

# An International Multicenter Evaluation of Type 5 Long QT Syndrome

## A Low Penetrant Primary Arrhythmic Condition

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**BACKGROUND:** Insight into type 5 long QT syndrome (LQT5) has been limited to case reports and small family series. Improved understanding of the clinical phenotype and genetic features associated with rare *KCNE1* variants implicated in LQT5 was sought through an international multicenter collaboration.

**METHODS:** Patients with either presumed autosomal dominant LQT5 (N = 229) or the recessive Type 2 Jervell and Lange-Nielsen syndrome (N = 19) were enrolled from 22 genetic arrhythmia clinics and 4 registries from 9 countries. *KCNE1* variants were evaluated for ECG penetrance (defined as QTc >460 ms on presenting ECG) and genotype-phenotype segregation. Multivariable Cox regression was used to compare the associations between clinical and genetic variables with a composite primary outcome of definite arrhythmic events, including appropriate implantable cardioverter-defibrillator shocks, aborted cardiac arrest, and sudden cardiac death.

**RESULTS:** A total of 32 distinct *KCNE1* rare variants were identified in 89 probands and 140 genotype positive family members with presumed LQT5 and an additional 19 Type 2 Jervell and Lange-Nielsen syndrome patients. Among presumed LQT5 patients, the mean QTc on presenting ECG was significantly longer in probands (476.9±38.6 ms) compared with genotype positive family members (441.8±30.9 ms,  $P<0.001$ ). ECG penetrance for heterozygous genotype positive family members was 20.7% (29/140). A definite arrhythmic event was experienced in 16.9% (15/89) of heterozygous probands in comparison with 1.4% (2/140) of family members (adjusted hazard ratio [HR] 11.6 [95% CI, 2.6–52.2];  $P=0.001$ ). Event incidence did not differ significantly for Type 2 Jervell and Lange-Nielsen syndrome patients relative to the overall heterozygous cohort (10.5% [2/19]; HR 1.7 [95% CI, 0.3–10.8],  $P=0.590$ ). The cumulative prevalence of the 32 *KCNE1* variants in the Genome Aggregation Database, which is a human database of exome and genome sequencing data from now over 140 000 individuals, was 238-fold greater than the anticipated prevalence of all LQT5 combined (0.238% vs 0.001%).

**CONCLUSIONS:** The present study suggests that putative/confirmed loss-of-function *KCNE1* variants predispose to QT prolongation, however, the low ECG penetrance observed suggests they do not manifest clinically in the majority of individuals, aligning with the mild phenotype observed for Type 2 Jervell and Lange-Nielsen syndrome patients.

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## Clinical Perspective

### What Is New?

- Rare loss-of-function *KCNE1* variants are weakly penetrant and do not manifest with a long QT syndrome phenotype in a majority of individuals.
- QT prolongation and arrhythmic risk associated with Type 2 Jervell and Lange-Nielsen syndrome are mild in comparison with the more malignant phenotype observed for Type 1 Jervell and Lange-Nielsen syndrome.

### What Are the Clinical Implications?

- All individuals possessing a rare loss-of-function *KCNE1* variant should be counseled to avoid QT-prolonging medication and undergo a meticulous clinical evaluation to screen for a long QT syndrome phenotype.
- In the absence of a long QT syndrome phenotype, more intensive measures such as  $\beta$ -blockade and exercise restriction may not be merited.

Long QT syndrome (LQTS) is an inherited channelopathy characterized by impaired cardiac repolarization that confers an increased risk of syncope and sudden cardiac death (SCD) secondary to torsades de pointes.<sup>1</sup> The prevalence of LQTS is approximately 1 in 2000, and 17 genes have been implicated in its pathogenesis, though the majority of cases stem from mutations within *KCNQ1* (type 1 LQTS; LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3); considered the major LQTS genetic subtypes.<sup>2–4</sup> The *KCNQ1* gene encodes the Kv7.1  $\alpha$ -subunit responsible for the slow component of the delayed rectifier potassium current ( $I_{Ks}$ ), whereas the Kv11.1  $\alpha$ -subunit of the rapid component of the delayed rectifier potassium current ( $I_{Kr}$ ) is encoded by *KCNH2*.<sup>5–7</sup> Loss-of-function mutations within these voltage-gated potassium channels impair ventricular repolarization during Phase 3 of the cardiac action potential leading to LQT1 and LQT2.<sup>8,9</sup>

Type 5 LQTS (LQT5) is a minor LQTS genetic subtype accounting for approximately 1% to 2% of LQTS cases. LQT5 develops secondary to loss-of-function variants within *KCNE1*, which encodes minK, a voltage-gated potassium channel  $\beta$ -subunit felt to primarily interact with the Kv7.1  $\alpha$ -subunit responsible for  $I_{Ks}$ , though reports have also suggested a role for minK in  $I_{Kr}$  through an interaction with the Kv11.1  $\alpha$ -subunit.<sup>5,10–12</sup> The most intensively investigated *KCNE1* rare variant, p.Asp76Asn, has been implicated in both congenital and drug-induced forms of LQTS.<sup>10,13</sup> The relative rarity of LQT5 has led to limited insight into its clinical and genetic attributes, and management is often extrapolated from knowledge of the canonical LQT1–3 subtypes.

Recent work has revealed that loss-of-function variants in *KCNE2*, another voltage-gated potassium channel  $\beta$ -subunit, are more aptly characterized as arrhythmia-predisposing variants or functional risk alleles, leading to the recognition that LQT6 is not a monogenic form of LQTS and a corresponding alteration to the treatment approach for individuals possessing these variants.<sup>14,15</sup> The *KCNE2* and *KCNE1* genes have many similarities, though only *KCNE1* loss-of-function homozygotes and compound heterozygotes manifest with sensorineural deafness in association with QT prolongation, referred to as Type 2 Jervell and Lange-Nielsen syndrome (JLNS2).<sup>16–18</sup> Notably, in contrast to the severe and often complete loss-of-function observed for pathogenic *KCNQ1* and *KCNH2* mutations, the reductions in cardiac potassium currents observed on experimental in vitro patch clamp analysis for *KCNE2* and *KCNE1* variants have been modest.<sup>10,19,20</sup>

The growing recognition that each genetic LQTS subtype may require a tailored approach to management led to the pursuit of an international multicenter collaboration to further define the clinical and genetic features of LQT5.<sup>21–25</sup>

## METHODS

### Transparency and Openness Promotion

Data that support the findings of this study are available from the corresponding author upon reasonable request.

### Study Population

The study population consisted of 4 LQTS registries, including the Canadian LQTS registry, the Rochester (New York) LQTS registry, the Japanese LQTS registry, and the National Cardiac Inherited Disease Registry of New Zealand, along with 22 inherited arrhythmia clinics from 9 countries. Care was taken to ensure that no study participants were included twice through consultation with study investigators. Inclusion criteria for living probands required the presence of a rare *KCNE1* variant, defined as an allele frequency <0.1% in the Genome Aggregation Database (gnomAD; a database comprised of 141 456 individuals from multiple population-based and disease-specific genetic cohort studies),<sup>26</sup> and presence of a resting QTc >460 ms on a surface ECG. An allele frequency of <0.1% was chosen, as this rate may be sufficiently rare to contribute to a low penetrant form of LQTS. Genotype positive family members identified on cascade screening, which refers to clinical and genetic evaluation with variant-specific genetic testing of blood relatives at risk of being affected, were also included.

Cases of SCD that remained unexplained following cardiac autopsy were eligible for inclusion when molecular autopsy identified a rare *KCNE1* variant that had been observed in at least one living proband in our study that possessed a QTc >460 ms on ECG. Homozygotes and compound heterozygotes of rare *KCNE1* variants that exhibited sensorineural deafness consistent with JLNS2 were also eligible for the study. All living probands presenting with an arrhythmic event

were required to have undergone clinical testing with an ECG, exercise treadmill test, and an echocardiogram at minimum, and exhibit no evidence of another channelopathy or cardiomyopathy. Probands entered into the study were also required to have undergone screening of all exons and associated exon-intron boundaries within the *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes.

Exclusion criteria for living probands and genotype positive family members consisted of a pathogenic, or likely pathogenic, mutation, as per American College of Medical Genetics and Genomics (ACMG) guidelines, in another LQTS gene and deceased probands were excluded when a pathogenic, or likely pathogenic, mutation was identified in a gene known to be causative for either a cardiac channelopathy or cardiomyopathy.<sup>27</sup> Individuals possessing the known loss-of-function, proarrhythmic risk allele *KCNE1*-p.Asp85Asn in isolation were not included because of its presence in 0.1% to 2.5% of the general population (depending on ancestry; 1.6% in European ancestry subjects) and it being considered too common to function as a monogenic culprit for LQTS.<sup>15,28</sup>

The following variables were collected retrospectively for all living probands and genotype positive family members: date of birth, date of initial presentation, reason for presentation, sex, familial status (proband versus family member), Bazett corrected QT-intervals (QTc) recorded on ECGs at initial presentation and during follow-up, date at the time of cardiac events (including presumed cardiac syncope, appropriate implantable cardioverter-defibrillator shock, aborted cardiac arrest [ACA] requiring resuscitation, and SCD with normal cardiac autopsy), activity at the time of the cardiac event, secondary QT stressors present at the time of the cardiac event (including QT-prolonging medication, electrolyte abnormality, and heart block), and details of  $\beta$ -blocker usage, including dates of initiation and discontinuation, if applicable. Genetic details of the *KCNE1* variant, including the nucleotide and amino acid change, were obtained for each case.

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. All study participants provided informed consent for their clinical and genetic data to be used for research.

### Assessment of ECG Penetrance and Genotype-Phenotype Segregation

ECG penetrance was assessed in genotype positive family members. Consistent with prior work, an electrocardiographically manifest (penetrant) LQTS phenotype was defined as a QTc value on the presenting ECG  $>460$  ms.<sup>22</sup> Evaluation for genotype-phenotype segregation was performed in each family to clarify the role of rare *KCNE1* variants in predisposing to QT prolongation and was considered present if 2 or more individuals possessing the variant were phenotype positive.

### Evaluation of *KCNE1* Variants

All *KCNE1* variants included in the study were subjected to computer-based analyses and their prevalence in the general population and among individuals of European ancestry in isolation was assessed using gnomAD.<sup>26</sup> Computer modeling predicting effects of mutations on protein function was performed

using Polymorphism Phenotyping v2, Sorting Intolerant From Tolerant, and Combined Annotation Dependent Depletion.<sup>29–31</sup> Prior in vitro functional analyses of *KCNE1* variants reported in the literature were reviewed. Variants were presumed to be loss-of-function if they manifested with sensorineural deafness consistent with a JLNS2 phenotype when present in a homozygous or compound heterozygous state.

Although variant classification was performed according to ACMG guidelines, this was ultimately deemed inappropriate secondary to the low level of penetrance observed for *KCNE1* variants; ACMG criteria have been designed for classification of highly penetrant variants.<sup>27</sup>

### Statistical Analysis

Continuous variables are presented as means $\pm$ standard deviation and those exhibiting normal and nonnormal distributions were compared using the Student *t* test and the Wilcoxon rank-sum test, respectively. The comparison of categorical values was performed using Fisher exact test. Cox proportional hazards models were used to estimate the associations between clinical and genetic variables and age at first presumed primary arrhythmic event (composite of presumed cardiac syncope, appropriate implantable cardioverter-defibrillator shock, ACA, or SCD with normal autopsy; subsequently referred to as the composite arrhythmic outcome with syncope) and the first definite primary arrhythmic event (composite of appropriate implantable cardioverter-defibrillator shock, ACA, or SCD with normal autopsy; subsequently referred to as the composite arrhythmic outcome without syncope) among heterozygotes possessing rare *KCNE1* variants and JLNS2 patients.

Variables evaluated in both uni-/multivariable analyses included familial status (proband versus family member), sex, QTc on initial presenting ECG,  $\beta$ -blocker therapy, and missense variant location (extracellular, transmembrane, intracellular) in the *KCNE1*-encoded  $\beta$ -subunit. The QTc on the initial presenting ECG was treated as a categorical variable divided into tertiles ( $<470$  ms,  $\geq 470$  ms but  $\leq 500$  ms, and  $>500$  ms). Cumulative years on  $\beta$ -blocker therapy was treated as a time-dependent covariable to account for patients starting and stopping treatment throughout their lifetime and enabled comparison of event rates during the time on  $\beta$ -blocker therapy relative to time off  $\beta$ -blocker therapy. Risk of arrhythmic events was also evaluated based on *KCNE1*-p.Asp76Asn variant status (*KCNE1*-p.Asp76Asn carriers versus carriers of another *KCNE1* variant). Robust standard errors were used to account for familial relatedness. Because of minimal missing data, which only consisted of ECG values and age at LQTS diagnosis among 2 SCD cases identified to possess *KCNE1* variants on molecular autopsy, complete case analysis was used. Two-tailed *P* values  $<0.05$  were considered statistically significant. Statistical analyses were performed using Stata version 16 (College Station, TX, USA).

## RESULTS

### Study Population

Eighty-nine probands heterozygous for a rare *KCNE1* variant in the setting of a phenotype compatible with

LQTS and 140 genotype positive family members were enrolled in the study (Table 1). The mean age at the time of first ECG was 25.4±19.7 years and 61.6% were female. The mean QTc on the presenting ECG among probands was significantly longer relative to genotype positive family members (476.9±38.6 ms vs 441.8±30.9 ms,  $P < 0.001$ ).  $\beta$ -blocker therapy was used at some point in 78.7% of probands and 55.0% of genotype positive family members. A total of 41.6% of probands experienced a presumed cardiac event during their lifetime, defined as presumed cardiac syncope, appropriate implantable cardioverter-defibrillator shock, ACA, or SCD, compared with only 5.7% of *KCNE1* variant-positive family members ( $P < 0.001$ ). The number of individuals that experienced each of these events is provided in Table 1. Within the overall heterozygous cohort, the median ages of onset of the composite arrhythmic outcomes with and without syncope were 23.5 (interquartile range [IQR], 14.2–43.4) and 27.0 (IQR, 15.2–45.4) years, respectively.

The *KCNE1*-p.Asp76Asn variant was present in 98 of 229 heterozygous individuals (42.8%), and the mean QTc among carriers (455.1±35.5 ms) was similar to the mean QTc value observed among the remaining individuals in the heterozygous cohort (455.9±40.2 ms,  $P = 0.873$ ). An additional 19 JLNS2 individuals, including

15 homozygotes and 4 compound heterozygotes, were enrolled in the study and their clinical features are reported in Table 1. The composite arrhythmic outcome with syncope was experienced in a total of 13.3% (2/15) of homozygotes and 50% (2/4) of compound heterozygotes.

Among *KCNE1* heterozygotes, only 2 genotype positive family members had definite arrhythmic events; their details are provided in the [online-only Data Supplement](#). The median age at the time of last follow up for the overall heterozygous cohort was 27.3 years (IQR, 15.2–45.6).

### Disease Penetrance and Genotype-Phenotype Segregation

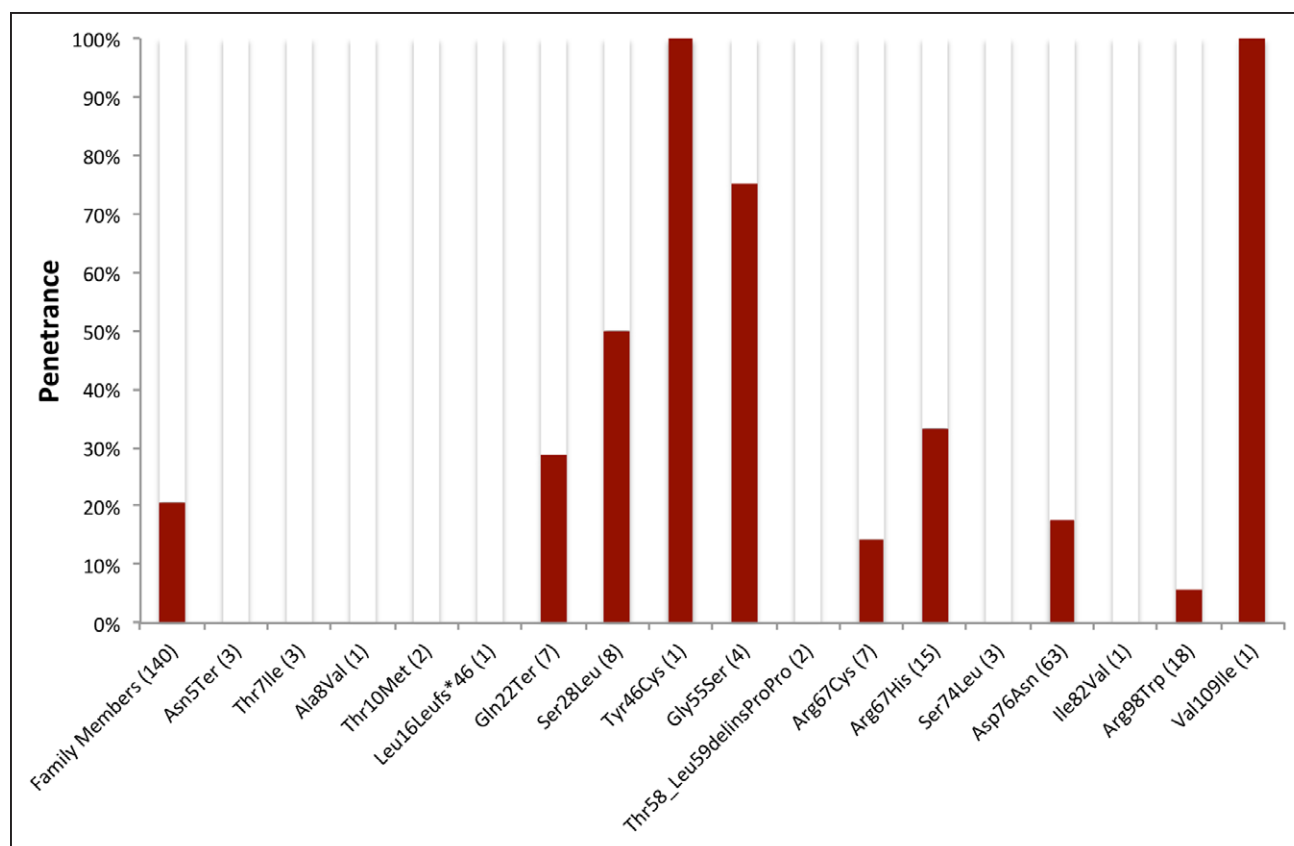
Disease penetrance was assessed in genotype positive family members based on the definition for an electrocardiographically manifest LQTS phenotype being a QTc value  $> 460$ ms on presenting ECG. The overall penetrance was 20.7% (29/140). Penetrance values for each *KCNE1* variant possessed in a heterozygous state by a family member are illustrated in Figure 1. Among the 10 *KCNE1* variants possessed by  $\geq 3$  individuals, penetrance values ranged from 0% (p.Asn5Ter and p.Thr7Ile) to 75% (p.Gly55Ser). The *KCNE1*-p.

**Table 1. Clinical Features of Probands and Genotype-Positive Family Members Possessing Rare *KCNE1* Variants**

Clinical Variable	LQT5			P Value*	JLNS2
	Overall n=229	Probands n=89	Genotype-Positive Family Members n=140		n=19
Age at first ECG, y	25.4 (19.7)	26.8 (19.2)	24.5 (19.9)	0.174	14.6 (14.0)
Female, %	141 (61.6)	59 (66.3)	82 (58.6)	0.211	9 (47.4)
European ancestry, %	219 (95.6)	83 (93.3)	136 (97.1)	0.016	16 (84.2)
QTc on presenting ECG, ms	455.6 (38.2)	476.9 (38.6)	441.8 (30.9)	$<0.001$	471.1 (43.5)
Males	448.5 (36.2)	469.3 (38.2)	437.7 (30.2)	$<0.001$	468.9 (53.5)
Females	460.1 (38.8)	480.8 (38.6)	444.8 (31.3)	$<0.001$	473.6 (32.0)
Atrial fibrillation	7 (3.1)	6 (6.7)	1 (0.7)	0.017	0 (0)
Treatment					
$\beta$ -Blocker	147 (64.2)	70 (78.7)	77 (55.0)	0.001	8 (42.1)
LCSD	5 (2.2)	2 (2.2)	3 (2.1)	1.000	1 (5.3)
ICD	28 (12.2)	23 (25.8)	5 (3.6)	$<0.001$	0 (0)
Cardiac event					
Syncope	31 (13.5)	25 (28.1)	6 (4.2)	$<0.001$	3 (15.8)
Appropriate ICD shock	4 (1.8)	3 (3.4)	1 (0.7)	0.304	0 (0)
Aborted cardiac arrest	12 (5.2)	12 (13.5)	0 (0)	$<0.001$	1 (5.3)
Sudden cardiac death	4 (1.8)	3 (3.4)	1 (0.7)	0.304	1 (5.3)
CAO with syncope	45 (19.7)	37 (41.6)	8 (5.7)	$<0.001$	4 (21.1)
CAO without syncope	17 (7.4)	15 (16.9)	2 (1.4)	$<0.001$	2 (10.5)

Data are n (%) or mean (SD). CAO indicates composite arrhythmic outcome; ICD, implantable cardioverter-defibrillator; JLNS2, Type 2 Jervell and Lange-Nielsen syndrome; LCSD, left cardiac sympathetic denervation; LQT5, Type 5 Long QT syndrome; and ms, milliseconds.

\*P value compares LQT5 probands and family members.



**Figure 1. ECG penetrance of rare *KCNE1* variants.**

Penetrance is defined as a QTc >460 ms on their presenting ECG. (N) indicates the number of genotype positive family members with the *KCNE1* variant.

Asp76Asn variant, present in a heterozygous state in 63 family members, exhibited an overall penetrance of 17.5%. Among JLNS2 patients, the electrocardiographic penetrance was 66.7% (10/15) in homozygotes and 75% (3/4) in compound heterozygotes.

Genotype-phenotype segregation was assumed to be present if at least 2 individuals in a single family were phenotype positive. Thirteen of 52 (25%) families with at least 2 genotype positive individuals possessed evidence of genotype-phenotype segregation (Table I in the online-only Data Supplement). Genotype-phenotype segregation was observed for 8 *KCNE1* variants (KCNE1-p.Gln22Ter, -p.Ser28Leu, -p.Tyr46Cys, -p.Gly55Ser, -p.Arg67Cys, p.Arg67His, -p.Asp76Asn, and -p.Val109Ile; Table I in the online-only Data Supplement).

## Arrhythmic Risk Associations

### Univariable Analyses

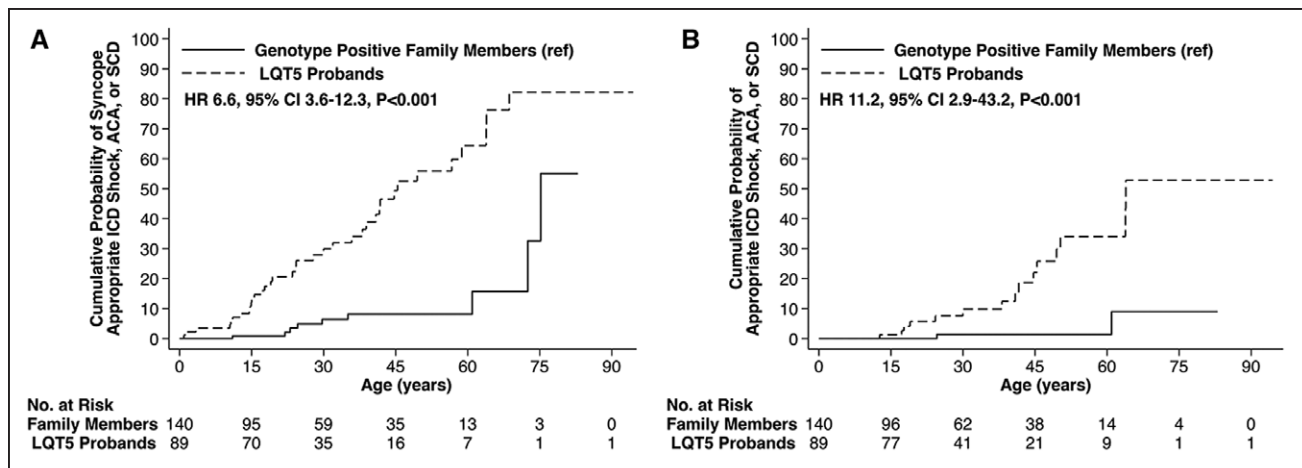
Proband possessing a rare *KCNE1* variant had a 6.6-fold (95% CI, 3.6–12.3;  $P < 0.001$ ) higher hazard of experiencing the composite arrhythmic outcome with syncope relative to genotype positive family members (Figure 2A and Table 2) and a 11.2-fold (95% CI, 2.9–43.2;  $P < 0.001$ ) higher hazard of the composite arrhythmic outcome without syncope (Figure 2B and Table 2).

Evaluation of QTc values on presenting ECG revealed that the upper 2 tertiles were both associated with a higher risk of the composite arrhythmic outcome with syncope, whereas only the QTc >500 ms tertile exhibited a statistically significant association for the composite arrhythmic outcome without syncope (Table 2 and Figure I in the online-only Data Supplement). Sex (Figure 3),  $\beta$ -blocker therapy, and missense variant location within the *KCNE1*-encoded Kv7.1  $\beta$  subunit (Figure II in the online-only Data Supplement) were not associated with altered risk of the composite arrhythmic outcomes based on univariable analysis (Table 2). The arrhythmic risk associated with the p.Asp76Asn variant, the most prevalent *KCNE1* variant in the cohort carried by 42.8% of heterozygotes, did not differ statistically relative to the collective remainder of the *KCNE1* variants evaluated (Figure III in the online-only Data Supplement).

Univariable analyses for probands in isolation revealed measures of association that were generally consistent with the overall heterozygous cohort with no point estimates that extended beyond the 95% CI boundaries (Table II in the online-only Data Supplement).

### Multivariable Analysis

A multivariable Cox regression model was constructed including the variables for familial status, sex, QTc tertile



**Figure 2.** Arrhythmic events among probands and genotype positive family members possessing a rare *KCNE1* variant.

Outcomes of (A) syncope, appropriate ICD shock, ACA, or SCD, and (B) appropriate ICD shock, ACA, or SCD. ACA indicates aborted cardiac arrest; HR, hazard ratio; ICD, implantable cardioverter-defibrillator; LQT5, long QT syndrome type 5; ref, reference; and SCD, sudden cardiac death.

on presenting ECG,  $\beta$ -blocker therapy, and location of the missense variant within the *KCNE1*-encoded Kv7.1  $\beta$  subunit. Following adjustment, familial status was the only predictor that continued to exhibit a statistically significant association for the arrhythmic outcomes (Table 2). Similar results were obtained for probands in isolation with no point estimates that extended beyond the 95% CI boundaries for the overall heterozygous cohort (Table II in the online-only Data Supplement).

## JLNS2 Arrhythmic Outcomes

The mean QTc values on presenting ECG in JLNS2 patients trended towards being longer relative to individuals possessing a *KCNE1* variant in a heterozygous state but did not reach statistical significance ( $471.1 \pm 43.5$  ms vs  $455.6 \pm 38.2$  ms,  $P=0.050$ ; Table 1). JLNS2 patients

had event rates that also did not exhibit statistically significant differences relative to *KCNE1* heterozygotes for the composite arrhythmic outcomes including syncope (hazard ratio [HR] 1.2 [95% CI, 0.2–6.4],  $P=0.800$ ; Figure 4A) and excluding syncope (HR 1.7 [95% CI, 0.3–10.8],  $P=0.590$ ; Figure 4B). The median age at the time of last follow up for the JLNS2 cohort was 27.2 years (IQR, 15.8–38.0).

## Secondary QT Stressors and Triggers for Cardiac Events

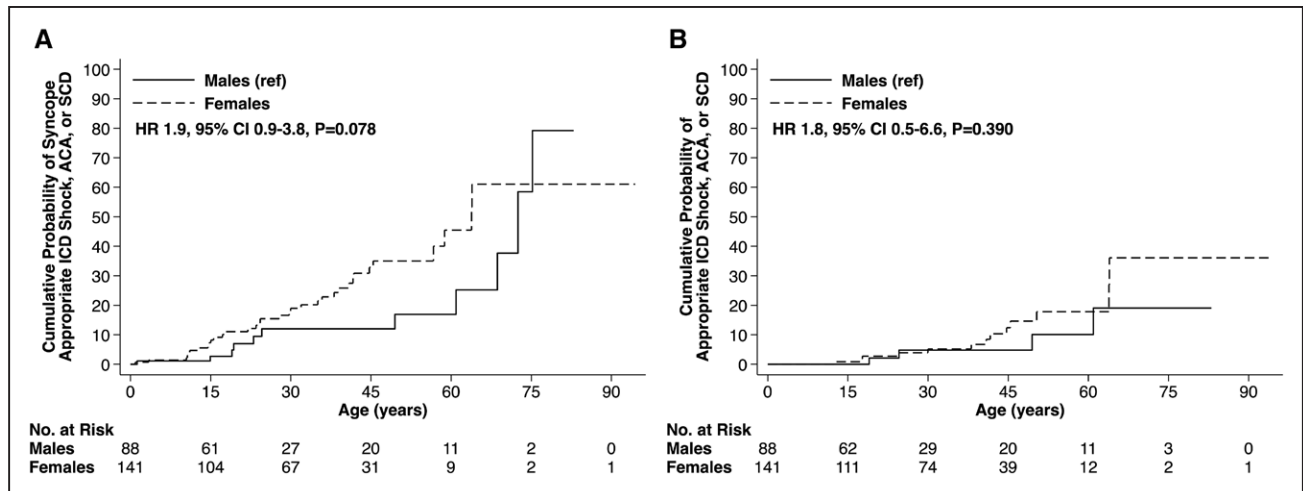
A total of 62 cardiac events were experienced among the entire cohort during a collective 7844 patient-years beginning from birth. Three events were reported to have occurred in the setting of a QT-prolonging medication, 1 in the context of a severe electrolyte abnormality,

**Table 2.** Association of Clinical and Genetic Variables With Cardiac Events Among Individuals Heterozygous for Rare *KCNE1* Variants

Clinical and Genetic Variables	Composite of Syncope, Appropriate ICD Shock, ACA, SCD				Composite of Appropriate ICD Shock, ACA, SCD			
	Unadjusted HR (95% CI)	P Value	Adjusted HR (95% CI)	P Value	Unadjusted HR (95% CI)	P Value	Adjusted HR (95% CI)	P Value
Familial status	6.6 (3.5–12.3)	<0.001	4.7 (1.9–11.7)	<0.001	11.2 (2.9–43.2)	<0.001	11.6 (2.6–52.2)	0.001
Female sex	1.9 (0.9–3.8)	0.08	0.9 (0.4–1.8)	0.75	1.8 (0.5–6.6)	0.39	0.4 (0.1–1.3)	0.13
QTc tertiles, ms								
<470	Reference	—	—	—	Reference	—	—	—
470–500	3.6 (1.8–7.2)	<0.001	1.8 (0.8–4.4)	0.17	2.1 (0.6–7.3)	0.23	0.9 (0.2–3.9)	0.90
>500	3.4 (1.5–7.9)	0.004	1.3 (0.4–4.6)	0.65	7.9 (2.4–25.3)	<0.001	3.3 (0.7–15.7)	0.13
Time on $\beta$ -blocker*	1.0 (0.9–1.2)	0.53	1.0 (0.9–1.2)	0.69	1.0 (0.9–1.1)	0.75	1.0 (0.9–1.1)	0.80
Variant location								
Extracellular	Reference	—	—	—	Reference	—	—	—
Transmembrane	1.6 (0.4–6.9)	0.51	1.4 (0.4–5.4)	0.65	1.1 (0.1–10.0)	0.93	0.5 (0.1–5.0)	0.55
Intracellular	0.9 (0.3–2.9)	0.88	0.7 (0.2–2.2)	0.53	0.5 (0.1–2.7)	0.44	0.3 (0.1–1.8)	0.21

ACA indicates aborted cardiac arrest; HR, hazard ratio; ICD, implantable cardioverter-defibrillator; ms, milliseconds; and SCD, sudden cardiac death.

\* $\beta$ -blocker treated as a time-dependent covariable.



**Figure 3.** Arrhythmic events among males and females possessing a rare *KCNE1* variant. Outcomes of (A) syncope, appropriate ICD shock, ACA, or SCD, and (B) appropriate ICD shock, ACA, or SCD. ACA indicates aborted cardiac arrest; HR, hazard ratio; ICD, implantable cardioverter-defibrillator; ref, reference; and SCD, sudden cardiac death.

and 1 was attributed to torsades de pointes in the setting of complete heart block. No secondary QT-prolonging stressors were identified in association with the remaining events. Activities reported at the time of events included awake at rest in 37 (60.0%), exertion in 17 (27.4%), auditory stimuli in 2 (3.2%), postexertion in 1 (1.6%), sleep in 1 (1.6%), and the activity at the time of the event was unknown in 4 (6.5%).

### Evaluation of *KCNE1* Variants

#### Population Allele Frequencies

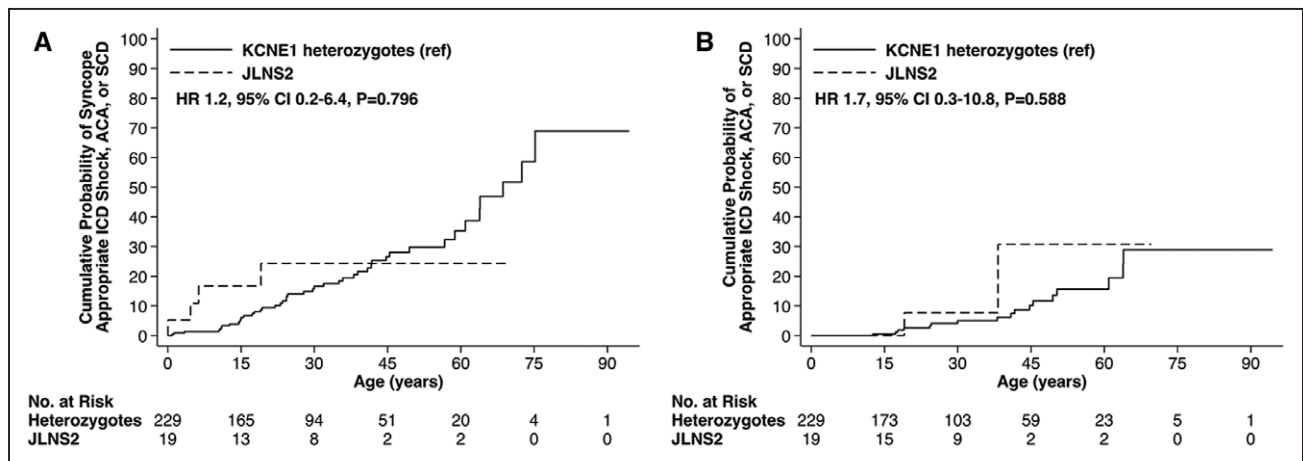
Among the 32 *KCNE1* variants possessed by the study participants, 22 were observed in gnomAD, with individual allele frequencies ranging up to 0.02094% for the Thr10Met variant (0.02134% when restricted to European ancestry; Table III in the online-only Data Supplement). The collective prevalence of these variants in the overall gnomAD cohort was 0.238% and 0.169%

among the European ancestry subgroup. Based on the assumptions that the prevalence of LQTS is 0.05% and LQT5 accounts for 2% of LQTS, its prevalence is estimated at 0.001%. The collective prevalence of *KCNE1* variants implicated in LQT5 is 238-fold the anticipated prevalence of LQT5 when the overall gnomAD cohort is considered and 169-fold when the analysis is restricted to individuals of European ancestry.

Eight of the 32 *KCNE1* variants were observed in JLNS2, confirming their status as loss-of-function given their being causative for sensorineural deafness (Table III in the online-only Data Supplement). The collective prevalence of *KCNE1* variants identified in the context of JLNS2 in the overall gnomAD cohort was 0.0162% and 0.0240% among Europeans.

#### Computer-Based and Previously Reported In Vitro Analyses

Computer-based analysis of *KCNE1* variants possessed by study participants was performed using



**Figure 4.** Arrhythmic events among Type 2 Jervell and Lange-Nielsen syndrome patients and *KCNE1* heterozygotes. Outcomes of (A) syncope, appropriate ICD shock, ACA, or SCD, and (B) appropriate ICD shock, ACA, or SCD. ACA indicates aborted cardiac arrest; HR, hazard ratio; ICD, implantable cardioverter-defibrillator; JLNS2, Type 2 Jervell and Lange-Nielsen syndrome; ref, reference; and SCD, sudden cardiac death.

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Polymorphism Phenotyping v2, Sorting Intolerant From Tolerant, and Combined Annotation Dependent Depletion (Table III in the online-only Data Supplement). Polymorphism Phenotyping v2 and Sorting Intolerant From Tolerant both identified 14 of 24 missense variants as probably/possibly damaging or damaging, respectively. A total of 18 of 27 single nucleotide variants had a Combined Annotation Dependent Depletion score greater than 20, predicting them being among the top 1% of most damaging variants within the genome.<sup>31</sup> Classification of the variants using the 2015 ACMG guidelines identified 3 as pathogenic, 5 as likely pathogenic, 17 as a variant of unknown significance, and 7 as likely benign (Table III in the online-only Data Supplement). Assignment of likely benign status to 7 variants was primarily driven by their minor allele frequencies being greater than the anticipated prevalence of LQT5 (0.001%), which is not considered appropriate when variant penetrance is anticipated to be low. On review of the literature, *in vitro* patch-clamping analysis using heterologous expression of mutant *KCNE1* in association with wild-type *KCNQ1* had been performed for only 4 of 25 *KCNE1* missense variants (Table III in the online-only Data Supplement) and each was consistent with a loss-of-function.<sup>10,19,20</sup>

## DISCUSSION

This international multicenter study represents the first large-scale evaluation of rare *KCNE1* variants implicated as monogenic culprits for LQTS. Their low ECG penetrance in family members, coupled with their excess prevalence in gnomAD, suggests that loss-of-function *KCNE1* variants do not manifest clinically in a majority of individuals. The benign phenotype observed in the vast majority of genotype positive family members strongly suggests that loss-of-function *KCNE1* variants require additional genetic or nongenetic factors to manifest with a positive LQTS phenotype. However, in contrast to *KCNE2*<sup>14</sup>, QT prolongation and clinical events occurred in the overwhelming majority of individuals in the absence of an identifiable QT-prolonging stressor, suggesting that LQT5 should be viewed as a low penetrant primary arrhythmic condition rather than an exclusively provoked syndrome. These findings, which align with the conclusions drawn for *KCNE1* from the recent Clinical Genome Resource Consortium reappraisal of LQTS genes, have important clinical implications for probands and genotype positive family members.<sup>32</sup>

Evaluation of arrhythmic events among probands initially suggested that LQT5 may be a highly malignant disorder, however, mirroring prior work in LQTS, the striking event rate observed among probands differed dramatically relative to the findings among genotype positive family members.<sup>33</sup> The contrasting arrhythmic

profiles of probands and genotype positive family members, coupled with clinical and genetic evidence suggesting *KCNE1* variants do not manifest clinically in the majority of individuals, strongly suggests that the high event rate observed among LQT5 probands was secondary to selection bias. Although operative in all forms of LQTS, the impact of selection bias is expected to be more extreme for low penetrant variants when the contribution of genomic background and environmental influences on arrhythmic events and QT prolongation is anticipated to be much greater. This concept is effectively illustrated by a recent study that identified hazard ratios ranging from 2.48 to 3.21 for a composite outcome of syncope, ACA, or SCD among probands relative to family members in the major LQTS genetic subtypes (1–3), in comparison to the unadjusted 6.6-fold increased HR reported here for LQT5.<sup>34</sup>

Aside from familial status, no other intrinsic clinical or genetic factors, including QTc on presenting ECG, sex,  $\beta$ -blocker therapy, and missense variant location, were associated with an altered risk of events on multivariable analyses (Table 2). Notably, only 64.2% of individuals were treated with  $\beta$ -blocker during their lifetime, and the mean QTc of those administered  $\beta$ -blockade was  $464.4 \pm 39.0$  ms in comparison with a mean value of  $439.4 \pm 30.8$  ms for those not treated ( $P < 0.001$ ). These findings suggest that patients with milder phenotypes were not treated, which is anticipated to lead to biased measures of association secondary to confounding by indication. It is possible that confounding by indication, coupled with the low event rate, may have led to the lack of an apparent protective effect with  $\beta$ -blockers.

Although the findings from the current study serve as strong evidence that many *KCNE1* variants are insufficient in isolation to cause LQTS, it could be argued that only a minority of these variants have undergone functional work and hence the physiological relevance for the majority is unclear. Eight of the 32 variants were observed among cases of JLNS2 providing definitive evidence for their being loss-of-function. Penetrance of these variants was 15.7% among family members, which was consistent with findings from the overall sample (20.7%). In addition, QTc values and event rates among study participants possessing the most prevalent *KCNE1* variant (p.Asp76Asn), known to be loss-of-function and present in 98 of the 229 heterozygous individuals, were consistent with those from the remainder of the cohort (Figure II in the online-only Data Supplement).<sup>10,19</sup>

Attempted evaluation of the *KCNE1* variants using ACMG criteria was ultimately deemed inappropriate because of their low penetrance given that ACMG criteria are tailored for highly penetrant variants.<sup>27</sup> Notably, the *KCNE1*-p.Asp76Asn variant has a prevalence among individuals with European ancestry of 0.02212%, which exceeds the anticipated prevalence of LQT5 (0.001%)



by >22-fold. A greater than expected allele frequency for the disorder being evaluated is considered a strong ACMG criterion for classifying a variant as benign. Although the p.Asp76Asn variant had sufficient additional supporting evidence to still receive a likely pathogenic designation, 7 *KCNE1* variants were demoted to likely benign status primarily owing to their prevalence being greater than anticipated for LQT5 (Table III in the online-only Data Supplement). In the collective view of the investigators, given that *KCNE1*-p.Asp76Asn is an established genetic culprit for LQT5; it is not felt that demotion of other variants with similar allele frequencies to likely benign status based on their apparent excess prevalence is appropriate.<sup>15</sup>

The study also builds upon prior work and provides additional insight into the JLNS2 phenotype.<sup>18</sup> In contrast to JLNS1, an autosomal recessive condition secondary to homozygous or compound heterozygous *KCNQ1* loss-of-function mutations and characterized by marked QT prolongation and a highly malignant arrhythmic phenotype, the phenotype of JLNS2 appeared surprisingly mild, which aligns with earlier work.<sup>18</sup> Although the apparent lack of an effect on phenotypic severity for increasing gene dosage may be secondary to inadequate power given that only 19 JLNS2 patients were included in the study, the finding that JLNS2 has a relatively mild phenotype lends further support to dysfunction of the *KCNE1*-encoded  $\beta$ -subunit often being clinically concealed.

Although a functional copy of *KCNE1* is necessary for sensorineural hearing, the findings from this study suggest that the *KCNE1*-encoded  $\beta$ -subunit may either exert a modest role in cardiac repolarization or the heart, in contrast to the inner ear, may have established a redundancy for  $\beta$ -subunits that allows for effective compensation in response to the loss of 1 constituent. The notion that a single  $\beta$ -subunit may be able to interact interchangeably with multiple pore-forming  $\alpha$ -subunits is alluded to by evidence that minK not only contributes to  $I_{Ks}$  but also  $I_{Kr}$  through an interaction with the Kv11.1  $\alpha$ -subunit.<sup>5,11,12</sup>

Whereas possessing a pathogenic mutation causative for the major genetic LQTS subtypes results in a diagnosis of LQTS, and most often triggers initiation of a  $\beta$ -blocker regardless of phenotype,<sup>35</sup> evidence from the current study suggests that an alternative approach to management for individuals possessing a *KCNE1* rare variant in the absence of an LQTS phenotype may be desired. While it is felt that all individuals possessing a loss-of-function *KCNE1* variant should be advised to avoid QT-prolonging drugs,<sup>13</sup> in the presence of a normal phenotype intensive measures such as  $\beta$ -blockade and exercise restriction may not be merited. Although a protective effect of  $\beta$ -blockade was not observed in the study, given the potential limitations highlighted above that may have

led to both biased and underpowered results, it is felt that  $\beta$ -blocker therapy should still be recommended in the presence of a positive LQTS phenotype. Because of the presence of study participants that experienced presumed arrhythmic events despite QTc values considered within normal limits on presenting ECG, highlighting the limitations of a single ECG to assess disease penetrance, it is advocated that all individuals possessing true loss-of-function variants be followed for serial monitoring of QTc values. Routine use of cascade screening for these variants is also advocated given their potential to manifest with a malignant LQTS phenotype, as highlighted by the natural history of the probands in the study.

## Limitations

Although the largest dedicated evaluation for rare *KCNE1* variants to date, the study may be underpowered to detect statistically significant associations between relevant clinical and genetic factors and arrhythmic risk. As an observational study, it is also vulnerable to various unavoidable forms of bias. The cohort consisted of probands referred to specialized inherited arrhythmia clinics because of worrisome clinical findings and likely led to the selection of a malignant subset of *KCNE1* heterozygotes and a correspondingly inflated arrhythmic event rate. In addition, evaluation for a potential protective effect of  $\beta$ -blocker therapy will unavoidably be biased secondary to confounding by indication.

## Conclusions

The present study reveals that *KCNE1* loss-of-function variants are weakly penetrant and individuals manifesting with an LQTS phenotype in the presence of a loss-of-function *KCNE1* variant likely possess additional genetic or environmental factors that predispose to QT prolongation. In contrast to *KCNE2*, the overwhelming majority of probands and genotype positive family members manifesting with QT prolongation and arrhythmic events did so in the absence of a QT-prolonging stressor suggesting that LQT5 should be viewed as a low penetrant primary arrhythmic condition rather than an exclusively provoked syndrome. Following the identification of a rare *KCNE1* loss-of-function variant, clinical management should consist of meticulous evaluation for an LQTS phenotype and counseling regarding the avoidance of QT-prolonging drugs.

## ARTICLE INFORMATION

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## Disclosures

None.

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