1   **An International Multi-Center Evaluation of Type 5 Long QT Syndrome:**

2   **A Low Penetrant Primary Arrhythmic Condition**

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5   **Short Title**: Evaluation of *KCNE1*-Associated LQT5

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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 effects of clinical and genetic predictors on 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 rates did not differ significantly for JLNS2 patients relative to the

45   overall heterozygous cohort (10.5% [2/19]; HR: 1.7, 95% CI: 0.3-10.8, p=0.590). The

46   cumulative prevalence of the 32 *KCNE1* variants in gnomAD, a human database of

47   exome and genome sequencing, was 119-fold greater than the anticipated prevalence of

48   all LQT5 combined (0.119% vs. 0.001%).

49   **Conclusions**: The present study suggests that putative/confirmed loss-of-function

50   *KCNE1* variants predispose to QT-prolongation, however the low ECG penetrance

51   observed suggests they do not manifest clinically in the majority of individuals, aligning

52   with the mild phenotype observed for JLNS2 patients.

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Introduction

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70   Long QT syndrome (LQTS) is an inherited channelopathy characterized by

71   impaired cardiac repolarization that confers an increased risk of syncope and sudden

72   cardiac death (SCD) secondary to torsades de pointes.[1] The prevalence of LQTS is 1 in

73   2,000 and 17 genes have been implicated in its pathogenesis, though the majority of cases

74   are due to mutations within *KCNQ1* (LQT1)*, KCNH2* (LQT2)*,* and *SCN5A* (LQT3),

75   considered the major LQTS genetic subtypes.[2–4] The *KCNQ1* gene encodes the Kv7.1 α-

76   subunit responsible for the slow component of the delayed rectifier potassium current

77   (I_{Ks}), whereas the Kv11.1 α-subunit of the rapid component of the delayed rectifier

78   potassium current (I_{Kr}) is encoded by *KCNH2*.[5–7] Loss-of-function mutations within

79   these voltage-gated potassium channels impair ventricular repolarization during Phase 3

80   of the cardiac action potential leading to LQT1 and LQT2.[8,9] In contrast, gain-of-

81   function mutations within *SCN5A*, the gene encoding the α-subunit of $Na_v1.5$ responsible

82   for mediating the cardiac sodium current (I_{Na}), cause LQT3 secondary to pathological

83   increases in late inward sodium current that prolongs repolarization.[10] Treatment with

84   mexiletine, a sodium channel blocker that reduces late inward sodium current, has been

85   shown to effectively shorten the QT-interval and reduce arrhythmic events in LQT3.[11,12]

86   LQT5 is the 4th most common LQTS genetic subtype and is felt to account for ~

87   1-2% of LQTS cases. LQT5 develops secondary to loss-of-function variants within

88   *KCNE1*, which encodes minK, a voltage-gated potassium channel β-subunit felt to

89   primarily interact with the Kv7.1 α-subunit responsible for I_{Ks}, though reports have also

90   suggested a role for minK in I_{Kr} through an interaction with the Kv11.1 α-subunit.[5,13–15]

91   The most intensively investigated KCNE1 rare variant, p.Asp76Asn, has been implicated

92   in both canonical and drug-induced forms of LQTS.[13,16] The relative rarity of LQT5 has

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93 led to limited insight into its clinical and genetic attributes and management is often

94 extrapolated from knowledge of the major LQTS subtypes. Recent work has revealed

95 that loss-of-function variants in *KCNE2*, another voltage-gated potassium channel β-

96 subunit, are more aptly characterized as arrhythmia predisposing variants or functional

97 risk alleles, leading to recognition that LQT6 is not a monogenic form of LQTS and a

98 corresponding alteration to the treatment approach for individuals possessing these

99 variants.[17,18] The *KCNE2* and *KCNE1* genes have many similarities, though only *KCNE1*

100 loss-of-function homozygotes and compound heterozygotes manifest with sensorineural

101 deafness in association with QT-prolongation, referred to as Type 2 Jervell and Lange-

102 Nielsen syndrome (JLNS2).[19–21] Notably, in contrast to the severe and often complete

103 loss-of-function observed for pathogenic *KCNQ1* and *KCNH2* mutations, the reductions

104 in cardiac potassium currents observed on experimental *in vitro* patch clamp analysis for

105 *KCNE2* and *KCNE1* variants have been modest.[13,22,23]

106   The growing recognition that each genetic LQTS subtype may require its own

107 tailored approach to management led to the pursuit of an international multi-center

108 collaboration to further define the clinical and genetic features of LQT5.[11,12,24–26]

109 **Methods**

110 Study Population

111   The study population consisted of 4 LQTS registries, including the Canadian

112 LQTS registry, the Rochester (New York) LQTS registry, the Japanese LQTS registry,

113 and the National Cardiac Inherited Disease Registry of New Zealand, along with 22

114 inherited arrhythmia clinics from 9 countries. Care was taken to ensure that no study

115 participants were included twice through consultation with study investigators. Inclusion

116   criteria for living probands required the presence of a rare *KCNE1* variant, defined as an

117   allele frequency < 0.1% in the Genome Aggregation Database (gnomAD; a database

118   comprised of 141,456 individuals from multiple population-based and disease-specific

119   genetic cohort studies),[27] and presence of a resting QTc >460ms on a surface ECG. A

120   threshold for allele frequency < 0.1% was chosen to restrict variants to those with a

121   prevalence that could be compatible with a low penetrant form of LQTS. Genotype

122   positive family members identified on cascade screening, which refers to clinical and

123   genetic evaluation of blood relatives at risk of being affected, were also included.

124   Cases of SCD that remained unexplained following cardiac autopsy were eligible

125   for inclusion when molecular autopsy identified a rare *KCNE1* variant that had been

126   observed in at least one living proband in our study that possessed a QTc > 460ms on

127   ECG. Homozygotes and compound heterozygotes of rare *KCNE1* variants that exhibited

128   sensorineural deafness consistent with JLNS2 were also eligible for the study. All living

129   probands presenting with an arrhythmic event were required to have undergone clinical

130   testing with an ECG, exercise treadmill test, and echocardiogram, at minimum, and

131   exhibit no evidence of another channelopathy or cardiomyopathy. Probands entered into

132   the study were also required to have undergone screening of all exons and associated

133   exon-intron boundaries within the *KCNQ1, KCNH2, SCN5A, KCNE1,* and *KCNE2* genes.

134   Exclusion criteria for living probands and genotype positive family members

135   consisted of a pathogenic or likely pathogenic mutation, as per American College of

136   Medical Genetics and Genomics (ACMG) guidelines, in another LQTS gene and

137   deceased probands were excluded when a pathogenic or likely pathogenic mutation was

138   identified in a gene known to be causative for either a cardiac channelopathy or

139 cardiomyopathy.[28] Individuals possessing the known loss-of-function, pro-arrhythmic

140 risk allele KCNE1-p.Asp85Asn in isolation were not included due to its presence in 0.1-

141 2.5% of the general population (depending on ancestry; 1.6% in European ancestry

142 subjects) and its being considered too common to function as a monogenic culprit for

143 LQTS.[18,29]

144   The following variables were collected retrospectively for all living probands and

145 genotype positive family members when available: date of birth, date of initial

146 presentation, reason for presentation, sex, familial status (proband versus family

147 member), Bazett corrected QT-intervals (QTc) recorded on ECGs at initial presentation

148 and during follow-up, date at the time of cardiac events (including presumed cardiac

149 syncope, appropriate implantable cardioverter defibrillator [ICD] shock, aborted cardiac

150 arrest [ACA] requiring resuscitation, and SCD with normal cardiac autopsy), activity at

151 the time of the cardiac event, secondary QT stressors present at the time of the cardiac

152 event (including QT prolonging medication, electrolyte abnormality, and heart block),

153 and details of β-blocker usage, including dates of initiation and discontinuation, if

154 applicable. Genetic details of the *KCNE1* variant, including the nucleotide and amino

155 acid change, were obtained for each case.

156   The study was performed as part of a protocol approved by the research ethics

157 boards of Western University, London, Ontario, Canada and the collaborating

158 institutions. All study participants provided informed consent for their clinical and

159 genetic data to be used for research.

160 Assessment of ECG Penetrance and Genotype-Phenotype Segregation

161   ECG penetrance was assessed in genotype positive family members. Consistent

162   with prior work, an electrocardiographically manifest (penetrant) LQTS phenotype was

163   defined as a QTc value on the presenting ECG > 460ms.[24] Evaluation for genotype-

164   phenotype segregation was performed in each family in an effort to clarify the role of rare

165   *KCNE1* variants in predisposing to QT-prolongation and was considered present if 2 or

166   more individuals possessing the variant were phenotype positive.

167   Evaluation of *KCNE1* Variants

168   All *KCNE1* variants included in the study were subjected to computer-based

169   analyses and their prevalence in the general population and among individuals of

170   European ancestry in isolation was assessed using gnomAD.[27] Computer model

171   predicting effects of mutations on protein function was performed using Polymorphism

172   Phenotyping v2 (PolyPhen-2), Sorting Intolerant From Tolerant (SIFT), and Combined

173   Annotation Dependent Depletion (CADD).[30–32] Prior *in vitro* functional analyses of

174   *KCNE1* variants reported in the literature were reviewed. Variants were presumed to be

175   loss-of-function if they manifested with sensorineural deafness consistent with a JLNS2

176   phenotype when present in a homozygous or compound heterozygous state.

177   Although variant classification was performed according to ACMG guidelines,

178   this was ultimately deemed inappropriate secondary to the low level of penetrance

179   observed for *KCNE1* variants; ACMG criteria have been designed for classification of

180   highly penetrant variants.[28]

181   Statistical Analysis

182   Continuous variables are presented as means $\pm$ standard deviation and those

183   exhibiting normal and non-normal distributions were compared using Student's t-test and

184   the Wilcoxon rank-sum test, respectively. Comparison of categorical values was

185   performed using Fisher's exact test. Cox proportional hazards models were used to

186   estimate the associations between clinical and genetic variables and age at first presumed

187   primary arrhythmic event (composite of presumed cardiac syncope, appropriate ICD

188   shock, ACA, or SCD with normal autopsy; subsequently referred to as the composite

189   arrhythmic outcome with syncope) and the first definite primary arrhythmic event

190   (composite of appropriate ICD shock, ACA, or SCD with normal autopsy; subsequently

191   referred to as the composite arrhythmic outcome without syncope) among heterozygotes

192   possessing rare *KCNE1* variants and JLNS2 patients.

193   Variables evaluated in both uni-/multivariable analyses included familial status

194   (proband versus family member), sex, QTc on initial presenting ECG, β-blocker therapy,

195   and missense variant location (extracellular, transmembrane, intracellular) in the *KCNE1*-

196   encoded β-subunit. The QTc on the initial presenting ECG was treated as a categorical

197   variable divided into tertiles (<470 ms, $\geq$ 470 ms but $\leq$ 500 ms, and > 500 ms).

198   Cumulative years on β-blocker therapy was treated as a time-dependent covariable in

199   order to account for patients starting and stopping treatment throughout their lifetime and

200   enabled comparison of event rates during time on β-blocker therapy relative to time off β-

201   blocker therapy. Risk of arrhythmic events was also evaluated based on KCNE1-

202   p.Asp76Asn variant status (KCNE1-p.Asp76Asn carriers versus carriers of another

203   *KCNE1* variant). Robust standard errors were used to account for familial relatedness.

204   Two-tailed p-values < 0.05 were considered statistically significant. Statistical analyses

205   were performed using Stata version 16 (College Station, TX, USA).

206   **Results**

Study Population

208   Eighty-nine probands heterozygous for a rare *KCNE1* variant in the setting of a

209   phenotype compatible with LQTS and 140 genotype positive family members were

210   enrolled into the study (**Table 1**). The mean age at the time of first ECG was 25.4 ± 19.7

211   years and 61.6% were female. The mean QTc on the presenting ECG among probands

212   was significantly longer relative to genotype positive family members (476.9 ± 38.6ms

213   vs. 441.8 ± 30.9ms, p< 0.001). β-blocker therapy was used at some point in 78.7% of

214   probands and 55.0% of genotype positive family members. A total of 41.6% of probands

215   experienced a presumed cardiac event during their lifetime, defined as presumed cardiac

216   syncope, appropriate ICD shock, ACA, or SCD, compared to only 5.7% of *KCNE1*

217   variant-positive family members (p<0.001). The number of individuals that experienced

218   each of these events is provided in Table 1. Within the overall heterozygous cohort, the

219   median ages of onset of the composite arrhythmic outcomes with and without syncope

220   were 24.6 and 40.9 years, respectively.

221   The KCNE1-p.Asp76Asn variant was present in 98 of 229 heterozygous

222   individuals (42.8%) and the mean QTc among carriers (455.1 ± 35.5ms) was similar to

223   the mean QTc value observed among the remaining individuals in the heterozygous

224   cohort (455.9 ± 40.2ms, p = 0.873). An additional 19 JLNS2 individuals, including 15

225   homozygotes and 4 compound heterozygotes, were enrolled into the study and their

226   clinical features are reported in **Table 1**. The composite arrhythmic outcome with

227   syncope was experienced in a total of 13.3% (2/15) of homozygotes and 50% (2/4) of

228   compound heterozygotes.

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229   Among *KCNE1* heterozygotes, only 2 genotype positive family members had

230   definite arrhythmic events. One was an asymptomatic male diagnosed with LQTS at 15

231   years of age following cascade screening for the KCNE1-p.Gly55Ser variant (gnomAD

232   allele frequency in Europeans = 0.003582%). His presenting QTc was 488ms and he

233   subsequently underwent ICD implantation due to family preference following the ACA

234   of his sister. He was initiated on atenolol and had an appropriate ICD shock for torsades

235   de pointes while at rest at 19 years of age in the absence of a QT-prolonging stressor. His

236   QTc at the time of the event was 505ms and his QTc values following his initial

237   presentation have ranged from 476ms to 512ms.

238   The second family member that had a definite arrhythmic event was a previously

239   asymptomatic male that possessed the KCNE1-p.Asp76Asn variant (gnomAD allele

240   frequency in Europeans = 0.01106%) and was diagnosed with LQT5 as part of cascade

241   screening at 50 years of age. His QTc on ECG at the time of diagnosis was 431ms and

242   no subsequent ECGs were available for review. A β-blocker was not initiated and he

243   died suddenly during long distance running at 61 years of age, had a normal cardiac

244   autopsy (including normal coronary arteries), and history from family indicated he had

245   not been exposed to a QT-prolonging drug. His fatal event at an older age may serve to

246   illustrate the persistent arrhythmic risk that LQTS confers throughout a lifetime,[33] though

247   it is also acknowledged that a normal autopsy does not completely exclude other potential

248   cardiac etiologies that may manifest clinically as SCD.

249   Disease Penetrance and Genotype-Phenotype Segregation

250   Disease penetrance was assessed in genotype positive family members based on

251   the definition for an electrocardiographically manifest LQTS phenotype being a QTc

252 value > 460ms on presenting ECG. The overall penetrance was 20.7% (29/140).

253 Penetrance values for each individual *KCNE1* variant possessed in a heterozygous state

254 by a family member are illustrated in **Figure 1**. Among the 10 *KCNE1* variants

255 possessed by ≥ 3 individuals, penetrance values ranged from 0% (p.Asn5Ter and

256 p.Thr7Ile) to 75% (p.Gly55Ser). The KCNE1-p.Asp76Asn variant, present in a

257 heterozygous state in 63 family members, exhibited an overall penetrance of 17.5%.

258 Among JLNS2 patients, the electrocardiographic penetrance was 66.7% (10/15) in

259 homozygotes and 75% (3/4) in heterozygotes.

260 Genotype-phenotype segregation was assumed to be present if at least 2

261 individuals in a single family were phenotype positive. Thirteen of 52 (25%) families

262 with at least 2 genotype positive individuals possessed evidence of genotype-phenotype

263 segregation (**Supplemental Table 1**). Genotype-phenotype segregation was observed for

264 8 *KCNE1* variants (KCNE1-p.Gln22Ter, -p.Ser28Leu, -p.Tyr46Cys, -p.Gly55Ser, -

265 p.Arg67Cys, p.Arg67His, -p.Asp76Asn, and -p.Val109Ile; **Supplemental Table 1**).

266 <u>Predictors of Arrhythmic Risk</u>

267 *Univariable Analyses*

268 Probands possessing a rare *KCNE1* variant had a 6.63-fold (95% confidence

269 intervals [CI]: 3.6-12.3, p< 0.001) higher hazard of experiencing the composite

270 arrhythmic outcome with syncope relative to genotype positive family members (**Figure

271 2A** and **Table 2**) and a 11.2-fold (95% CI: 2.9-43.2, p<0.001) higher hazard of the

272 composite arrhythmic outcome without syncope (**Figure 2B** and **Table 2**). Evaluation of

273 QTc values on presenting ECG revealed that the upper 2 tertiles were both associated

274 with a higher risk of the composite arrhythmic outcome with syncope, whereas only the

275 QTc > 500ms tertile exhibited a statistically significant association for the composite

276 arrhythmic outcome without syncope, respectively (**Table 2** and **Supplemental Figure**

277 **1**). Neither sex (**Figure 3**), nor β-blocker therapy, nor missense variant location within

278 the KCNE1-encoded Kv7.1 β subunit (**Supplemental Figure 2)** were associated with an

279 altered risk of the composite arrhythmic outcomes on univariable analysis (**Table 2**). The

280 arrhythmic risk associated with the p.Asp76Asn variant, the most prevalent *KCNE1*

281 variant in the cohort carried by 42.8% of heterozygotes, did not differ statistically relative

282 to the collective remainder of the *KCNE1* variants evaluated (**Supplemental Figure 3**).

283   Univariable analyses for probands in isolation revealed measures of association

284 that were generally consistent with the overall heterozygous cohort with no point

285 estimates that extended beyond the 95% CI boundaries (**Supplemental Table 2**).

286 <u>Multivariable Analysis</u>

287   A multivariable Cox regression model was constructed including the variables for

288 familial status, sex, QTc tertile on presenting ECG, β-blocker therapy, and location of the

289 missense variant within the *KCNE1*-encoded Kv7.1 β subunit. Following adjustment,

290 familial status was the only predictor that continued to exhibit a statistically significant

291 association for the arrhythmic outcomes (**Table 2**). Similar results were obtained for

292 probands in isolation with no point estimates that extended beyond the 95% CI

293 boundaries for the overall heterozygous cohort (**Supplemental Table 2**).

294 <u>JLNS2 Arrhythmic Outcomes</u>

295   The mean QTc values on presenting ECG in JLNS2 patients trended towards

296 being longer relative to individuals possessing a *KCNE1* variant in a heterozygous state,

297 but did not reach statistical significance (471.1 ± 43.5ms versus 455.6 ± 38.2ms, p =

298 0.050) (**Table 1**). JLNS2 patients had event rates that also did not exhibit statistically

299 significant differences relative to *KCNE1* heterozygotes for the composite arrhythmic

300 outcomes including syncope (HR = 1.2, 95% CI 0.2-6.4, p= 0.800, **Figure 4A**) and

301 excluding syncope (HR = 1.7, 95% CI 0.3-10.8, p= 0.590, **Figure 4B**).

302 <u>Secondary QT Stressors and Triggers for Cardiac Events</u>

303   A total of 62 cardiac events were experienced among the entire cohort during a

304 collective 7,844 patient years beginning from birth. Three events were reported to have

305 occurred in the setting of a QT-prolonging medication, 1 in the context of a severe

306 electrolyte abnormality, and 1 was attributed to torsades de pointes in the setting of

307 complete heart block. No secondary QT-prolonging stressors were identified in

308 association with the remaining events. Activities reported at the time of events included

309 awake at rest in 37 (60.0%), exertion in 17 (27.4%), auditory stimuli in 2 (3.2%), post-

310 exertion in 1 (1.6%), sleep in 1 (1.6%), and the activity at the time of the event was

311 unknown in 4 (6.5%).

312 <u>Evaluation of *KCNE1* Variants</u>

313 *Population Allele Frequencies*

314   Among the 32 *KCNE1* variants possessed by the study participants, 22 were

315 observed in gnomAD, with individual allele frequencies ranging up to 0.02094% for the

316 Thr10Met variant (0.02134% when restricted to European ancestry; **Table 3**). The

317 collective prevalence of these variants in the overall gnomAD cohort was 0.119% and

318 0.084% among the European ancestry subgroup. Based on the assumptions that the

319 prevalence of LQTS is 0.05% and LQT5 accounts for 2% of LQTS, its prevalence is

320 estimated at 0.001%. The collective prevalence of *KCNE1* variants implicated in LQT5

321   is 119-fold the anticipated prevalence of LQT5 when the overall gnomAD cohort is

322   considered and 84-fold when the analysis is restricted to individuals of European

323   ancestry.

324   Eight of the 32 *KCNE1* variants were observed in JLNS2, confirming their status

325   as loss-of-function given their being causative for sensorineural deafness (**Table 3**). The

326   collective prevalence of *KCNE1* variants identified in the context of JLNS2 in the overall

327   gnomAD cohort was 0.0081% and 0.0120% among Europeans.

328   *Computer-Based and Previously Reported In Vitro Analyses*

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

330   performed using PolyPhen-2, SIFT, and CADD (**Table 3**). PolyPhen-2 and SIFT both

331   identified 14 of 24 missense variants as probably/possibly damaging or damaging,

332   respectively. A total of 18 of 27 single nucleotide variants had a CADD score greater

333   than 20, predicting their being among the top 1% of most damaging variants within the

334   genome.[32] Classification of the variants using the 2015 ACMG guidelines identified 3 as

335   pathogenic, 5 as likely pathogenic, 17 as a variant of unknown significance, and 7 as

336   likely benign (**Supplemental Table 3**). Assignment of likely benign status to 7 variants

337   was primarily driven by their minor allele frequencies being greater than the anticipated

338   prevalence of LQT5 (0.001%), which is not considered appropriate when variant

339   penetrance is anticipated to be low. On review of the literature, *in vitro* patch-clamping

340   analysis using heterologous expression of mutant *KCNE1* in association with wild-type

341   *KCNQ1* had been performed for only 4 of 25 *KCNE1* missense variants (**Table 3**) and

342   each was consistent with a loss-of-function.[13,22,23]

343   **Discussion**

344   This international multicenter study represents the first large-scale evaluation of

345   rare *KCNE1* variants implicated as monogenic culprits for LQTS. Their low ECG

346   penetrance in family members, coupled with their excess prevalence in gnomAD,

347   suggests that loss-of-function *KCNE1* variants do not manifest clinically in a majority of

348   individuals. The benign phenotype observed in the vast majority of genotype positive

349   family members differs markedly from the more severe phenotype observed in probands

350   and strongly suggests that loss-of-function *KCNE1* variants require additional genetic

351   and/or non-genetic factors to manifest with a positive LQTS phenotype. However in

352   contrast to *KCNE2*[17], QT-prolongation and clinical events occurred in the overwhelming

353   majority of individuals in the absence of an identifiable QT prolonging stressor,

354   suggesting that LQT5 should be viewed as a low penetrant primary arrhythmic condition

355   rather than an exclusively provoked syndrome. These findings have important clinical

356   implications for probands and genotype positive family members.

357   Evaluation of arrhythmic events among probands revealed that 41.6% of probands

358   experienced the composite arrhythmic outcome including syncope and 16.9% had

359   suffered a confirmed, potentially lethal ventricular arrhythmia. These findings initially

360   suggested that LQT5 may be a highly malignant disorder, however mirroring prior work

361   in LQTS, the striking event rate observed among probands differed dramatically relative

362   to the findings among genotype positive family members.[34] Among 140 genotype

363   positive family members evaluated, 6 had experienced syncope and only 2 (1.4%) had

364   suffered a definite arrhythmic event in the form of an appropriate ICD shock or SCD over

365   a total of 4,670 patient years of follow up. This disparate natural history was mirrored by

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the markedly greater QTc values observed on the presenting ECGs in probands relative to

genotype positive family members (476.9ms versus 441.8ms, p<0.001).

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 and not reflective

of the true arrhythmic risk intrinsic to *KCNE1* loss-of-function variants. 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

between a 2.48- to 3.21-fold increased hazard of 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 observed in the LQT5

cohort in this study.[35]

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

389   confounding by indication, coupled with the low event rate, may have led to the lack of

390   an apparent protective effect with β-blocker.

391   Although the findings from the current study serve as strong evidence that many

392   *KCNE1* variants are insufficient in isolation to cause LQTS, it could be argued that only a

393   minority of these variants have undergone functional work and hence the physiological

394   relevance for the majority is unclear. Eight of the 32 variants were observed among cases

395   of JLNS2 providing definitive evidence for their being loss-of-function. Penetrance of

396   these variants was 15.7% among family members, which was consistent with findings

397   from the overall sample (20.7%). In addition, QTc values and event rates among study

398   participants possessing the most prevalent *KCNE1* variant (p.Asp76Asn), known to be

399   loss-of-function and present in 98 of the 229 heterozygous individuals, were consistent

400   with those from the remainder of the cohort (**Supplemental Figure 2**).[13,22]

401   Attempted evaluation of the *KCNE1* variants using ACMG criteria was ultimately

402   deemed inappropriate due to their low penetrance given that ACMG criteria are tailored

403   for highly penetrant variants.[28] Notably, the KCNE1-p.Asp76Asn variant has a

404   prevalence among individuals with European ancestry of 0.01106%, which exceeds the

405   anticipated prevalence of LQT5 (0.001%) by >11-fold. A greater than expected allele

406   frequency for the disorder being evaluated is considered a strong ACMG criterion for

407   classifying a variant as benign. Although the p.Asp76Asn variant had sufficient

408   additional supporting evidence to still receive a likely pathogenic designation, 7 *KCNE1*

409   variants were demoted to likely benign status primarily owing to their prevalence being

410   greater than anticipated for LQT5 (**Supplemental Table 3**). In the collective view of the

411   investigators, given that KCNE1-p.Asp76Asn is an established genetic culprit for LQT5,

412   it is not felt that demotion of other variants with similar allele frequencies to likely

413   benign status on the basis of their apparent excess prevalence is appropriate.[18]

414   The study also builds upon prior work and provides additional insight into the

415   JLNS2 phenotype.[21] In contrast to JLNS1, an autosomal recessive condition secondary to

416   homozygous or compound heterozygous *KCNQ1* loss-of-function mutations and

417   characterized by marked QT prolongation and a highly malignant arrhythmic phenotype,

418   the phenotype of JLNS2 appeared surprisingly mild, which aligns with earlier work.[21]

419   Although the apparent lack of an effect on phenotypic severity for increasing gene dosage

420   may be secondary to inadequate power given that only 19 JLNS2 patients were included

421   in the study, the finding that JLNS2 has a relatively mild phenotype lends further support

422   to dysfunction of the *KCNE1*-encoded β-subunit often being clinically concealed..

423   Although a functional copy of *KCNE1* is necessary for sensorineural hearing, the

424   findings from this study suggest that the *KCNE1*-encoded β-subunit may either exert a

425   modest role in cardiac repolarization or, alternatively, the heart, in contrast to the inner

426   ear, may have established a redundancy for β-subunits that allows for effective

427   compensation in response to the loss of one constituent. The notion that a single β-

428   subunit may be able to interact interchangeably with multiple pore forming α-subunits is

429   alluded to by evidence that minK not only contributes to $I_{Ks,}$ but also I_{Kr} through an

430   interaction with the Kv11.1 α-subunit.[5,14,15]

431   Whereas possessing a pathogenic mutation causative for the major genetic LQTS

432   subtypes results in a diagnosis of LQTS and most often triggers initiation of a β-blocker

433   regardless of phenotype[36], evidence from the current study suggests that an alternative

434   approach to management for individuals possessing a *KCNE1* rare variant in the absence

435 of an LQTS phenotype may be desired. While it is felt that all individuals possessing a

436 loss-of-function *KCNE1* variant should be advised to avoid QT-prolonging drugs[16], in the

437 presence of a normal phenotype intensive measures such as β-blockade and exercise

438 restriction may not be merited. Although a protective effect of β-blockade was not

439 observed in the study, given the potential limitations highlighted above that may have led

440 to both biased and underpowered results, it is felt that β-blockade should still be

441 recommended in the presence of a positive LQTS phenotype. Due to the presence of

442 study participants that experienced presumed arrhythmic events despite QTc values

443 considered within normal limits on presenting ECG, highlighting the limitations of a

444 single ECG to assess disease penetrance, it is advocated that all individuals possessing

445 true loss-of-function variants be followed for serial monitoring of QTc values. Routine

446 use of cascade screening for these variants is also advocated given their potential to

447 manifest with a malignant LQTS phenotype, as highlighted by the natural history of the

448 probands in the study.

449 Limitations

450   Although the largest dedicated evaluation for rare *KCNE1* variants to date, the

451 study may be underpowered to detect statistically significant associations for relevant

452 clinical and genetic predictors. As an observational study, it is also vulnerable to various

453 unavoidable forms of bias. The cohort consisted of probands referred to specialized

454 inherited arrhythmia clinics due to worrisome clinical findings and likely led to selection

455 of a malignant subset of *KCNE1* heterozygotes and a correspondingly inflated arrhythmic

456 event rate. In addition, evaluation for a potential protective effect of β-blocker therapy

457 will unavoidably be biased secondary to confounding by indication.

458   Conclusions

459   The present study reveals that *KCNE1* loss-of-function variants are weakly

460   penetrant and individuals manifesting with an LQTS phenotype in the presence of a loss-

461   of-function *KCNE1* variant likely possess additional genetic or environmental factors that

462   predispose to QT prolongation. In contrast to *KCNE2*, the overwhelming majority of

463   probands and genotype positive family members manifesting with QT-prolongation and

464   arrhythmic events did so in the absence of a QT-prolonging stressor suggesting that

465   LQT5 should be viewed as a low penetrant primary arrhythmic condition rather than an

466   exclusively provoked syndrome. Following identification of a rare *KCNE1* loss-of-

467   function variant, clinical management should consist of meticulous evaluation for an

468   LQTS phenotype and counselling regarding the avoidance of QT prolonging drugs.

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751 **Figure Legends**

752 **Figure 1:** ECG Penetrance of Rare *KCNE1* Variants Based on QTc > 460ms on
753 Presenting ECG

754 (N) indicates the number of individuals with the *KCNE1* variant

755 **Figure 2:** Arrhythmic Events Among Probands and Genotype Positive Family Members
756 Possessing a Rare *KCNE1* Variant

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

759 **Figure 3:** Arrhythmic Events Among Individuals Possessing a Rare *KCNE1* Variant
760 Stratified by QTc Tertiles

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

763 **Figure 4:** Arrhythmic Events Among Type 2 Jervell and Lange-Nielsen Syndrome
764 Patients and *KCNE1* Heterozygotes

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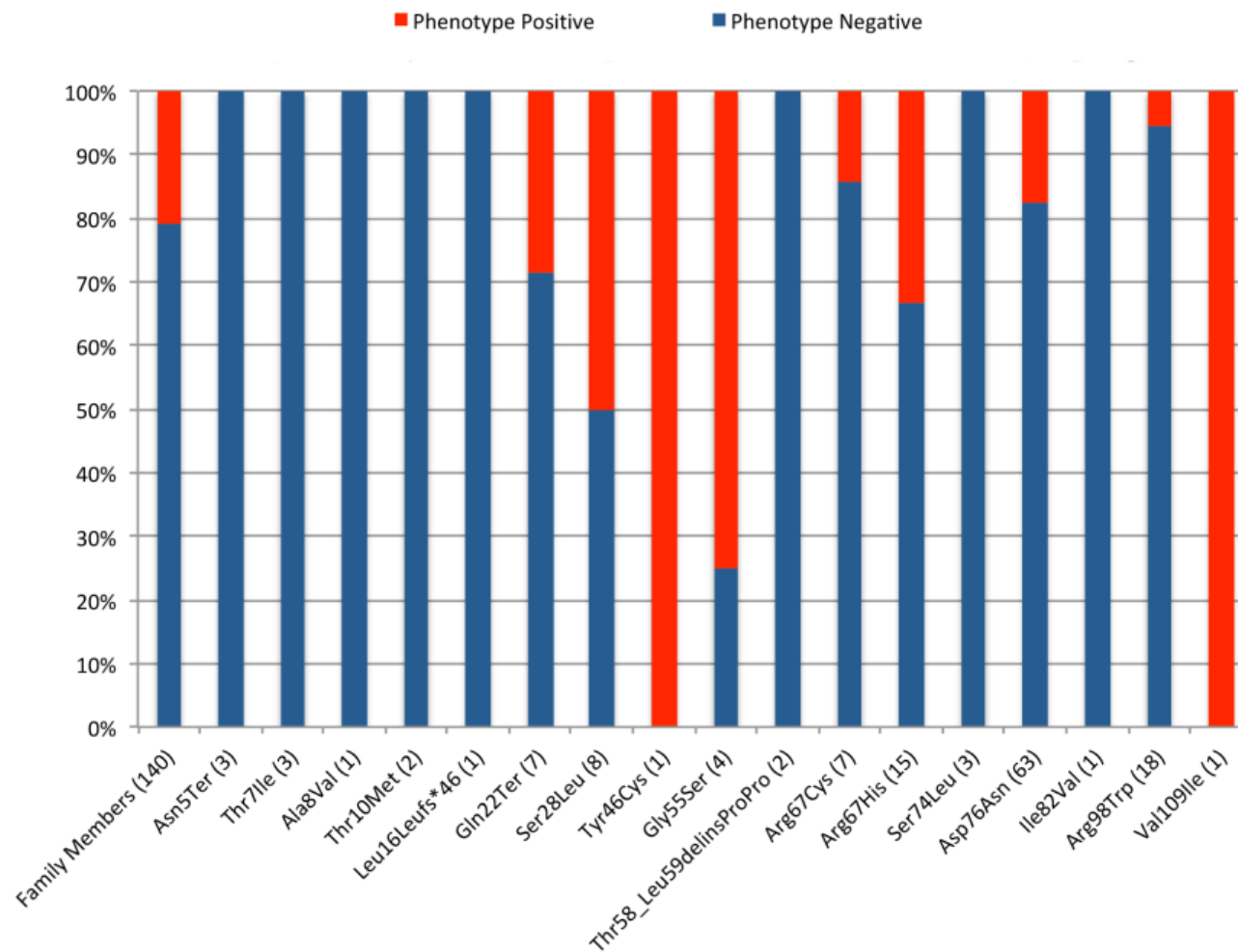767 HR = hazard ratio, CI = confidence intervals.

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Figure 1: ECG Penetrance of Rare *KCNE1* Variants Based on QTc > 460ms on Presenting ECG. (N) indicates the number of individuals with the *KCNE1* variant.
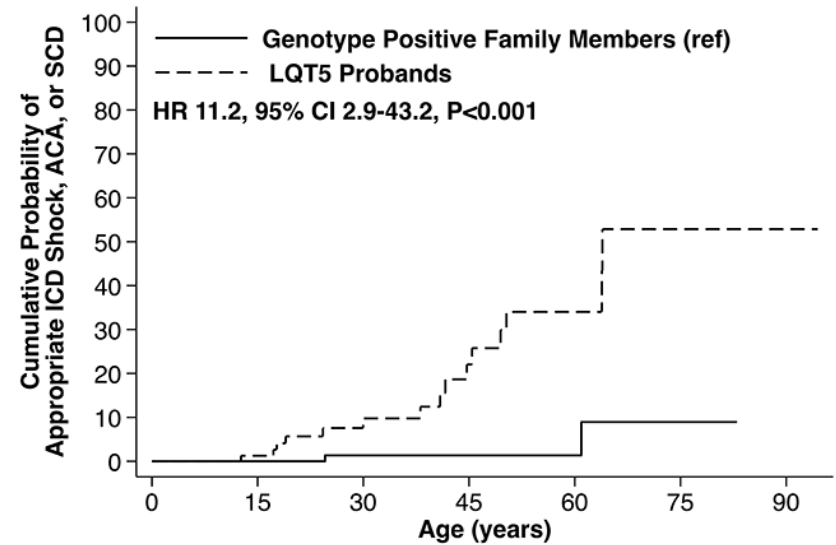
Figure 2: Arrhythmic Events Among Probands and Genotype Positive Family Members Possessing a Rare *KCNE1* Variant

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

ICD = implantable cardioverter-defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, ref = reference, HR = hazard ratio, CI = confidence intervals.
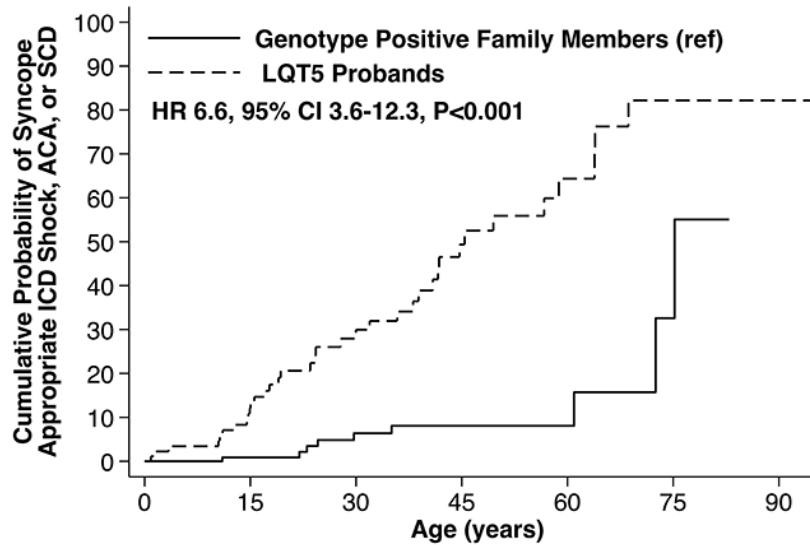
Figure 4: Arrhythmic Events Among Type 2 Jervell and Lange-Nielsen Syndrome Patients and *KCNE1* Heterozygotes

JLNS2 = Type 2 Jervell and Lange- Nielsen syndrome, ICD = implantable cardioverter-defibrillator, ACA = aborted cardiac arrest, SCD = sudden cardiac death, ref = reference, HR = hazard ratio, CI = confidence intervals.
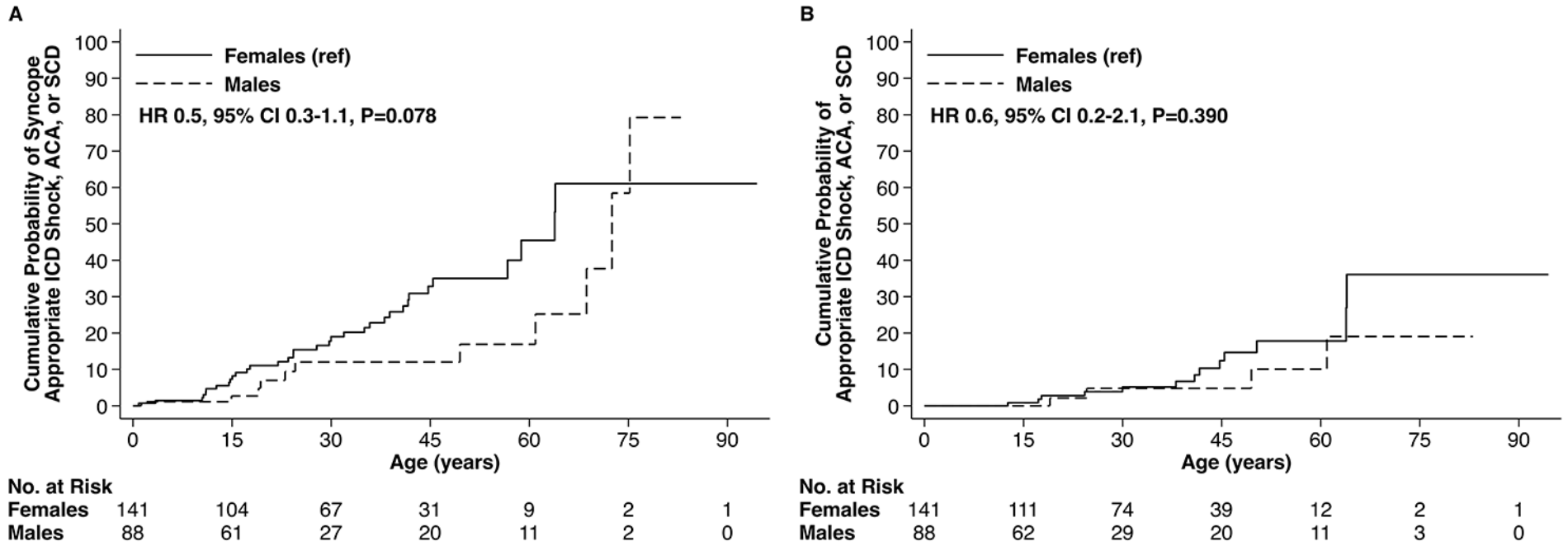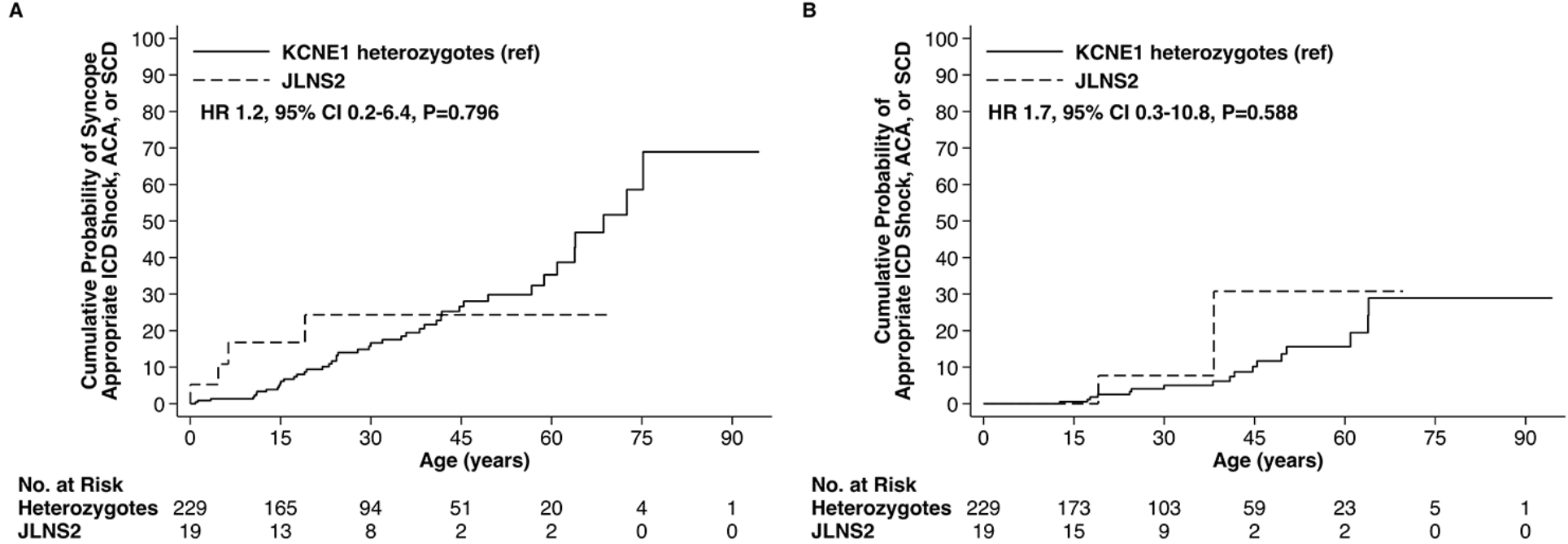
Table 1: Clinical Features of Probands and Genotype Positive Family Members Possessing Rare *KCNE1* Variants

Clinical Variable	Overall n = 229	LQT5 Probands n = 89	Genotype +ve FM n = 140	p value*	JLNS2 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)
Male (%)	88 (38.4)	30 (33.7)	58 (41.4)	0.211	10 (52.6)
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
Male Sex	0.5 (0.3-1.1)	0.08	1.1 (0.5-2.3)	0.75	0.6 (0.2-2.1)	0.39	2.5 (0.8-8.1)	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.

Table 3: Evaluation of Rare *KCNE1* Variants Implicated in Type 5 Long QT Syndrome.

| KCNE1 Variant | | Channel | gnomAD AF (%) | | In Silico Analysis | | | Functional Work | Documented JLNS2 |
Nucleotide	Amino Acid	Location	OA	EA	PP2	SIFT	CADD	(Ref)	Culprit
21:35,821,283-35,884,669*	Whole gene deletion	N/A	-	-	-	-	-	-	-
c.12dup	Asn5Ter	N/A	-	-	-	-	-	-	Y
c.20C>T	Thr7Ile	E	0.0004065	-	PrD	D	22.5	-	Y
c.23C>T	Ala8Val	E	0.01155	0.003952	B	T	4.126	-	-
c.29C>T	Thr10Met	E	0.02094	0.02134	B	T	0.007	-	-
c.48delG	Leu16LeufsTer46	N/A	-	-	-	-	-	-	Y
c.50G>A	Trp17Ter	N/A	0.0004063	-	-	-	37	-	Y
c.51G>A	Trp17Ter	N/A	0.0004063	0.0008960	-	-	36	-	Y
c.64C>T	Gln22Ter	N/A	-	-	-	-	36	-	-
c.83C>T	Ser28Leu	E	0.005414	0.007110	B	D	16.03	-	-
c.98G>T	Arg33Met	E	-	-	PoD	T	22.3	-	-
c.123G>C	Lys41Asn	E	0.0008123	-	B	T	14.01	-	-
c.137A>G	Tyr46Cys	T	0.003232	-	PrD	D	26.0	-	-
c.139G>T	Val47Phe	T	-	-	PoD	D	23.3	22	Y
c.152_153delinsAT	Leu51His	T	-	-	-	-	-	-	Y
c.158T>G	Phe53Cys	T	-	-	PrD	D	25.3	-	-
c.163G>A	Gly55Ser	T	0.01218	0.003582	PoD	T	23.6	-	-

c.172_177 delACCCCTGinsCCCCCT	Thr58_Leu59 delinsProPro	T	0.001443	0.002369	-	-	-	-	-
c.181A>G	Ile61Val	T	0.003232	0.006675	B	T	19.74	-	-
c.199C>T	Arg67Cys	I	0.002844	0.001792	PrD	D	33	-	-
c.200G>A	Arg67His	I	0.005774	0.004738	PrD	D	31	-	-
c.200G>T	Arg67Leu	I	0.0004062	0.0008958	B	D	25.1	-	-
c.209A>T	Lys70Met	I	0.0004062	-	PrD	D	26.5	-	-
c.221C>T	Ser74Leu	I	0.001804	0.001580	PrD	D	25.4	13	-
c.226G>A	Asp76Asn	I	0.006856	0.01106	PoD	D	24.0	13,22	Y
c.238G>A	Val80Ile	I	0.005412	0.004737	B	T	13.95	-	-
c.244A>G	Ile82Val	I	-	-	PrD	D	23.7	-	-
c.292C>T	Arg98Trp	I	0.002886	0.002368	PrD	D	25.2	-	-
c.293G>A	Arg98Gln	I	0.004468	0.001791	PoD	D	24.2	-	-
c.295G>C	Val99Leu	I	-	-	B	T	7.132	-	-
c.325G>A	Val109Ile	I	0.01408	0.005535	B	T	0.014	23	-
c.374C>T	Thr125Met	I	0.01414	0.003976	B	T	0.004	-	-

*GRCh37 Chr:position, AF = allele frequency, OA = overall, EA = European ancestry, JLNS2 = Type 2 Jervell and Lange-Nielsen Syndrome, E = extracellular, T = transmembrane, I = intracellular, PP2 = PolyPhen-2, Ref = reference, N/A = not applicable, Y = yes, PrD = probably damaging, D = damaging, B = benign, T = tolerated, PoD = possibly damaging.