Original Article

Loss-of-Function *KCNE2* Variants

True Monogenic Culprits of Long-QT Syndrome or Proarrhythmic Variants Requiring Secondary Provocation?

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Background—Insight into type 6 long-QT syndrome (LQT6), stemming from mutations in the *KCNE2*-encoded voltage-gated channel β-subunit, is limited. We sought to further characterize its clinical phenotype.

Methods and Results—Individuals with reported pathogenic *KCNE2* mutations identified during arrhythmia evaluation were collected from inherited arrhythmia clinics and the Rochester long-QT syndrome (LQTS) registry. Previously reported LQT6 cases were identified through a search of the MEDLINE database. Clinical features were assessed, while reported *KCNE2* mutations were evaluated for genotype–phenotype segregation and classified according to the contemporary American College of Medical Genetics guidelines. Twenty-seven probands possessed reported pathogenic *KCNE2* mutations, while a MEDLINE search identified 17 additional LQT6 cases providing clinical and genetic data. Sixteen probands had normal resting QTc values and only developed QT prolongation and malignant arrhythmias after exposure to QT-prolonging stressors, 10 had other LQTS pathogenic mutations, and 10 did not have an LQTS phenotype. Although the remaining 8 subjects had an LQTS phenotype, evidence suggested that the *KCNE2* variant was not the underlying culprit. The collective frequency of *KCNE2* variants implicated in LQT6 in the Exome Aggregation Consortium database was 1.4%, in comparison with a 0.0005% estimated clinical prevalence for LQT6.

Conclusions—On the basis of clinical phenotype, the high allelic frequencies of LQT6 mutations in the Exome Aggregation Consortium database, and absence of previous documentation of genotype–phenotype segregation, our findings suggest that many *KCNE2* variants, and perhaps all, have been erroneously designated as LQTS-causative mutations. Instead, *KCNE2* variants may confer proarrhythmic susceptibility when provoked by additional environmental/acquired or genetic factors, or both. **(*Circ Arrhythm Electrophysiol*. 2017;10:e005282. DOI: 10.1161/CIRCEP.117.005282.)**

Key Words: exome ■ genetics ■ long QT syndrome ■ mutation ■ prevalence

Long-QT syndrome (LQTS) is an inherited arrhythmia syndrome associated with an increased risk of sudden cardiac death secondary to torsades de pointes (TdP).[1] To date, 16 different LQTS-susceptibility genes have been identified, and many of the genetic subtypes possess unique phenotypic features.[2] Type 6 long-QT syndrome (LQT6), estimated to have an overall prevalence of ≈0.0005%, has been attributed to mutations within *KCNE2,* a gene that encodes an accessory or β-subunit that modulates the activity of multiple different voltage-gated ion channels (Figure 1).[3] Contrasting with its low prevalence, many *KCNE2* variants implicated in LQT6 have higher than anticipated frequencies within population-based exome cohorts.[4]

It is also notable that reported LQT6 cases have almost invariably had normal baseline QT intervals and only experienced arrhythmic events in the setting of an additional QT-prolonging insult.[5–7] In contrast with the Mendelian inheritance patterns often observed with canonical forms of LQTS, familial genotype–phenotype segregation has never been previously documented for a *KCNE2* mutation and an LQTS phenotype, potentially reflecting a need for secondary provocation. Consistent with this theme, in the seminal

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WHAT IS KNOWN

- Mutations within the *KCNE2*-encoded voltage-gated channel β-subunit have been implicated as causative for LQT6 (type 6 long-QT syndrome); however, insight into the clinical phenotype is limited.
- Initial reports suggested that *KCNE2* rare variants required additional stressors to manifest a clinical phenotype; however, current medical literature includes LQT6 as a canonical form of LQTS (long-QT syndrome).

WHAT THE STUDY ADDS

- Loss-of-function *KCNE2* rare variants do not seem sufficient in isolation to cause LQTS, but may confer proarrhythmic susceptibility when provoked by additional environmental or genetic factors.
- Clinical management of individuals with loss-of-function *KCNE2* variants and normal phenotypes should focus primarily on the avoidance of secondary stressors associated with QT prolongation.

study implicating *KCNE2* in LQTS, the authors concluded: "A mechanism for acquired arrhythmia is revealed: genetically based reduction in potassium currents that remains clinically silent until combined with additional stressors".[5] Although highlighted in initial reports, the notion that *KCNE2* is an arrhythmia-susceptibility, rather than disease-causing gene seems largely unappreciated, as highlighted by contemporary reviews and textbooks listing *KCNE2* as a cause of canonical LQTS.[8,9] Such misclassification, assuming that initial reports were accurate, has the potential to lead to widespread inappropriate clinical care, particularly given the emergence of large-scale exome sequencing initiatives within healthcare systems.[10] We sought to further clarify the alleged LQT6 phenotype through a novel case series composed of individuals identified to possess reported pathogenic *KCNE2* mutations during clinical evaluation and a systematic review of previous literature reported cases.

Methods

Novel Case Series

Inherited arrhythmia clinics and the Rochester LQTS registry provided details on individuals evaluated for arrhythmic disorders possessing possible or presumed pathogenic *KCNE2* mutations.[11] The following variables were recorded for each case: age at presentation, sex, Bazett-corrected QT interval (QTc), family history of LQTS or sudden cardiac death, history of a cardiac event and any potential secondary stressors, panel of genes screened, the presumed *KCNE2* culprit mutation, and presence of other potentially pathogenic mutations relevant to cardiac arrhythmias. Whenever possible, evidence for genotype–phenotype segregation was sought. When available, treadmill testing (QTc supine, on standing, at peak exertion, and at 4 minutes into recovery) and cardiac imaging data were collected. Study participants were also assessed for sinus node dysfunction and other arrhythmic features (details provided in the Data Supplement). The study was performed as part of a protocol approved by the research ethics boards of Western University, London, Ontario, Canada, and the collaborating institutions. Study participants provided informed consent.

Systematic Literature Review of Reported Cases

The MEDLINE electronic database was searched for articles in the English language published before November 2016 using the medical subject headings KCNE2 and long QT. Identified articles were reviewed for reported cases of LQTS attributed to *KCNE2* mutations. Published cases were eligible for inclusion if the clinical context of the diagnosis and cardiac event were provided. Cases reported as part of genetic compendiums of LQTS in the absence of clinical details were excluded.

Evaluation of Reported Pathogenic *KCNE2* Mutations

All *KCNE2* mutations implicated in LQT6, including those reported in ClinVar (a public archive of genetic variants and associated diseases),[12] were subjected to in silico analyses and variant classification according to current American College of Medical Genetics guidelines.[13] Their prevalence within the general population was assessed using the Exome Aggregation Consortium (ExAC), a database comprised 60 706 nonrelated individuals derived from multiple population-based and disease-specific genetic cohort studies.[4] In silico prediction was performed using Polymorphism Phenotyping v2,[14] Sorting Intolerant From Tolerant,[15] and Mutation Taster.[16] The literature was also reviewed for previous in vitro functional analyses.

Results

Phenotype of Subjects With Reported Pathogenic *KCNE2* Mutations

Among 15 inherited arrhythmia clinics and the Rochester LQTS Registry, we identified 48 individuals from 28 families possessing a rare *KCNE2* variant classified as likely pathogenic or pathogenic. All probands were at minimum screened for mutations in *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* (Table I in the Data Supplement). Thirty-two of the 48 study participants had undergone cardiac imaging, and, aside from 2 individuals, all those possessing *KCNE2* variants had normal biventricular size and function (Table II in the Data Supplement). Importantly, in no instance did the variant segregate with a phenotype consistent with LQTS (Table 1). The probands from 7 families (Table 1; Figure 2) had a normal resting QTc and another predisposing factor for QT prolongation at the time of their cardiac event or diagnosis.

Among the 13 probands with a typical LQTS phenotype, 9 possessed a pathogenic mutation in another *LQTS* gene (Table 1; Figure 2). In family 8, the proband that suffered a cardiac arrest had a prolonged QTc; however, genetic testing was negative. Subsequent screening of family members identified a daughter with a QTc of 464 ms who had a KCNE2-Asn6Ser variant that was absent from the cardiac arrest victim. The remaining probands with an LQTS phenotype were from Families 26 (Thr10Met), 27 (Ile57Thr), and 28 (Glu94Gly). Notably, all of the first-degree family members that had these *KCNE2* variants exhibited a normal QT phenotype (Table 1).

Among the remaining 8 probands (Table 1; Figure 2) possessing a *KCNE2* mutation, none had an LQTS phenotype on baseline ECG. Three of these 8 probands were evaluated with treadmill testing, and all exhibited normal QT behavior on standing from lying (Table III in the Data Supplement). The proband in family 25 exhibited mild QT prolongation during peak exercise and at 4 minutes in recovery (QTc values=479 and 476, respectively), although this was in the presence of right bundle branch block. His 3 children possessing

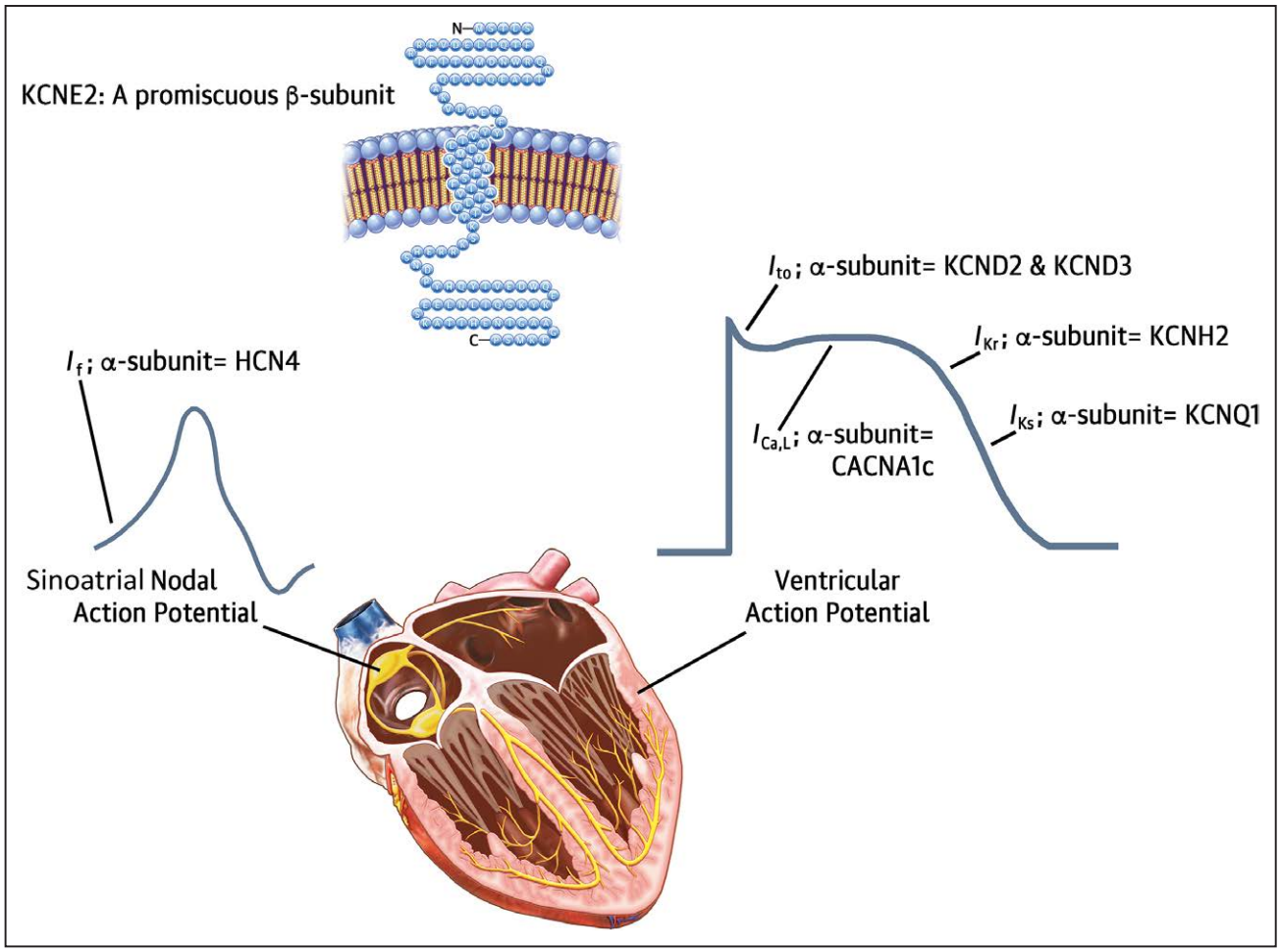

Figure 1. KCNE2 is a β-subunit for multiple cardiac ion currents.

the KCNE2-Leu101Ter variant had normal QT behavior during treadmill testing and normal QTc values on serial surface ECGs (Table 1; Table III in the Data Supplement). The proband in family 21 had QTc values ranging from 430 to 465 ms and bidirectional ventricular tachycardia during exercise, while the proband in family 23 (Ser74Tyr) had recurrent polymorphic ventricular tachycardia triggered by short-coupled premature ventricular beats refractory to β-blockade, but responsive to quinidine. In family 24, the cause of aborted cardiac arrest in the proband has been attributed to arrhythmic mitral valve prolapse syndrome.[17] The remaining 4 probands (Families 18, 19, 20, and 22) had no arrhythmic phenotype, and all had their LQTS diagnoses removed by a specialized inherited arrhythmia clinic (Table 1).

None of the cases were reported to have clinically significant sinus node dysfunction or intolerance to β-blockade secondary to bradycardia. A single patient had transient complete atrioventricular block during cooling and ventricular preexcitation (Proband, family 5). Profound bradycardia complicated by TdP was observed in 3 probands (Families 5, 6, and 26) during systemic hypothermia initiated for neuroprotection after cardiac arrest.

Among the 28 reported families, excluding the 9 that possessed a pathogenic mutation in another *LQTS* gene, identification of a rare *KCNE2* variant led to a diagnosis of LQT6 and initiation of β-blocker therapy by the initial treating team in 15 of the remaining 19 families. Cascade screening was performed in 8 of these 15 families, and 14 first-degree family members with normal phenotypes found to possess the *KCNE2* variant were labeled with LQT6 and treated with a β-blocker. In family 14, the *KCNE2* Met54Thr variant, and not the *KCNH2* Arg148Trp mutation, was used for cascade screening of family members.

Literature Reported Cases

Among 36 index cases of LQTS attributed to *KCNE2* mutations identified through the systematic literature review, 17 met prespecified inclusion criteria (Table 2). One individual was a compound heterozygote (case 11—Ile20Asn and Arg27His),[20] while the Thr8Ala, Met54Thr, and Ile57Thr mutations were each present in 3 cases.[5–7,19,23] None of the reports provided evidence for familial genotype–phenotype segregation.

A secondary precipitant or additional genetic variant contributing to QT prolongation was documented in 10 cases (59%; Table 2). Eight had normal baseline QT intervals and only developed QT prolongation or TdP with a QT-prolonging medication (Table 2). Of the 2 remaining, a baseline QT interval was not provided for case 8 (Arg77Trp), who developed TdP during complete atrioventricular block.[18] Case 10 (Phe60Ala) suffered an aborted arrest at 1 month of age, however, also

Table 1. Index Cases and Families Identified to Possess Putative Pathogenic *KCNE2* Mutations Among 15 Surveyed Inherited Arrhythmia Clinics and the Rochester Long-QT Syndrome Registry

Family	KCNE2 Mutation	Familial Status	Age*	Sex	QTc Range, ms; ECG#	FHx	Cardiac Event	Secondary Precipitant or Predisposition	Additional Features
LQTS phenotype secondary to QT-prolonging stressor									
1	Thr8Ala	Proband	40	F	410–460; 9	Negative	ACA	QTc=500 with severe hypo-K, -Mg, and -Ca	…
		Asymp first DFM	25	F	390–460; 12	ACA	…	…	…
2	Thr8Ala	Proband	60	M	434–493; 6	SCD	TdP	KCNQ1-Thr224Met	…
								SCN5A-Ala572Asp	
								QTc=800 on Amiodarone	
3	Thr8Ala	Proband	73	F	456; 1	Negative	Syncope	Hydroxyzine	…
								Fluoxetine	
4	Leu11fsX46	Proband	45	M	422; 1	Negative	Presyncope	QTc=500 ms with severe hypokalemia	…
5	Ile57Thr	Proband	33	F	458–515; 4	Negative	ACA	Third-degree AV block	Third-degree AV block and TdP during cooling
								Preexcitation	
6	Ile57Thr	Proband	58	F	420–460; 3	Negative	ACA	QTc=510 ms with desipramine	TdP during cooling
		Asymp first DFM	57	F	426–450; 4	ACA	…	…	…
7	Met121Lys	Proband	43	F	390–441; 2	Negative	Asymp	QTc=518 with antibiotic	…
		Asymp first DFM	9	M	440–450; 2	Negative	Asymp	…	…
LQTS phenotype but proband does not have the variant									
8	Asn6Ser	Proband	56	F	445–477; 7	Negative	ACA	Proband does not have the variant	…
		Asymp first DFM	38	F	464–485; 3	ACA	…	…	…
		Asymp first DFM	19	M	417–432; 2	ACA	…	…	…
LQTS phenotype and pathogenic mutation in another *LQTS* gene									
9	c.-13+5 G>A (IVS1+5 G>A)	Proband	10	F	440–470; 4	LQTS/SCD	Syncope	KCNH2-Phe805Cys	*KCNE2* variant does not segregate with LQTS phenotype
		Asymp first DFM	6	1	420–430; 2	LQTS/SCD	Asymp	*KCNH2* mutation absent	
10	Thr8Ala	Proband	14	F	495–545; 6	Negative	Syncope	KCNH2-Asp501Asn	…
11	Thr8Ala	Proband	24	F	464–472; 2	Negative	None	KCNH2-Arg752Trp	Incidental finding of long QT
								KCNQ1-Thr153Met	
12	Thr10Met	Proband	21	M	415–448; 4	Negative	None	KCNQ1-Arg594Gln	Long QT observed on screening for sports participation
13	Arg27Ser	Proband	50	F	503–537; 3	Negative	None	KCNQ1-Gly168Arg	…
								SCN5A-Arg1897Trp	
14	Met54Thr	Proband	14	F	†	Negative	SCD	Not tested for *KCNH2* mutation	SCD during sleep and gastroenteritis
		First DFM (M)	49	F	490–520; 3	SCD	Asymp	KCNH2-Arg148Trp	Does not carry KCNE2-Met54Thr
		First DFM (F)	51	M	460–476; 4	SCD	Idiopathic DCM	Bifascicular block; QRS duration=178 ms	…

(*Continued*)

Table 1. Continued

Family	KCNE2 Mutation	Familial Status	Age*	Sex	QTc Range, ms; ECG#	FHx	Cardiac Event	Secondary Precipitant or Predisposition	Additional Features
15	Ile57Thr	Proband	8	F	480–497; 3	Negative	None	*KCNH2* IVS5-1 G>A	Incidental finding of long QT
		Asymp first DFM	41	F	452–471; 3	Negative	…	*KCNH2* IVS5-1 G>A	…
16	Ile57Thr	Proband	41	F	484; 1	Negative	Syncope	KCNH2-Ala422Asp	…
17	Pro123fsTer16	Proband	22	F	…	Negative	SCD	KCNH2 Gln376=	SCD during sleep and auditory stimulus
		Asymp first DFM	52	M	410–440; 3	SCD/LQTS	Asymp	…	*KCNE2* variant does not segregate with LQTS phenotype
		Asymp first DFM	28	M	422–446; 3	SCD/LQTS	Asymp	…	
		Asymp first DFM	11	F	352–376; 3	SCD/LQTS	Asymp	…	
Non-LQTS phenotype									
18	Thr8Ala	Proband	9	F	430; 1	Negative	Possible syncope	…	LQTS diagnosis removed after evaluation
19	Thr8Ala	Proband	49	F	418–452; 2	Negative	None	…	Abnormal ECG identified on employee physical
20	Thr8Ala	Proband	51	F	415; 1	SCD	None	…	LQTS diagnosis removed after evaluation
21	Arg16Gln Ter124SerextTer1	Proband	24	F	430–465;4	Negative	None	None identified	Frequent PVCs and bidirectional VT
22	Met54Thr	Proband	15	F	420; 1	Negative	None	…	LQTS diagnosis removed after evaluation
23	Ser74Tyr	Proband	61	F	440–457; 7	Negative	Syncope	None identified	SC PVC induced PVT
		Asymp first DFM	88	F	436–450; 3	Negative	…	…	…
		Asymp first DFM	29	M	413; 1	Negative	…	…	…
24	Arg77Trp	Proband	47	F	407–437; 2	SCD	ACA	Levofloxacin Hypokalemia	Primary diagnosis of arrhythmic MVPS
25	Leu101Ter	Proband	62	M	420–450; 9	Negative	ACA	None	…
		Asymp first DFM	22	M	360–415; 6	ACA	…	…	…
		Asymp first DFM	18	F	380–400; 8	ACA	…	…	…
		Asymp first DFM	18	F	350–420; 8	ACA	…	…	…
LQTS phenotype in absence of another predisposing factor									
26	Thr10Met	Proband	72	M	480–495; 2	Negative	ACA	None identified	TdP during cooling
		Asymp first DFM	43	M	405; 1	ACA	…	…	…
27	Ile57Thr	Proband	39	F	462–470; 2	Negative	Palpitations	None identified	…
		Asymp first DFM	67	M	397; 1	Negative	…	…	…
		Asymp first DFM	36	F	417–422; 2	Negative	…	…	…
28	Glu94Gly	Proband	38	M	500; 1	SCD	SCD	None identified	…
		Asymp first DFM	10	F	417–437; 5	SCD	…	…	…
		Asymp first DFM	4	M	356–412; 5	SCD	…	…	…

# indicates number of surface ECGs performed in the absence of QT prolonging stressors; ACA, aborted cardiac arrest; Asymp first DFM, asymptomatic first-degree family member; AV, atrioventricular; F, female; FHx, family history; LQTS, long-QT syndrome; M, male; MVPS, mitral valve prolapse syndrome; PVC, premature ventricular contraction; PVT, polymorphic VT; SC, short coupled; SCD, sudden cardiac death; TdP, torsades de pointes; and VT, ventricular tachycardia.

*Age at first presentation.

†Premortem ECG unavailable.

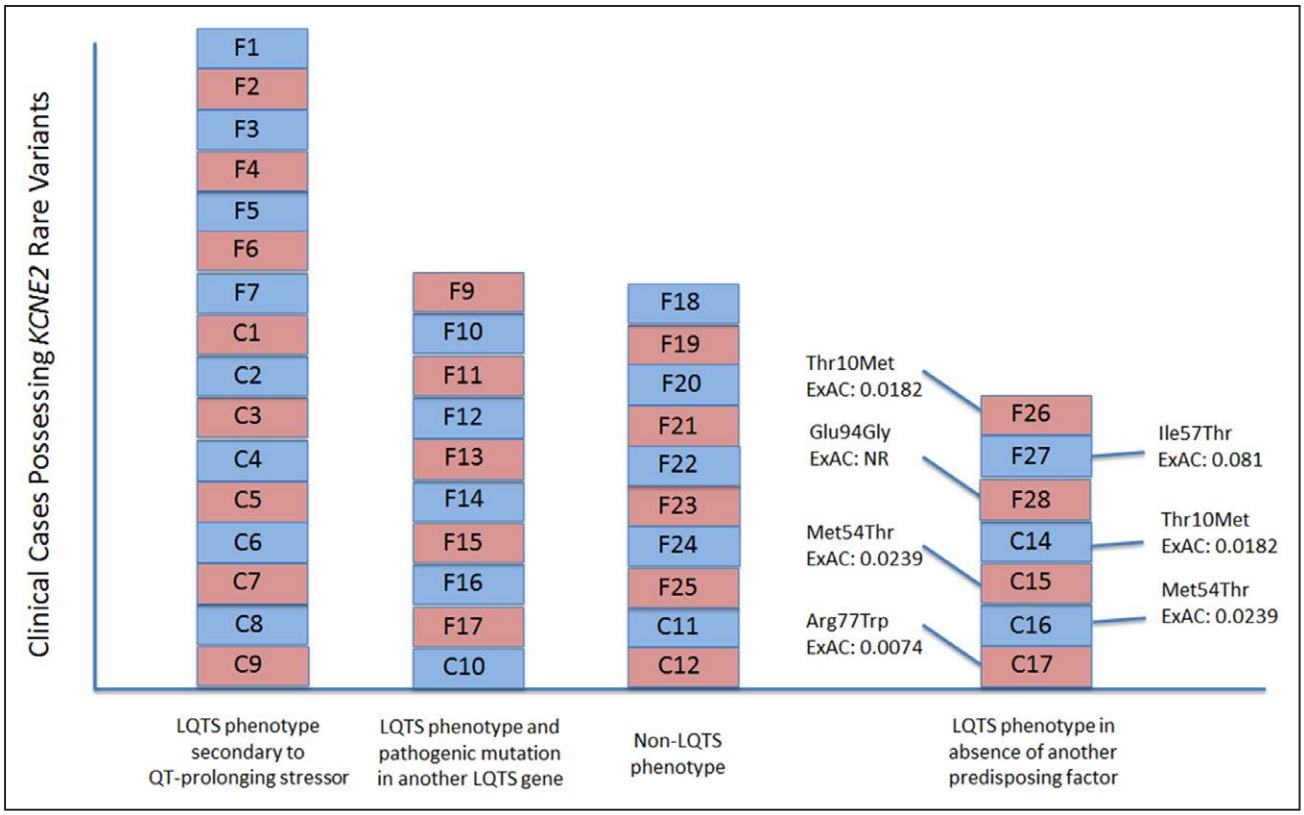

Figure 2. Spectrum of clinical phenotypes observed among individuals with rare *KCNE2* variants from a novel case series and previous literature reported cases. Family 7 (proband did not carry the variant) and case 13 (LQTS phenotype did not segregate with *KCNE2* variant) are not included. C indicates literature reported case number; ExAC, Exome Aggregation Consortium allele frequency (%); F, family number from novel case series; and LQTS, long-QT syndrome.

possessed the well-established LQT3-causative mutation, SCN5A-Arg1623Gln (Table 2).[19,24] Case 14 (Thr10Met) suffered an aborted cardiac arrest in the context of hypokalemia and hypocalcemia, although had QTc prolongation at baseline (505 ms).[22] It is conceivable that her baseline QT interval prolongation was secondary to the Thr10Met mutation in isolation; however, 3 Thr10Met-positive family members had normal QT intervals, and patch-clamp analysis revealed only mild changes in I_{Kr} when KCNE2-Thr10Met was coexpressed with wild-type KCNH2.[22]

Of the 6 cases without a secondary precipitant (Table 2), case 11, a 19-day-old infant that passed away secondary to recurrent episodes of ventricular fibrillation, was a compound heterozygote for Ile20Asn and Arg27His.[20] Although the QTc was 465 ms, her phenotype of recurrent ventricular fibrillation is atypical for LQTS (TdP not reported), although could have been a manifestation of her *KCNE2* mutations. Case 12 had no evidence of QT prolongation (QTc=385 ms) and was diagnosed with LQT6 after identification of a KCNE2-Ile57Thr mutation as part of cascade screening for a family history of sudden cardiac death.[19] Case 13 was diagnosed with LQT6 in the context of syncope, a borderline QT interval (QTc=460 ms), and a novel KCNE2-Val65Met mutation. However, subsequent cascade screening revealed that this novel variant did not segregate with the QT prolongation observed in other family members.[21]

The remaining 3 cases had phenotypes consistent with LQTS. A KCNE2-Met54Thr mutation was identified as the culprit in case 15 after an aborted arrest and a treadmill test that revealed QTc values ranging from 390 to 500 ms.[5] Case 16 (Met54Thr) was a 55-year-old asymptomatic male with a QTc =476 ms,[23] while case 17 (Arg77Trp) was a 67-year-old male with syncope and a QTc =514 ms.[19]

In summary, of 44 probands possessing *KCNE2* rare variants identified and reviewed in our study, only 7 (18%), including 4 of 17 (24%) from the literature (Table 2; Figure 2) and 3 of 27 (11%) in our novel case series (Table 1; Figure 2), had LQTS phenotypes in the absence of other genetic mutations or precipitating factors associated with QT prolongation. However, even in each of these cases, clinical and genetic findings suggested that the *KCNE2* variant was not the underlying culprit.

Evaluation of *KCNE2* Rare Variants

Population Allele Frequencies

Among the 26 *KCNE2* mutations reported as presumed or possibly pathogenic, 15 were in ExAC (Table 3; Figure 3). The allele frequencies for the 3 *KCNE2* rare variants most often identified as culprits for LQT6, Thr8Ala (0.3804%), Met54Thr (0.0239%), and Ile57Thr (0.0881%) are much higher than anticipated for mutations causative for a rare autosomal dominant disorder. Allelic frequencies of the remaining *KCNE2* mutations linked to LQT6 are provided in Table 3. Current estimates suggest that the LQTS prevalence is ≈0.05%, while LQT6 is anticipated to account for 1% of LQTS, corresponding

Table 2. Literature Documented Index Cases of Alleged Long-QT Syndrome Type 6 or Drug-Induced LQTS Reported to Possess Culprit Pathogenic *KCNE2* Mutations

LQT6 Case (Reference)	KCNE2 Mutation	Age at Diagnosis, y	Sex	Baseline; QTc, ms	FHx	Cardiac Event	Secondary Precipitant/Other Genetic Variant
LQTS phenotype secondary to QT-prolonging stressor							
1 [5]	Thr8Ala	NR	NR	Normal	N	TdP	QTc=500 ms on quinidine
2 [7]	Thr8Ala	12	M	420	N	TdP	Amiodarone
3 [6]	Thr8Ala	45	M	Normal	N	Asymptomatic	QTc >600 ms on TMP/SMX
4 [5]	Gln9Glu	76	F	460	N	ACA	QTc=540 ms in setting of clarithromycin and K=2.8 mmol/L
5 [6]	Met54Thr	Normal	NR	Normal	N	Asymptomatic	QTc >600 ms on procainamide
6 [5]	Ile57Thr	NR	NR	Normal	N	TdP	QTc=500 ms on quinidine
7 [6]	Ile57Thr	NR	NR	Normal	N	Asymptomatic	QTc >600 ms on oxatomide
8 [18]	Arg77Trp	NR	NR	NR	NR	TdP	Complete atrioventricular block
9 [6]	Ala116Val	55	F	Normal	N	Asymptomatic	QTc >600 ms on quinidine
LQTS phenotype and pathogenic mutation in another *LQTS* gene							
10 [19]	Phe60Ala	1 month	M	460	NR	ACA	SCN5A-Arg1623Gln
Non-LQTS phenotype							
11 [20]	Ile20Asn, Arg27His	19 days	F	465	N	Recurrent VF	None identified
12 [19]	Ile57Thr	NR	F	385	SCD	Asymptomatic	Not applicable
KCNE2 variant did not segregate with LQTS phenotype							
13 [21]	Val65Met	17	F	480	LQTS	Syncope	Variant did not segregate with familial LQTS phenotype
LQTS phenotype in absence of another predisposing factor							
14 [22]	Thr10Met	24	F	505	N	ACA	QTc=530 ms in setting of hypokalemia and hypocalcemia
15 [5]	Met54Thr	38	F	Normal	N	ACA	None; atypical QT behavior on exercise
16 [23]	Met54Thr	55	M	476	SCD	Sinus bradycardia	None
17 [19]	Arg77Trp	67	M	514	NR	Syncope	Bradycardia

ACA indicates aborted cardiac arrest; F, female; FHx, family history; LQTS, long-QT syndrome; M, male; N, nil; NR, not reported; TdP, torsades de pointes; TMP/SMX, trimethoprim/sulfamethoxazole; and VF, ventricular fibrillation.

to a prevalence of 0.0005%.[26,30,31] Overall, 859 individuals within ExAC were positive for a *KCNE2* variant previously implicated in LQT6 corresponding to an overall prevalence of 1.4%. This is 2800× the anticipated prevalence of LQT6.

Physicochemical and In Vitro Biophysical Analysis

In silico analysis of *KCNE2* mutations implicated in LQT6 was performed using Polymorphism Phenotyping v2, Sorting Intolerant From Tolerant, and Mutation Taster (Table 3). Mutation Taster identified each variant as disease causing except Gln9Glu, Val14Ile, Arg16Gln, Met121Lys, and Ter124Serext-Ter1. Comparable results were observed when missense mutations were analyzed with Polymorphism Phenotyping v2 (14 of 21 labeled as probably damaging) and Sorting Intolerant From Tolerant (14 of 21 labeled as damaging). Classification of mutations using the American College of Medical Genetics guidelines identified 18 as uncertain significance, 5 as likely pathogenic, and 3 as likely benign.

In vitro biophysical analysis using patch clamping with heterologously expressed channels had been performed on 9 of 26 *KCNE2* rare variants (Table 3). For 7 of the 9 variants, experimental findings were consistent with a loss-of-function, whereas functional work suggested that KCNE2-Arg27Cys resulted in a gain-of-function in I_{Ks},[29] and no electrophysiological changes in I_{Kr} were observed for Arg77Trp.[18] Among the 7 loss-of-function mutations, the in vitro studies demonstrated a negligible reduction in potassium currents, in contrast to the severe and complete loss of function often observed with *KCNQ1* and *KCNH2* mutations implicated in LQT1 and LQT2, respectively.[5,6,18,21,22,32,33]

Discussion

Our investigation into the association between *KCNE2* and LQTS has revealed that, when a *KCNE2* variant was felt to be the primary culprit, the arrhythmic phenotype only manifested with a secondary stressor. We postulate that many *KCNE2* variants,

Table 3. Evaluation of Reported Pathogenic *KCNE2* Mutations Implicated in Type 6 Long-QT Syndrome

KCNE2 Mutation	Source (Reference)	ExAC; AF (%)	Channel Location	In Silico Analysis			Functional Work (Reference)	ACMG Classification
				PolyPhen-2	SIFT	Mut Taster		
c.-13+5 G>A	CR, ClinVar [25]	…	…	…	…	Disease causing	…	Uncertain significance
Asn6Ser	CR	0.0017	Extracellular	0.999 (PD)	0.000 (D)	Disease causing	…	Uncertain significance
Thr8Ala	CR, ClinVar [5–7,18]	0.3804	Extracellular	0.999 (PD)	0.000 (D)	Disease causing	LoF [5,18]	Uncertain significance
Gln9Glu	ClinVar [5]	0.1452	Extracellular	0.000 (B)	0.280 (T)	Polymorphism	LoF [5]	Uncertain significance
Thr10Met	CR, ClinVar [22,26,27]	0.0182	Extracellular	0.952 (PD)	0.000 (D)	Disease causing	LoF [22]	Uncertain significance
Leu11fsX46	CR	…	Extracellular	N/A	Damaging	Disease causing	…	Likely pathogenic
Val14Ile	ClinVar [26]	0.0239	Extracellular	0.001 (B)	0.520 (T)	Polymorphism	…	Likely benign
Arg16Gln	CR	0.0025	Extracellular	0.000 (B)	0.240 (T)	Polymorphism	…	Likely benign
Ile20Asn	ClinVar [26]	…	Extracellular	0.055 (B)	0.000 (T)	Disease causing	…	Likely benign
Arg27Ser	CR	…	Extracellular	1.000 (PD)	0.000 (D)	Disease causing	…	Likely pathogenic
Arg27His	ClinVar [20,26]	0.0082	Extracellular	1.000 (PD)	0.000 (D)	Disease causing	…	Uncertain significance
Arg27Cys	ClinVar [29]	0.0074	Extracellular	1.000 (PD)	0.000 (D)	Disease causing	GoF [29]	Uncertain significance
Met54Thr	ClinVar [5,6,23,26,27]	0.0239	Transmembrane	0.959 (PD)	0.000 (D)	Disease causing	LoF [5,6]	Uncertain significance
Ile57Thr	ClinVar [6,26]	0.0881	Transmembrane	0.999 (PD)	0.010 (D)	Disease causing	LoF [6]	Uncertain significance
Phe60Leu	ClinVar [19]	…	Transmembrane	0.999 (PD)	0.000 (D)	Disease causing	…	Likely pathogenic
Val65Leu	ClinVar [26]	0.0025	Transmembrane	1.000 (PD)	0.560 (T)	Disease causing	…	Uncertain significance
Val65Met	ClinVar [21]	0.0025	Transmembrane	1.000 (PD)	0.050 (D)	Disease causing	LoF [21]	Uncertain significance
Ser74Tyr	CR	…	Cytosolic	1.000 (PD)	0.000 (D)	Disease causing	…	Likely pathogenic
Arg77Gln	ClinVar [26]	0.0016	Cytosolic	0.004 (B)	0.410 (T)	Disease causing	…	Uncertain significance
Arg77Trp	ClinVar [18,26]	0.0074	Cytosolic	0.995 (PD)	0.020 (D)	Disease causing	No Change to I_{Kr} [18]	Uncertain significance
Glu94Gly	CR, ClinVar [26]	…	Cytosolic	0.001 (B)	0.200 (T)	Disease causing	…	Uncertain significance
Leu101Ter	CR	…	Cytosolic	N/A	N/A	Disease causing	…	Uncertain significance
Ala116Val	ClinVar [6]	0.0017	Cytosolic	1.000 (PD)	0.000 (D)	Disease causing	LoF [6]	Uncertain significance
Met121Lys	CR, ClinVar	…	Cytosolic	0.009 (B)	0.980 (D)	Polymorphism	…	Uncertain significance
Pro123fsTer16	CR [26]	…	Cytosolic	N/A	N/A	Disease causing	…	Likely pathogenic
Ter124SerextTer1	CR	…	Cytosolic	N/A	N/A	Polymorphism	…	Uncertain significance

ACMG indicates American College of Medical Genetics; AF, allele frequency; B, benign; CR, current report; D, damaging; ExAC, Exome Aggregation Consortium; GoF, gain-of-function; LoF, loss-of-function; Mut Taster, Mutation Taster; N/A, not applicable; PD, probably damaging; and T, tolerated.

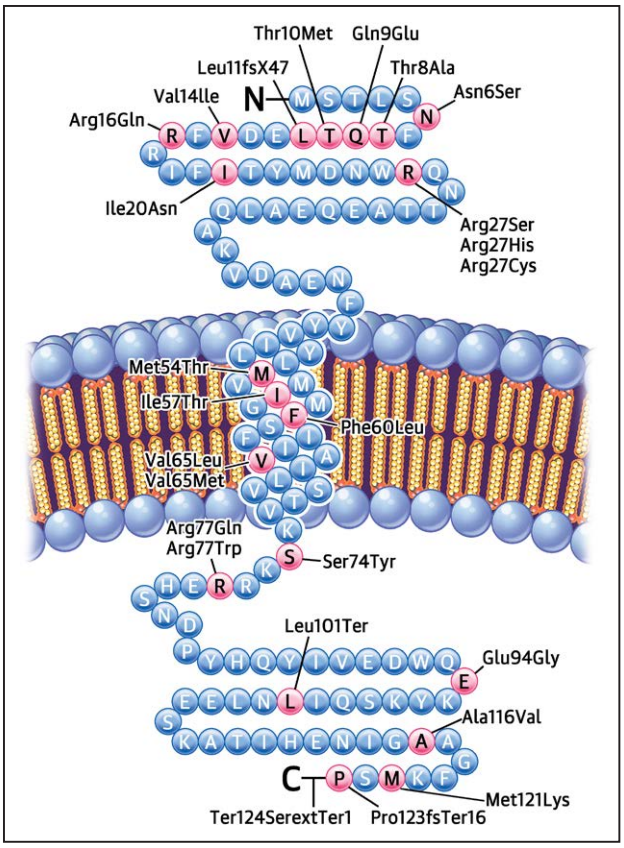

Figure 3. Structural topology of KCNE2 and location of putative pathogenic mutations implicated in long-QT syndrome type 6.

and perhaps all, have been designated erroneously as LQTS-causative mutations. Instead, the small subset of functionally disruptive, pathogenic *KCNE2* mutations seem to predispose to a phenotype more accurately reflective of an arrhythmia-susceptibility condition requiring secondary provocation, rather than a highly penetrant primary arrhythmic syndrome. This position is supported by the absence of a single reported family, either in the literature or our multicenter cohort, whereby a *KCNE2* variant segregated with an LQTS phenotype and is further reinforced by the high allelic frequencies observed for reported culprit *KCNE2* variants within ExAC.

Collectively, we think that our findings have important implications for the clinical management of patients with rare *KCNE2* variants previously designated as LQT6-causative mutations. Unlike LQT1 and LQT2, patients with loss-of-function *KCNE2* rare variants with a normal clinical and electrocardiographic phenotype should not be treated as concealed forms of LQTS. Instead, at most, they should be advised to avoid, when feasible, exposure to known QT-aggravating factors. Otherwise, 1.4% of the general population risks being labeled with a potentially lethal arrhythmia syndrome, treated with prophylactic β-blocker therapy, or worse, an implantable cardioverter–defibrillator, and restricted unnecessarily from sports. In other words, the majority of the *KCNE2* variants published as LQT6-causative mutations need to be demoted to being at most variants of uncertain significance.[28,34] The perceived value of cascade screening for these variants varies among investigators in this study, some advocating the benefit of education and knowledge for avoidance of QT-prolonging drugs, whereas others do not routinely use them to screen additional family members.

Within our study, 16 patients exclusively developed QT prolongation or TdP with a secondary stressor (Figure 2). Of cases that had phenotypes consistent with typical LQTS, 10 had pathogenic variants within other *LQTS* genes (Figure 2). Given our findings, we suspect that these mutations were responsible for the LQTS phenotype, while the *KCNE2* variants may have been modulators of arrhythmic risk or benign incidental findings. Eight of the index cases in our novel case series lacked an LQTS phenotype; however, identification of a *KCNE2* variant often led to an LQTS diagnosis and overtreatment.

Seven cases from our study exhibited an LQTS phenotype in the presence of a *KCNE2* mutation in isolation (Figure 2). Cases 15 and 16 from the literature (Table 2) possessed the Met54Thr mutation (ExAC allele frequency of 0.0239%). Although it is conceivable that Met54Thr may be contributing to their phenotype, its allele frequency in the general population is incompatible with a genetic culprit causative for a rare malignant arrhythmic syndrome. The same argument applies to the Ile57Thr-positive proband from family 27 (ExAC allele frequency: 0.0881%) whose first-degree family members also had normal QTc values (Table 1) and the Thr10Met-positive probands from family 26 and case 14 (ExAC allele frequency: 0.0182%). Case 17 (QT_c=514 ms) from the literature (Table 2) possessed the Arg77Trp mutation (ExAC allele frequency: 0.0074%), previously shown to have no impact on I_{Kr}.[18] The proband from family 28 (Glu94Gly; Table 1) had a QTc of 500 ms immediately after his subsequently fatal arrest. LQTS diagnoses in both of his children possessing the variant (QT_c values ranging from 417 to 437 ms and 356 to 412 ms) were removed following the assessment in a specialized inherited arrhythmia clinic.

It should also be noted that studies have begun to implicate *KCNE2* in nonarrhythmic conditions, including coronary artery disease, structural heart disease, and extracardiac disorders; however, our study was not designed to address these potential associations.[35,36]

Limitations

The current report combines a novel multicenter experience with all previously reported cases with alleged LQT6 status. Despite this, we acknowledge that the number of cases and families is limited, and it is not possible to draw definitive conclusions about the phenotype associated with all *KCNE2* variants. Meticulous evaluation of clinical phenotype remains paramount for ensuring delivery of optimal medical care. Clinical details were incomplete for multiple literature reported cases limiting inferences that could be drawn in those instances, while additional arrhythmic features, including sinus node dysfunction, preexcitation, and complete atrioventricular block, could not be systematically assessed in literature reported cases. Finally, the lack of evidence for genotype–phenotype segregation for *KCNE2* may potentially be limited by the presence of relatively small families and an inability to perform larger-scale cascade screening.

Conclusions

On the basis of the reported phenotypes of subjects with rare *KCNE2* variants, coupled with the unacceptably high allelic frequencies of these variants within the general population, the findings from our study suggest that loss-of-function *KCNE2* variants result in a phenotype more reflective of an arrhythmia-susceptibility condition requiring in the majority of cases secondary provocation by environmental or genetic factors, rather than representing a bona fide cause of monogenic LQTS. Clinical management of individuals with such loss-of-function *KCNE2* variants with normal clinical/electrocardiographic phenotypes should focus primarily on the avoidance of secondary stressors associated with QT prolongation, rather than the more intensive interventions pursued with the canonical forms of LQTS.

Appendix

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Disclosures

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Loss-of-Function *KCNE2* Variants: True Monogenic Culprits of Long-QT Syndrome or Proarrhythmic Variants Requiring Secondary Provocation?

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Supplemental Material

Assessment for Sinus Node Dysfunction and other Arrhythmic Features

Supplemental Table 1: Genes and *KCNE2* Mutations Screened Among Study Participants in Novel Case Series

Supplemental Table 2: Cardiac Imaging Evaluation for Structural Heart Disease Among Study Participants in Novel Case Series

Supplemental Table 3: QT behavior during Treadmill Testing Among Study Participants in Novel Case Series

Assessment for Sinus Node Dysfunction and other Arrhythmic Features

Given prior case reports and observations that KCNE2 also functions as the β−subunit for HCN4, the treating inherited arrhythmia specialist was asked if the study participant had exhibited clinical evidence for sinus node dysfunction, while intolerance to β-blockade and presence of a pacemaker were also documented. All study participants were also screened for other arrhythmic features, including complete atrioventricular block and pre-excitation through review of clinical history and surface ECGs.

Supplemental Table 1: Genes and *KCNE2* Mutations Screened Among Study Participants in Novel Case Series

Family	Familial Status	Genes and *KCNE2* Mutations Screened
LQTS phenotype secondary to QT-prolonging stressor		
1	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1*
	Asy 1st DFM	*KCNE2* Thr8Ala
2	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, GIRK4, CALM1, CALM2*
3	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, GIRK4, CALM1, CALM2*
4	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
5	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5*
6	Proband	*AKAP9, ANK2, CACNA1C, CACNB2, CASQ2, CAV3, DSC2, DSG2, DSP, GPD1L, HCN4, JUP, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, NKX2.5, PKP2, RANGRF, RyR2, SCN1B, SCN3B, SCN4B, SCN5A, SNTA1, TMEM43*
	Asy 1st DFM	*KCNE2* Ile57Thr
7	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9*
	Asy 1st DFM	*KCNE2* Met121Lys
LQTS phenotype but Proband does NOT have the variant		
8	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* Asn6Ser
LQTS phenotype and pathogenic mutation in another LQTS gene		
9	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* c.-13+5 G>A
10	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
11	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
12	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5*
13	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1*

14	Proband	*KCNE2* Met54Thr
	1st DFM (M)	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	1st DFM (F)	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
15	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1*
	Asy 1st DFM	*KCNE2* Ile57Thr
16	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
17	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* Pro123fs+16X and *KCNH2* Gln476spl
	Asy 1st DFM	*KCNE2* Pro123fs+16X and *KCNH2* Gln476spl
	Asy 1st DFM	*KCNE2* Pro123fs+16X and *KCNH2* Gln476spl
	Non-LQTS phenotype	
18	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
19	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
20	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5*
21	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, RyR2, CASQ2*
22	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5, CACNB2, CASQ2, DSC2, DSG2, DSP, GPD1L, HCN4, JUP, KCNE3, KCNJ8, NKX25, PKP2, RANGRF, RyR2, SCN3B, TMEM43*
23	Proband	*ABCA1, ABCC6, ABCC9, ACTA2, ACTC1, ACTN2, ADRB1, ADRB2, ADRB3, AGL, AKAP9, ANK2, ANKRD1, APOA1, APOB, APOE, BAG3, BRAF, CACNA1C, CACNA1D, CACNA2D1, CACNB2, CALR3, CASQ2, CAV3, CBL, COL3A1, CRYAB, CSRP3, BTF1, DES, DMD, DPP6, DSC2, DSG2, DSP, DTNA, EFEMP2, EMD, ENPP1, EYA4, FBLN5, FBN1, FBN2, FHL1, FHL2, FKTN, FLNC, FXN, GAA, GJA1, GJA5, GLA, GPD1L, HCN1, HCN4, HRAS, ILK, JPH2, JUP, KCNA5, KCND3, KCNE1, KCNE1L, KCNE2, KCNE3, KCNE4, KCNH2, KCNJ11, KCNJ12, KCNJ2, KCNJ3, KCNJ5, KCNJ8, KCNQ1, KCNQ2, KRAS, LAMA4, LAMP2, LDB3, LDLR, LMNA, LPL, LRP6, MAP2K1, MAP2K2, MEF2A, MYBPC3, MYH11, MYH6, MYH7, MYL2, MYL3, MYLK, MYLK2, MYOT, MYOZ2, MYPN, NEBL, NEXN, NOTCH1, NPPA, NRAS, PCSK9, PDLIM3, PKP2, PKP4, PLEC, PLN, PNN, PRKAG2, PTPN11, RAF1, RANGRF, RBM20, RPSA, RyR2, SCN1B, SCN2B, SCN3B, SCN4B, SCN5A, SN10A, SCNN1B, SCNN1G, SDHA, SGCD, SHOC2, SLC25A4, SLC2A10, SMAD3, SNTA1, SOS1, SPRED1, SYNE1, SYNE2, TAZ, TCAP, TGFB3, TGFBR1, TGFBR2, TGFBR3, TMEM43, TMPO, TNNC1, TNNT2, TPM1, TRPM4, TTN, VCL*

	Asy 1st DFM	*KCNE2* Ser74Tyr
	Asy 1st DFM	*KCNE2* Ser74Tyr
24	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1, KCNJ5*
25	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* Leu101Ter
	Asy 1st DFM	*KCNE2* Leu101Ter
	Asy 1st DFM	*KCNE2* Leu101Ter
LQTS phenotype in absence of another predisposing factor		
26	Proband	*KCNQ1, KCNH2, SCN5A, ANK2, KCNE1, KCNE2, KCNJ2, CACNA1C, CAV3, SCN4B, AKAP9, SNTA1*
	Asy 1st DFM	*KCNE2* Thr10Met
27	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* Ile57Thr
	Asy 1st DFM	*KCNE2* Ile57Thr
28	Proband	*KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2*
	Asy 1st DFM	*KCNE2* Glu94Gly
	Asy 1st DFM	*KCNE2* Glu94Gly

Supplemental Table 2: Cardiac Imaging Evaluation for Structural Heart Disease Among Study Participants in Novel Case Series

Family	Familial Status	Cardiac Imaging		
		ECHO	cMRI	Findings
LQTS phenotype secondary to QT-prolonging stressor				
1	Proband	No	No	Not applicable
	Asy 1st DFM	No	No	Not applicable
2	Proband	Yes	No	Normal biventricular systolic function; remote mitral valve replacement
3	Proband	No	No	Not applicable
4	Proband	No	No	Not applicable
5	Proband	Yes	No	Structurally normal heart
6	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
7	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
LQTS phenotype but Proband does NOT have the variant				
8	Proband	Yes	Yes	Ischemic Cardiomyopathy (LVEF: 40-45%)
	Asy 1st DFM	No	No	Not applicable
	Asy 1st DFM	No	No	Not applicable
LQTS phenotype and pathogenic mutation in another LQTS gene				
9	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	No	No	Not applicable
10	Proband	Yes	No	Structurally normal heart
11	Proband	No	No	Not applicable
12	Proband	Yes	No	Structurally normal heart
13	Proband	Yes	No	Structurally normal heart
14	Proband	No	No	Autopsy revealed structurally normal heart
	1st DFM (M)	Yes	No	Structurally normal heart
	1st DFM (F)	Yes	No	Idiopathic Dilated Cardiomyopathy

15	Proband	No	No	Structurally normal heart
	Asy 1st DFM	No	No	Structurally normal heart
16	Proband	No	No	Not applicable
17	Proband	No	No	Autopsy revealed structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
	Asy 1st DFM	No	No	Not applicable
	Asy 1st DFM	No	No	Not applicable
Non-LQTS phenotype				
18	Proband	Yes	No	Structurally normal heart
19	Proband	Yes	No	Structurally normal heart
20	Proband	Yes	No	Structurally normal heart
21	Proband	Yes	Yes	Structurally normal heart
22	Proband	Yes	No	Structurally normal heart
23	Proband	Yes	Yes	Mild global LV systolic dysfunction (LVEF 45-50%; prior anthracycline therapy)
	Asy 1st DFM	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	Yes	Structurally normal heart
24	Proband	Yes	No	Bileaflet mitral valve prolapse; normal biventricular size and function
25	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart
LQTS phenotype in absence of another predisposing factor				
26	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	No	No	Not applicable
27	Proband	Yes	No	Structurally normal heart
	Asy 1st DFM	No	No	Not applicable
	Asy 1st DFM	No	No	Not applicable
28	Proband	Yes	No	Structurally normal heart

	Asy 1st DFM	Yes	No	Structurally normal heart
	Asy 1st DFM	Yes	No	Structurally normal heart

Supplemental Table 3: QT behavior during Treadmill Testing Among Study Participants in Novel Case Series

Family	Familial Status	QTc (ms)			
		Supine	**Standing**	**Peak Exercise**	**4/5 minute recovery***
LQTS phenotype secondary to QT-prolonging stressor					
1	Proband	-	-	-	-
	Asy 1st DFM	480	470	520	430
2	Proband	-	-	-	-
3	Proband	-	-	-	-
4	Proband	-	-	-	-
5	Proband	-	-	-	-
6	Proband	-	-	-	-
	Asy 1st DFM	-	-	-	-
7	Proband	410	430	440	410
	Asy 1st DFM	-	-	-	-
LQTS phenotype but Proband does NOT have the variant					
8	Proband	456	426	412	461
	Asy 1st DFM	443	446	353	412
	Asy 1st DFM	368	400	340	443
LQTS phenotype and pathogenic mutation in another LQTS gene					
9	Proband	-	-	-	-
	Asy 1st DFM	410	404	400	420
10	Proband	546	570	526	542
11	Proband	-	-	-	-
12	Proband	447	460	537	501
13	Proband	460	450	526	500
14	Proband	-	-	-	-
	1st DFM (M)	-	-	-	-
	1st DFM (F)	-	-	-	-

15	Proband	-	-	-	-
	Asy 1st DFM	-	-	-	-
16	Proband	-	-	-	-
17	Proband	-	-	-	-
	Asy 1st DFM	415	425	430	410
	Asy 1st DFM	-	-	-	-
	Asy 1st DFM	-	-	-	-
Non-LQTS phenotype					
18	Proband	427	412	460	424
19	Proband	-	-	-	-
20	Proband	-	-	-	-
21	Proband	-	-	-	-
22	Proband	417	410	469	441
23	Proband	-	-	-	-
	Asy 1st DFM	-	-	-	-
	Asy 1st DFM	-	-	-	-
24	Proband	*	*	*	*
25	Proband	420	428	479	476
	Asy 1st DFM	367	371	439	394
	Asy 1st DFM	380	394	402	406
	Asy 1st DFM	412	384	431	425
LQTS phenotype in absence of another predisposing factor					
26	Proband	-	-	-	-
	Asy 1st DFM	-	-	-	-
27	Proband	-	-	-	-
	Asy 1st DFM	-	-	-	-
	Asy 1st DFM	-	-	-	-
28	Proband	-	-	-	-
	Asy 1st DFM	425	408	394	430
	Asy 1st DFM	419	389	431	418

*QTc not assessed due to high burden ventricular ectopy.