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An International Multi-Center Evaluation of Type 5 Long QT Syndrome: A Low Penetrant Primary Arrhythmic Condition

Running Title: *Roberts et al.; Evaluation of KCNE1-Associated LQT5*

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The full author list is available on page 20.

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Abstract

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 multi-center collaboration.

Methods: Patients with either presumed autosomal dominant LQT5 (N = 229) or the recessive Type 2 Jervell and Lange-Nielsen syndrome (JLNS2, 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 > 460ms 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 JLNS2 patients. Among presumed LQT5 patients, the mean QTc on presenting ECG was significantly longer in probands (476.9 $\pm$ 38.6ms) compared to genotype positive family members (441.8 $\pm$ 30.9ms, 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% confidence interval [CI]: 2.6-52.2; p=0.001). Event incidence did not differ significantly for JLNS2 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 (gnomAD), 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 JLNS2 patients.

Key Words: long QT syndrome, genetics, penetrance, arrhythmia, sudden cardiac death

Non-standard Abbreviations and Acronyms:
long QT syndrome = LQTS
sudden cardiac death = SCD
Type 2 Jervell and Lange-Nielsen syndrome = JLNS2
Genome Aggregation Database = gnomAD
American College of Medical Genetics and Genomics = ACMG
implantable cardioverter defibrillator = ICD
aborted cardiac arrest = ACA
Polymorphism Phenotyping v2 = PolyPhen-2
Sorting Intolerant From Tolerant = SIFT
Combined Annotation Dependent Depletion = CADD
interquartile range = IQR
confidence intervals = CI
hazard ratio = HR.

Clinical Perspective

What is new?

- Rare loss-of-function *KCNE1* variants are weakly penetrant and do not manifest with an LQTS phenotype in a majority of individuals.
- QT-prolongation and arrhythmic risk associated with Type 2 Jervell and Lange-Nielsen syndrome is 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 an LQTS phenotype
- In the absence of an LQTS phenotype, more intensive measures such as β-blockade and exercise restriction may not be merited.

Introduction

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.[1] The prevalence of LQTS is approximately 1 in 2,000 and 17 genes have been implicated in its pathogenesis, though the majority of cases stem from mutations within *KCNQ1* (LQT1), *KCNH2* (LQT2), and *SCN5A* (LQT3), considered the major LQTS genetic subtypes.[2–4] The *KCNQ1* gene encodes the Kv7.1 α-subunit responsible for the slow component of the delayed rectifier potassium current (I_{Ks}), whereas the Kv11.1 α-subunit of the rapid component of the delayed rectifier potassium current (I_{Kr}) is encoded by *KCNH2*.[5–7] 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.[8,9]

LQT5 is a minor LQTS genetic subtype accounting for approximately 1-2% of LQTS cases. LQT5 develops secondary to loss-of-function variants within *KCNE1*, which encodes minK, a voltage-gated potassium channel β-subunit felt to primarily interact with the Kv7.1 α-subunit responsible for I_{Ks}, though reports have also suggested a role for minK in I_{Kr} through an interaction with the Kv11.1 α-subunit.[5,10–12] The most intensively investigated KCNE1 rare variant, p.Asp76Asn, has been implicated in both congenital and drug-induced forms of LQTS.[10,13] 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 β-subunit, are more aptly characterized as arrhythmia predisposing variants or functional risk alleles, leading to recognition that LQT6 is not a monogenic form of LQTS and a

corresponding alteration to the treatment approach for individuals possessing these variants.[14,15] 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).[16–18] 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.[10,19,20]

The growing recognition that each genetic LQTS subtype may require its own tailored approach to management led to the pursuit of an international multi-center collaboration to further define the clinical and genetic features of LQT5.[21–25]

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),[26] and presence of a resting QTc >460ms 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 > 460ms 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 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.[27] Individuals possessing the known loss-of-function, pro-arrhythmic risk allele KCNE1-p.Asp85Asn in isolation were not included due to its presence in 0.1-2.5% of the general population (depending on ancestry; 1.6% in European

ancestry subjects) and its being considered too common to function as a monogenic culprit for LQTS.[15,28]

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 [ICD] 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 β-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 > 460ms.[22] Evaluation for genotype-phenotype segregation was performed in each family in an effort 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.[26] Computer model predicting effects of mutations on protein function was performed using Polymorphism Phenotyping v2 (PolyPhen-2), Sorting Intolerant From Tolerant (SIFT), and Combined Annotation Dependent Depletion (CADD).[29–31] 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.[27]

Statistical Analysis

Continuous variables are presented as means ± standard deviation and those exhibiting normal and non-normal distributions were compared using Student's t-test and the Wilcoxon rank-sum test, respectively. Comparison of categorical values was performed using Fisher's 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 ICD 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 ICD 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, β-blocker therapy, and missense variant location (extracellular, transmembrane, intracellular) in the *KCNE1*-encoded β-subunit. The QTc on the initial presenting ECG was treated as a categorical variable divided into tertiles (<470 ms, ≥ 470 ms but ≤ 500 ms, and > 500 ms). Cumulative years on β-blocker therapy was treated as a time-dependent covariable in order to account for patients starting and stopping treatment throughout their lifetime and enabled comparison of event rates during time on β-blocker therapy relative to time off β-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. Due to 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 into 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 ± 38.6ms vs. 441.8 ± 30.9ms, p< 0.001). β-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 ICD shock, ACA, or SCD, compared to 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 (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 $\pm$ 35.5ms) was similar to the mean QTc value observed among the remaining individuals in the heterozygous cohort (455.9 $\pm$ 40.2ms, $p = 0.873$). An additional 19 JLNS2 individuals, including 15 homozygotes and 4 compound heterozygotes, were enrolled into 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 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 > 460ms on presenting ECG. The overall penetrance was 20.7% (29/140). Penetrance values for each individual *KCNE1* variant possessed in a heterozygous state by a family member are illustrated

in **Figure 1**. Among the 10 *KCNE1* variants possessed by ≥ 3 individuals, penetrance values ranged from 0% (p.Asn5Ter and p.Thr7Ile) to 75% (p.Gly55Ser). The KCNE1-p.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 (**Supplemental Table 1**). 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; **Supplemental Table 1**).

Arrhythmic Risk Associations

Univariable Analyses

Probands possessing a rare *KCNE1* variant had a 6.6-fold (95% confidence intervals [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 > 500ms tertile exhibited a statistically significant association for the composite arrhythmic outcome without syncope, respectively (**Table 2** and **Supplemental Figure 1**). Neither sex (**Figure 3**), nor β-blocker therapy, nor missense variant location within the KCNE1-encoded Kv7.1 β subunit (**Supplemental Figure 2)** were associated with an altered

risk of the composite arrhythmic outcomes 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 (**Supplemental Figure 3**).

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 (**Supplemental Table 2**).

Multivariable Analysis

A multivariable Cox regression model was constructed including the variables for familial status, sex, QTc tertile on presenting ECG, β-blocker therapy, and location of the missense variant within the *KCNE1*-encoded Kv7.1 β 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 (**Supplemental Table 2**).

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 $\pm$ 43.5ms versus 455.6 $\pm$ 38.2ms, 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 7,844 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, 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%), post-exertion 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; **Supplemental Table 3**). 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 (**Supplemental Table 3**). 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 PolyPhen-2, SIFT, and CADD (**Supplemental Table 3**). PolyPhen-2 and SIFT 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 CADD score greater than 20, predicting their being among the top 1% of most damaging variants within the genome.[31] 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 (**Supplemental Table 3**). 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 (**Supplemental Table 3**) and each was consistent with a loss-of-function.[10,19,20]

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 and/or non-genetic factors to manifest with a positive LQTS phenotype. However in contrast to *KCNE2*[14], 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.[32]

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.[33] 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 to 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 hazard ratio reported here for LQT5.[34]

Aside from familial status, no other intrinsic clinical or genetic factors, including QTc on presenting ECG, sex, β-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 β-blocker during their lifetime and the mean QTc of those administered β-blockade was 464.4 ± 39.0 ms in comparison with a mean value of 439.4 ± 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 β-blocker.

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 (**Supplemental Figure 2**).[10,19]

Attempted evaluation of the *KCNE1* variants using ACMG criteria was ultimately deemed inappropriate due to their low penetrance given that ACMG criteria are tailored for

highly penetrant variants.[27] 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 (**Supplemental Table 3**). 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 on the basis of their apparent excess prevalence is appropriate.[15]

The study also builds upon prior work and provides additional insight into the JLNS2 phenotype.[18] 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.[18] 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 β-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 β-subunit may either exert a modest role in cardiac repolarization or, alternatively, the heart, in contrast to the inner ear, may have established a redundancy for β-subunits that allows for effective compensation in response to the

loss of one constituent. The notion that a single β-subunit may be able to interact interchangeably with multiple pore forming α-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 α-subunit.[5,11,12]

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 β-blocker regardless of phenotype[35], 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[13], in the presence of a normal phenotype intensive measures such as β-blockade and exercise restriction may not be merited. Although a protective effect of β-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 β-blocker therapy should still be recommended in the presence of a positive LQTS phenotype. Due to 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 due to worrisome clinical findings and likely led to selection of a malignant subset of *KCNE1* heterozygotes and a correspondingly inflated arrhythmic event rate. In addition, evaluation for a potential protective effect of β-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 identification of a rare *KCNE1* loss-of-function variant, clinical management should consist of meticulous evaluation for an LQTS phenotype and counselling regarding the avoidance of QT prolonging drugs.

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

	LQT5				JLNS2
Clinical Variable	**Overall n = 229**	**Probands n = 89**	**Genotype +ve FM n = 140**	**p value***	**n = 19**
Age at First ECG (years)	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					
β-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). *p-value compares LQT5 probands and family members. LQT5 = Type 5 Long QT syndrome, JLNS2 = Type 2 Jervell and Lange-Nielsen Syndrome, Genotype +ve FM = genotype positive family members, ms = milliseconds, LCSD = left cardiac sympathetic denervation, ICD = implantable cardioverter defibrillator, CAO = composite arrhythmic outcome

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 β-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

* β-blocker treated as a time dependent covariable. ICD = implantable cardioverter defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, HR = hazard ratio, CI = confidence interval, ms = milliseconds.

Figure Legends

Figure 1. ECG Penetrance of Rare *KCNE1* Variants. Penetrance is defined as a QTc > 460ms on their presenting ECG. (N) indicates the number of individuals with the *KCNE1* variant

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. ICD = implantable cardioverter-defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, ref = reference, HR = hazard ratio, CI = confidence intervals.

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. ICD = implantable cardioverter-defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, ref = reference, HR = hazard ratio, CI = confidence intervals.

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. JLNS2 = Type 2 Jervell and Lange-NieIsen syndrome, ICD = implantable cardioverter-defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, ref = reference, HR = hazard ratio, CI = confidence intervals.