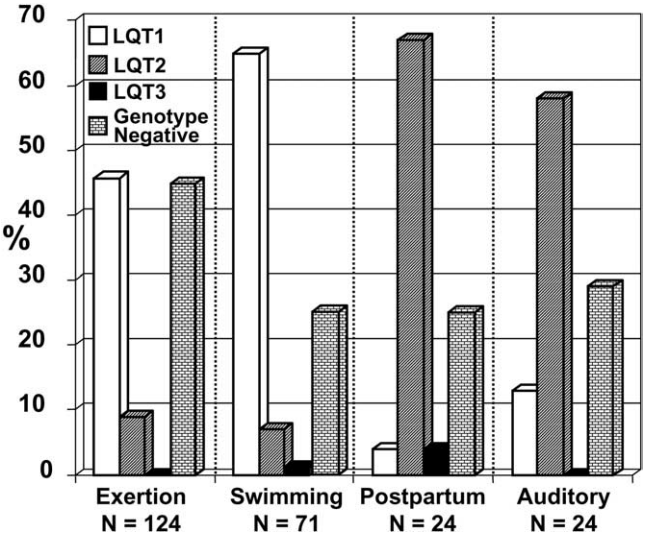

Figure 1 Arrhythmogenic triggers and long QT syndrome genotype. Depicted is a summary of the spectrum and frequency of genotype positive (LQT1–LQT3) status and genotype negative status associated with particular triggers, including personal history of exertional syncope or aborted cardiac arrest during exertion, personal or family history of a swimming-triggered cardiac event, personal or family history of syncope, aborted cardiac arrest, or sudden death occurring postpartum, and a personal history of auditory-triggered syncope or aborted cardiac arrest. LQT1 genotype is indicated as *open rectangles,* LQT2 as *diagonal-filled rectangles,* LQT3 in *black-filled rectangles,* and genotype negative in *brick-filled rectangles.*

Table 2 Summary of *KCNQ1* LQT1-associated variants

No.	Exon	Nucleotide	Variant	Location	No. of patients
1	1	153 C→G	Y51X*	N-terminus	1
2	1	del 211–219	AAP71-73del	N-terminus	1
3	1	217 C→A	P73T*	N-terminus	1
4	1	del C 287	S95fs/141*	N-terminus	1
5	1	344 A→G	E115G*	N-terminus	1
6	1	Ins G 2025–2026	K121fs/629*	N-terminus	1
7	1	365 G→A	C122Y*	N-terminus	1
8	2	397 G→A	V133I*	S1	1
9	2	407 G→T	C136F*	S1	1
10	2	477+5 G→A	M159sp	S2	2
11	2	477+5 G→C	M159sp*	S2	1
12	3	478 G→A	E160K	S2	1
13	3	del T 488	V162fs/73*	S2	1
14	3	502 G→A	G168R	S2	2
15	3	513 C→G	Y171X	S2–S3	1
16	3	520 C→T	R174C	S2–S3	2
17	3	569 G→A	R190Q	S2–S3	2
18	3	del 572–576	L191fs/90	S2–S3	7
19	4	610 A→T	I204F*	S3	1
20	4	674 C→T	S225L	S4	3
21	5	704 T→A	I235N*	S4	1
22	5	724 G→A	D242N	S4–S5	1
23	5	727 C→T	R243C	S4–S5	3
24	5	760 G→A	V254M	S4–S5	2
25	5	del 760–768	VVF254–256del*	S4–S5	1
26	5	775 C→T	R259C	S4–S5	1
27	5	776 G→T	R259L*	S4–S5	2
28	6	783 G→C	E261D	S4–S5	1
29	6	797 T→C	L266P	S5	4
30	6	805 G→A	G269S	S5	4
31	6	806 G→A	G269D	S5	3
32	6	817 C→T	L273F	S5	1
33	6	818 T→G	L273R*	S5	1
34	6	del 826–828	S276del	S5	1
35	6	830 C→T	S277L	S5	1
36	6	832 T→C	Y278H*	S5	1
37	6	868 G→A	E290K*	S5-PORE	1
38	6	875 G→A	G292D*	S5-PORE	1
39	6	877 C→T	R293C*	S4–S5	1
40	6	905 C→T	A302V*	PORE	1
41	6	910 T→C	W304R*	PORE	1
42	6	914 G→C	W305S	PORE	1
43	7	935 C→T	T312I	PORE	2
44	7	940 G→A	G314S	PORE	2
45	7	940 G→C	G314R*	PORE	1
46	7	941 G→A	G314D*	PORE	2
47	7	944 A→G	Y315C	PORE	1
48	7	946 G→A	G316R	PORE	1
49	7	964 A→G	T322A*	PORE-S6	1
50	7	del 1015–1017	F339del	S6	1
51	7	1022 C→T	A341V	S6	3
52	7	1027 C→T	P343S*	S6	1
53	7	1031 C→A	A344E*	S6	1
54	7	1031 C→T	A344V	S6	3
55	7	1032 G→A	SP/A344/G-A	S6	6
56	8	1034 G→A	G345E	S6	1
57	8	1046 C→G	S349W	S6	1
58	8	1058 T→C	L353P	S6	1
59	8	1085 A→G	K362R*	C-terminus	2
60	8	1096 C→T	R366W	C-terminus	3
61	8	1121 T→A	L374H*	C-terminus	1
62	8	del 1124–1127	L374/fs43*	C-terminus	1

Table 2 (continued)

No.	Exon	Nucleotide	Variant	Location	No. of patients
63	8	1128+1 G→T	Q376sp*	C-terminus	1
64	9	1140 G→T	R380S*	C-terminus	1
65	9	1166 C→A	S389Y*	C-terminus	1
66	9	ins C 1201–1202	P400fs/62*	C-terminus	1
67	10	ins A 1265–1266	K422fs/39*	C-terminus	1
68	10	ins C 1343–1344	P448fs/13*	C-terminus	1
69	10	1354 C→T	R452W*	C-terminus	1
70	12	1552 C→T	R518X	C-terminus	5
71	12	1571 T→G	V524G*	C-terminus	7
72	12	1576 A→G	K526E*	C-terminus	1
73	12	1588 C→T	Q530X	C-terminus	1
74	13	1608 C→A	Y536X*	C-terminus	1
75	13	1615 C→T	R539W	C-terminus	6
76	13	1637 C→T	S546L*	C-terminus	3
77	13	1663 C→T	R555C	C-terminus	1
78	13	1664 G→A	R555H	C-terminus	2
79	14	1697 C→A	S566Y*	C-terminus	1
80	14	1700 T→G	I567S*	C-terminus	1
81	14	1702 G→A	G568R*	C-terminus	1
82	15	1760 C→T	T587M	C-terminus	1
83	15	1768 G→A	A590T	C-terminus	1
84	15	1772 G→A	R591H	C-terminus	1
85	15	1781 G→A	R594Q	C-terminus	2
86	16	1855 T→A	L619M*	C-terminus	1
87	16	1876 G→A	G626S*	C-terminus	1
88	16	ins G 2025–2026	G675fs/17*	C-terminus	1

The number in the first column corresponds to the variant's location shown on the channel topology figure (Figure 2).

Deletion variants are indicated as del, splice site variants are designated by sp, and frameshift mutations are annotated for example as S95fs/141. Here, the last normal amino acid in this 676-amino-acid protein is the serine (S) at position 95 followed by 141 "scrambled" amino acids before premature truncation.

*A novel variant, unique to this cohort.

encoded calcium release channel.[18] Twenty-four patients had a personal or family history of syncope, aborted cardiac arrest, or sudden death postpartum, of which two thirds were LQT2 and one fourth were genotype negative. Finally, there were 24 unrelated patients who were documented to have syncope (N = 18) or aborted cardiac arrest (N = 6) associated with an auditory trigger, such as a door bell, alarm clock, or ringing phone. Here, 14 of 24 (58%) were LQT2, 13% LQT1, 0 LQT3, and 29% remained genotype negative. Five of the six patients with auditory-triggered aborted cardiac arrest had LQT2-associated mutations involving *KCNH2*.

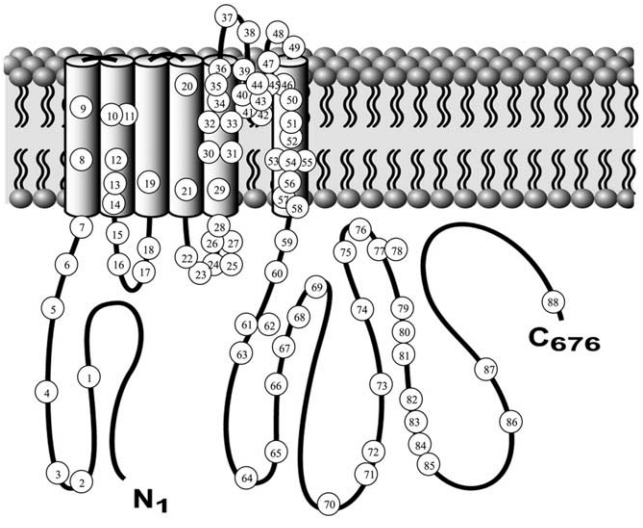

Figure 2 Channel topology of *KCNQ1* with LQT1-associated variants. The linear channel topology of the I_{Ks} α-subunit encoded by *KCNQ1* is shown, with the approximate location of the pathogenic LQT1-causing mutations indicated. The *number within the circle* corresponds to the case number in Table 2.

Overall, 211 putative LQTS-causing variants in *KCNQ1* (88; Table 2 and Figure 2), *KCNH2* (89; Table 3 and Figure 3), *SCN5A* (32; Table 4 and Figure 4), *KCNE1* (1), and *KCNE2* (1) were discovered in 272 unrelated patients (50%). More than half of the variants (125/211 [59%]) were novel to this cohort, including 52%, 66%, and 59% of the *KCNQ1*, *KCNH2,* and *SCN5A* variants, respectively. Consistent with the notion of family-specific LQTS-causing variants, only 45 of 211 variants (21%) were observed more than once in this cohort (Figure 5). The five most commonly observed LQTS-causing variants were L191fs/90-KCNQ1 seen in 7 unrelated patients, V524G-KCNQ1 in 7, SP/A344/G-A-KCNQ1 in 6, R539W-KCNQ1 in 6, and T613M-KCNH2 in 6 (Tables 2 and 3). The majority of the variants (155/211 [73%]) were missense mutations (Figure 6). Frameshift mutations composed 21% of the KCNH2 vari-

Table 3 Summary of *KCNH2* LQT2-associated variants

No.	Exon	Nucleotide	Variant	Location	No. of patients
1	2	77–78 GC→TT	S26I	N-terminus	1
2	2	87 C→A	F29L	N-terminus	1
3	2	92 T→G	I31S*	N-terminus	1
4	2	157 G→C	G53R	N-terminus	1
5	2	164 C→T	S55L*	N-terminus	1
6	2	193 A→C	T65P	N-terminus	1
7	2	209 A→G	H70R	N-terminus	1
8	2	del 221–251	R73fs/31	N-terminus	1
9	2	232 G→C	A78P	N-terminus	1
10	2	254 C→T	A85V*	N-terminus	1
11	2	299 G→A	R100Q*	N-terminus	1
12	3	del 395–456	V131fs/185*	N-terminus	1
13	3	ins CC 453–454	P151fs/14*	N-terminus	1
14	3	ins C 453–454	P151fs/179	N-terminus	2
15	4	ins GGTGCGGT 544–545	R181fs/20*	N-terminus	1
16	4	545 C→A	S182X*	N-terminus	1
17	4	685 G→T	E229X*	N-terminus	2
18	4	712 G→A	G238S*	N-terminus	1
19	4	916 G→T	G306W*	N-terminus	1
20	5	959 C→T	S320L*	N-terminus	1
21	5	del 981–991	R326fs/0*	N-terminus	1
22	5	982 C→T	R328C*	N-terminus	2
23	5	1096 C→T	R366X	N-terminus	1
24	5	1128 G→A	Q376sp	N-terminus	2
25	6	1259 A→G	Y420C*	S1	1
26	6	1262 C→T	T421M*	S1	1
27	6	1264 G→A	A422T*	S1	1
28	6	1280 A→C	Y427S*	S1–S2	1
29	6	1366 G→T	D456Y*	S2	1
30	6	del TAC 1423–1425	Y475del*	S2–S3	1
31	7	1600 C→T	R534C	S4	1
32	7	1655 T→C	L552S	S5	1
33	7	1681 G→A	A561T	S5	1
34	7	1682 C→T	A561V	S5	1
35	7	1685 A→C	H562P*	S5	1
36	7	1711 A→C	I571L*	S5	1
37	7	1714 G→A	G572S*	S5	2
38	7	1744 C→T	R582C	S5-PORE	2
39	7	1750 G→A	G584S	S5-PORE	1
40	7	1762 A→G	N588D	S5-PORE	1
41	7	1787 C→G	P596R*	S5-PORE	1
42	7	1810 G→A	G604S	S5-PORE	4
43	7	1838 C→T	T613M	PORE	6
44	7	1841 C→T	A614V	PORE	2
45	7	1864 C→T	L622F*	PORE	1
46	7	1868 C→T	T623I*	PORE	1
47	7	1882 G→A	G628S	PORE	2
48	7	1883 G→T	G628V*	PORE	1
49	7	1889 T→C	V630A	PORE	1
50	7	1898 A→G	N633S	PORE-S6	1
51	7	1904 A→T	N6351*	PORE-S6	1
52	7	1918 T→G	F640V*	S6	1
53	7	1922 C→T	S641F*	S6	1
54	8	del 2011–2025	671–675del*	S6-cNBD	1
55	8	2145 G→A	A715sp*	S6-cNBD	1
56	9	2162 C→T	P721L*	C-terminus	1
57	9	2320 G→T	D774Y*	C-terminus	1
58	9	2350 C→T	R784W	C-terminus	1
59	9	2364 G→C	E788D*	C-terminus	1
60	9	del C 2395	I798fs/10	C-terminus	1
61	9	2398+5 G→T	L799/sp*	C-terminus	3
62	9	2398+1 G→C	L799/sp	C-terminus	1

Table 3 (continued)

No.	Exon	Nucleotide	Variant	Location	No. of patients
63	10	2414 T→G	F805C	C-terminus	1
64	10	2458 G→A	G820R*	C-terminus	1
65	10	2464 G→A	V822M	C-terminus	1
66	10	2510 A→G	D837G*	C-terminus	1
67	10	2587 C→T	R863X*	C-terminus	1
68	10	2592+3 G→A	D864/sp*	C-terminus	1
69	11	2626 G→T	E876X*	C-terminus	1
70	11	2660 G→A	R887H*	C-terminus	1
71	12	del C 2705	Q901fs/71*	C-terminus	1
72	12	del 2728–2762	P910fs/16*	C-terminus	1
73	12	2738 C→T	A913V*	C-terminus	2
74	12	del G 2762	R920fs/51	C-terminus	1
75	12	del G 2766	R922fs/50*	C-terminus	1
76	12	2773 G→A	G925R*	C-terminus	1
77	12	ins G 2785–2786	G928fs/10*	C-terminus	1
78	12	2948 C→T	T983I*	C-terminus	1
79	13	2987 A→T	N996I*	C-terminus	1
80	13	del 3014	R1005fs/50*	C-terminus	1
81	13	3040 C→T	R1014X	C-terminus	1
82	13	ins CG 3098–3099	R1033fs/23*	C-terminus	1
83	13	del 3101–3108	R1033fs/81*	C-terminus	1
84	13	del 3103	P1034fs/21*	C-terminus	1
85	13	dup 3106–3112	V1038fs/80*	C-terminus	1
86	13	3107 G→A	G1036D*	C-terminus	1
87	14	3157 G→T	E1053X*	C-terminus	1
88	14	ins T 3168	L1056fs/61*	C-terminus	1
89	14	ins G 3173	S1057fs/60*	C-terminus	1

The number in the first column corresponds to the variant's location shown on the channel topology figure (Figure 3).
Deletion variants are indicated as del, splice site variants are designated by "sp", and frameshift mutations are designated by "fs".
*A novel variant, unique to this cohort.

ants. Only nine of the 211 variants involved splicing domains. For KCNQ1 variants, 51 of 88 (58%) localized to the transmembrane-spanning domains compared with 7 of 88 (8%) in the N-terminus and 30 of 88 (34%) residing in the C-terminus (Figure 2). In contrast, nearly two thirds of KCNH2 variants localized outside of the transmembrane-spanning domains to either the N-terminus (24/89 [27%]) or the C-terminus (34/89 [38%]; Figure 3). For the 32 variants in cardiac sodium channel encoded by *SCN5A,* 2 localized to the N-terminus, 9 to the transmembrane-spanning domains, 14 to the interdomain cytoplasmic linkers, and 7 to the C-terminus (Figure 4).

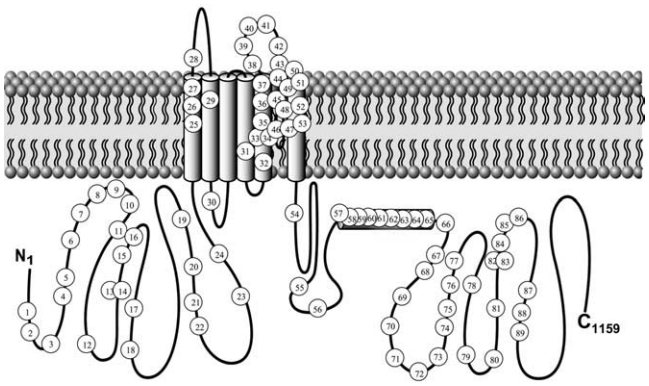

Figure 3 Channel topology of *KCNH2* with LQT2-associated variants. The linear channel topology of the I_{Kr} potassium channel encoded by *KCNH2* is shown, with the location of the pathogenic LQT2-causing variants indicated. The *number within the circle* corresponds to the case number in Table 3.

Among the 272 genotype positive patients, 243 patients had one identifiable mutation: LQT1 (120 patients), LQT2 (93), LQT3 (26), LQT5 (3, all D76N-KCNE1), and LQT6 (1, T10M-KCNE2). Twenty-nine patients (10.7% of genotype-positive patients and 5.4% overall) had two LQTS-causing variants: 9 with multiple *KCNQ1* variants, 7 with a *KCNQ1* and a *KCNH2* variant, 5 with a *KCNQ1* and a *SCN5A* variant, 2 with two *KCNH2* variants, 4 with a *KCNH2* and a *SCN5A* variant, and 2 with two *SCN5A* variants. Twenty-two of the 29 patients harboring multiple variants were white. None of the nine patients hosting double *KCNQ1* mutations displayed the phenotype of Jervell and Lange-Nielsen syndrome with clinical deafness, indicating that both mutations likely resided on the same allele.

QTc (506 ± 57 ms) trended greater among this subset of patients hosting multiple mutations compared with those hosting a single LQTS-causing variant (493 ± 50 ms) but did not achieve statistical significance. Patients with LQT2 had the longest QTc (507 ± 56 ms), followed by LQT3 (494 ± 61 ms) and LQT1 (483 ± 40 ms), but again without

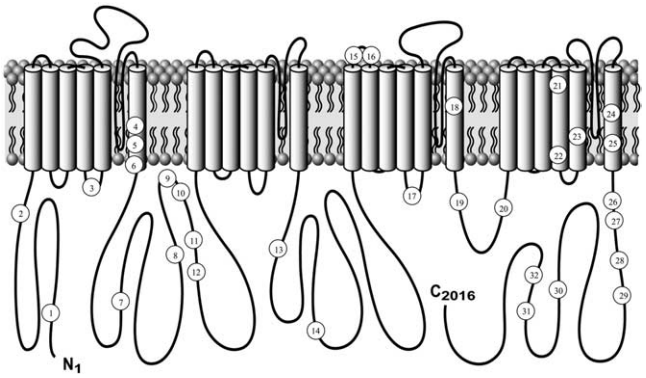

Figure 4 Channel topology of *SCN5A* with LQT3-associated variants. The linear channel topology of the NaV1.5 cardiac sodium channel encoded by *SCN5A* is shown, with the location of the pathogenic LQT3-causing variants indicated. The *number within the circle* corresponds to the case number in Table 4.

statistical significance (Table 1). QTc was significantly greater in patients with one or more identifiable mutations (genotype positive, 494 ± 51 ms) compared with the 269 patients who were genotype negative (470 ± 60 ms, $P <$ .0001; Table 1). In addition, the unrelated patients with more than one LQTS-causing variant were younger at diagnosis (15 ± 11 years) than either single-mutation individuals (24 ± 16 years) or genotype-negative individuals (25 ± 16 years, $P <$ .003; Table 1).

There was no statistical difference in the likelihood of a personal history of either syncope or aborted cardiac arrest or a positive family history between patients with and those without an identifiable LQTS-causing mutation (Table 1). Clinically, unrelated patients with LQT3 were less likely to have experienced syncope (31%) than patients with either LQT1 (50%) or multiple mutation status (57%, $P <$ .02; Table 1). For the cohort of unrelated patients within each LQTS genotype, we could not detect any phenotypic distinctions (i.e., degree of QT prolongation, age at diagnosis,

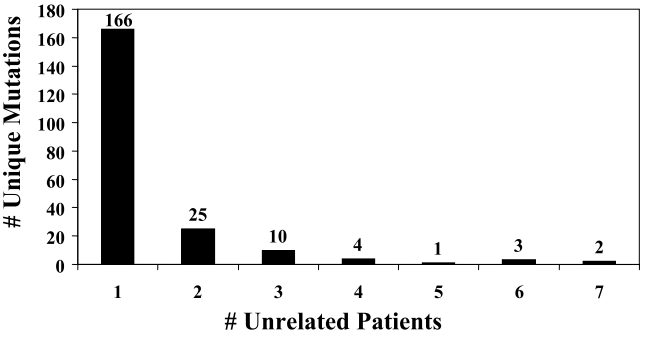

Figure 5 Long QT syndrome (LQTS)-associated variants as private family matters. Bar graph summarizes the distribution of specific mutations among unrelated patients. The y-axis depicts the number of distinct LQTS-associated variants. The x-axis represents the number of unrelated patients. For example, the *first column* indicates there were 166 unique variants, each represented only once. The *last column* indicates two different LQTS-associated variants were each seen in seven unrelated patients.

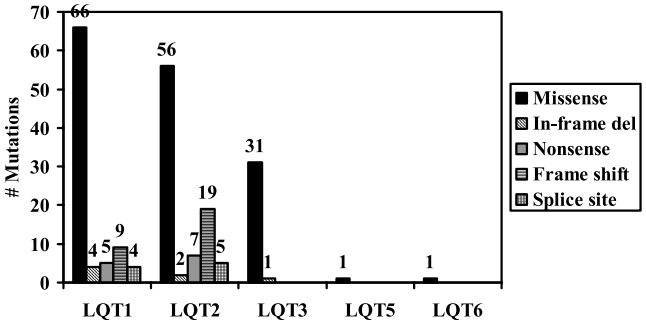

Figure 6 Summary of type of mutation for LQT1, LQT2, and LQT3. The distribution of mutation type (missense, frameshift, etc.) is summarized for the three most common genotypes.

presence of syncope or aborted cardiac arrest, degree of penetrance as estimated by positive family history) pursuant to either location (N-terminus, transmembrane, C-terminus) and/or type of mutation (missense, frameshift, splice site; data not shown).

Discussion

This study provides a compendium of LQTS-associated mutations derived from the largest series of consecutive, unrelated patients referred for LQTS genetic testing in a research environment. Previously, Splawski et al[7] performed mutational analysis of these five LQTS-causing channel genes in a cohort approximately half the size of the current study and identified putative LQTS-causing variants in 177 of 262 subjects (68%). The difference in overall yield (50% current study) can be accounted for by careful examination of the two cohorts, as the probability for a clinical diagnosis of LQTS was higher in the cohort reported by Splawski et al. The overall QTc was 492 ± 47 ms in their study compared with 482 ± 57 ms in this cohort, and LQTS-attributable symptoms were noted in 75% compared with 50% of this cohort. In fact, LQTS-causing mutations were detected in nearly three fourths of patients in our study having the highest clinical probability for the disease (i.e., Schwartz score ≥4; data not shown).

It will be interesting to glean the molecular underpinnings for the other half of the cohort that remains genotype negative. For instance, we demonstrated that one mimicker of LQTS, namely, CPVT, likely is dispersed among this cohort of patients referred explicitly for LQTS genetic testing.[18] To date, we have found nine patients in this cohort with a CPVT1-associated mutation involving the *RyR2*-encoded calcium release channel.[18] These patients all had experienced a personal or family history of a near-drowning or drowning and had a nondiagnostic QTc. We surmise that these patients were suspected of having concealed LQT1 based upon the previous association between LQT1 and swimming.[15,16]

As CPVT1 analysis thus far has been confined to the subset of 71 patients harboring a swimming phenotype, the

overall prevalence of CPVT1 in this entire cohort of patients referred specifically for LQTS genetic testing remains unknown. Already, CPVT1 with nine patients is twice as common in this referred LQTS cohort than both LQT5 and LQT6 combined at four patients. *KCNJ2* mutations responsible for type 1 Andersen-Tawil syndrome (ATS1)[28] have been identified in six patients (data not shown). However, all six had phenotypic features suggestive of ATS1. Thus, astute recognition of these various cardiac channelopathies, such as CPVT and ATS, which can masquerade in part as LQTS, will be essential to directing the patient's evaluation toward the proper molecular genetic test.

The prevalence of the first noncardiac channel genotype of LQTS (LQT4) pursuant to mutations in the *Ank2*-encoded ankyrin B was not investigated in this cohort but likely is rare. Mohler et al[29] reported that approximately 1% of a cohort of 664 patients selected for *Ank2* mutational analysis based on a suspected heritable arrhythmia syndrome had a putative disease-associated mutation, and a clinical phenotype of LQTS was seen in only three of the *Ank2*-positive probands. Finally, novel pathogenic mechanisms surely await discovery. In this cohort, approximately one fourth of subjects having compelling clinical evidence supporting the diagnosis of LQTS remain genotype negative following open reading frame/splice site mutational analysis of these five LQTS-associated channel genes. Currently, novel candidate gene exploration and expansion of pedigrees represented by the 34 genotype negative/phenotype positive unrelated cases is underway.

A host of genotype-phenotype correlations have been reported in LQTS.[10–20] Our particular interest in genotype-phenotype correlations has dealt with specific arrhythmogenic triggers. We previously reported from the first 388 unrelated patients composing this cohort a strong predilection for swimming-triggered events and LQT1 and events occurring in the postpartum period with LQT2 genotype.[17,18] Consistent with previous reports, exertionally mediated events predominate for LQT1, whereas events in the setting of rest, arousal, or auditory stimuli were more common in LQT2 and LQT3 (data not shown). However, nearly half (44%) of the patients referred for LQTS genetic testing were genotype negative despite documentation of syncope or aborted cardiac arrest during exertion. The genotype negative rate among those with a swimming, postpartum, or auditory phenotype was 25%, and 29% respectively. In our cohort, baseline QTc was slightly longer among patients with LQT2. Drawing from only eight individuals with LQT5 or LQT6, Splawski et al[7] suggested that QTc might be shorter than the other genotypes. Our cohort contained only three unrelated patients with LQT5 (all D76N-KCNE1) and one individual with LQT6, preventing any conclusions about the clinical phenotype, including QTc.

Unrelated individuals harboring multiple mutations presented at a younger age than those with a single mutation, findings consistent with a report by Westenskow et al[30] on 20 probands with "compound mutation status." The prevalence of compound or multiple mutation status was 5.4% for our cohort and 7.9% for the Keating cohort. However, of the 20 probands in the Keating study assigned as having multiple mutations, half possessed the D85N-KCNE1 common polymorphism as the "second hit." In our study, both variants had to be absent from more than 1,400 reference alleles to be considered a potential LQTS-associated variant. Thus far, in this cohort of *unrelated* individuals, we have been unable to detect a modifying effect of the common polymorphisms,[26,27] such as P448R-KCNQ1, K897T-HERG, H558R-SCN5A, G38S-KCNE1, or D85N-KCNE1, when comparing individuals with a single LQTS-associated variant and those with a single LQTS variant plus a polymorphism in terms of QTc, age at diagnosis, or presence of syncope (data not shown).

At variance with previous studies, we were unable to discern any particular phenotype associated with location of the mutation or type of mutation. Previously, Moss et al[31]demonstrated that, within families, patients with pore mutations in HERG (KCNH2) did more poorly than those with C-terminal mutations. Similar domain-specific phenotypes were reported in LQT1 family studies.[32] In contrast, among unrelated individuals rather than families, we were unable to discern any difference between individuals hosting missense mutations vs frameshift, nonsense, or splicing mutations (data not shown). In addition, the location of the mutation (N-terminus, transmembrane, pore, C-terminus) did not portend any phenotypic distinctions in terms of age at onset, symptoms, or degree of QT prolongation (data not shown). Again, the striking difference between our study and these previous observations is that our conclusions are drawn from a cohort of unrelated individuals seeking LQTS genetic testing rather than multigenerational family studies. In addition, our cohort of patients is mostly white, whereas the domain-specific phenotypes associated with KCNQ1 (LQT1) were drawn from families of Asian ethnicity.[32] It is possible that across families in general, and families of a given ethnicity in particular, mutation location and nature of mutation reveal distinguishing phenotypes. However, at the present time, clinical risk stratification for the next unrelated individual seen in the clinic should not be based upon the presence or absence of purported "malignant" mutations or "dangerous" domains.

After nearly a decade of LQTS genetic testing performed in research laboratories, elucidated variants continue to be predominantly novel, family-specific, missense mutations. This study adds 125 novel LQTS-associated variants to the web-based compendium of catalogued LQTS mutations that currently lists 328 published variants found in other cohorts, increasing the compendium by nearly 40%.[8] Comparing our latest 100 subjects to the first 100 subjects, the prevalence of novel variants continues to exceed 50%, suggesting that saturation of potential LQTS-associated variants has not yet been achieved (data not shown). The "hottest" mutations

Table 4 Summary of *SCN5A* LQT3-associated variants

No.	Exon	Nucleotide	Variant	Location	No. of patients
1	2	52 C→T	R18W*	N-terminus	1
2	3	373 G→C	V125L*	N-terminus	1
3	7	733 C→A	Q245K*	IS4–IS5	1
4	10	1211 T→A	L404Q*	IS6	1
5	10	1218 C→A	N406K*	IS6	1
6	10	1231 G→A	V411M	IS6	1
7	11	1384 G→A	E462K*	IS6–IIS1	1
8	12	1715 C→A	A572D	IS6–IIS1	3
9	12	1844 G→A	G615E	IS6–IIS1	1
10	12	1852 C→T	L618F	IS6–IIS1	2
11	13	1910 C→T	P637L*	IS6–IIS1	1
12	13	1943 C→T	P648L*	IS6–IIS1	1
13	17	2911 C→T	R971C*	IIS6–IIIS1	1
14	17	3206 C→T	T1069M*	IIS6–IIIS1	1
15	21	3673 G→A	E1225K*	IIS1–IIIS2	1
16	21	3691 G→A	E1231K	IIIS1–IIIS2	1
17	23	3974 A→G	N1325S	IIIS4–IIIS5	2
18	25	4373 C→A	S1458Y*	IIIS6	1
19	26	4442 G→A	G1481E*	IIIS6–IVS1	1
20	26	del 4511–4519	KPQ1505–1507del	IIIS6–IVS1	1
21	28	4868 G→T	R1623L	IVS4	1
22	28	4931 G→A	R1644H	IVS4	2
23	28	4999 G→A	V1667I*	IVS5	1
24	28	5287 G→A	V1763M*	IVS6	1
25	28	5296 A→C	M1766L	IVS6	1
26	28	5328 G→A	V1777M	C-terminus	1
27	28	5336 C→T	T1779M*	C-terminus	1
28	28	5350 G→A	E1784K	C-terminus	4
29	28	5384 A→G	Y1795C	C-terminus	1
30	28	5726 A→G	Q1909R*	C-terminus	1
31	28	5845 G→T	A1949S*	C-terminus	1
32	28	5873 G→A	R1958Q*	C-terminus	1

The number in the first column corresponds to the variant's location shown on the channel topology figure (Figure 4). Deletion variants are indicated as "del".

*A novel variant, unique to this cohort.

in our study were the L191fs/90- and V524G-KCNQ1 variants, each found in seven apparently unrelated individuals. However, formal genetic testing to eliminate the possibility of distant relatedness has not been performed. Excepting KCNH2, which hosts a significant minority of frameshift and nonsense variants, the vast majority of LQTS variants continue to be nonsynonymous single nucleotide substitutions or missense mutations. Consequently, comprehensive surveillance of the entire protein-encoding exons of the LQTS-associated genes appears to be the only suitable method of detection for the foreseeable future.

Conclusion

This comprehensive open reading frame analysis of the five cardiac channel genes implicated in LQTS revealed a disease-associated mutation in half of the patients composing this largest published cohort of consecutive, unrelated patients who were referred to a research laboratory for LQTS genetic testing. This yield was significantly greater (72%) among the subset with the highest clinical probability of LQTS. The majority of mutations continue to represent novel singletons. The novel mutations identified herein expand the compendium of LQTS-causing mutations by nearly 40%. Future studies involving expansion of the pedigrees represented by these genotype positive unrelated cases will help further define the variability of expression and extent of incomplete penetrance seen for this intriguing channelopathy. Hopefully, solving the phenotype positive/genotype negative mysteries will shed new insight into novel pathogenetic mechanisms underlying the rest of LQTS.

Acknowledgments

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ing the availability of a commercial molecular diagnostic test for LQTS. We are particularly indebted to the index cases and family members for their participation and patience in this study and trust that we have helped not only to advance the science but to heal the sick as well. We trust that families afflicted by LQTS will now become the true beneficiaries of the scientific discoveries of the past decade.

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