ESC European Society of Cardiology

Europace (2023) **25**, 1491–1499
https://doi.org/10.1093/europace/euac269

CLINICAL RESEARCH
Long QT syndrome

Non-missense variants of *KCNH2* show better outcomes in type 2 long QT syndrome

Takanori Aizawa [1], Yuko Wada [2,3]*, Kanae Hasegawa [3,4], Hai Huang [1], Tomohiko Imamura [1], Jingshan Gao [1], Asami Kashiwa [1], Hirohiko Kohjitani [1], Megumi Fukuyama [2], Koichi Kato [2], Eri Toda Kato [1], Takashi Hisamatsu [5], Seiko Ohno [6], Takeru Makiyama[1], Takeshi Kimura [7], and Minoru Horie [2]

[1]Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Department of Cardiovascular Medicine, Shiga University of Medical Science, Setatsukinowacho, Otsu, Shiga 520-2192, Japan; [3]Department of Medicine, Vanderbilt University Medical Center, 2215B Garland Ave, 1275 MRBIV, Nashville, TN 37232, USA; [4]Department of Cardiovascular Medicine, Faculty of Medical Sciences, University of Fukui, Fukui, Japan; [5]Department of Public Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama, Japan; [6]Department of Bioscience and Genetics, National Cerebral and Cardiovascular Center, Osaka, Japan; and [7]Division of Cardiology, Hirakata Kohsai Hospital, Osaka, Japan

Received 24 October 2022; accepted after revision 27 November 2022; online publish-ahead-of-print 2 March 2023

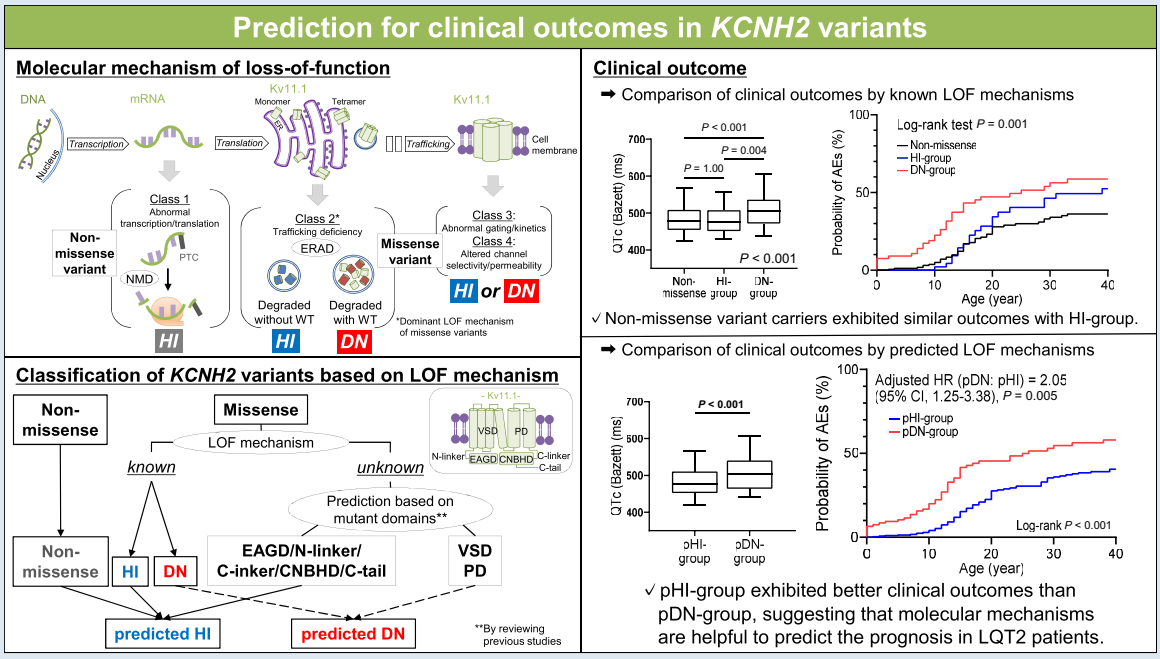
Aims More than one-third of type 2 long QT syndrome (LQT2) patients carry *KCNH2* non-missense variants that can result in haploinsufficiency (HI), leading to mechanistic loss-of-function. However, their clinical phenotypes have not been fully investigated. The remaining two-thirds of patients harbour missense variants, and past studies uncovered that most of these variants cause trafficking deficiency, resulting in different functional changes: either HI or dominant-negative (DN) effects. In this study, we examined the impact of altered molecular mechanisms on clinical outcomes in LQT2 patients.

Methods and results We included 429 LQT2 patients (234 probands) carrying a rare *KCNH2* variant from our patient cohort undergoing genetic testing. Non-missense variants showed shorter corrected QT (QTc) and less arrhythmic events (AEs) than missense variants. We found that 40% of missense variants in this study were previously reported as HI or DN. Non-missense and HI-groups had similar phenotypes, while both exhibited shorter QTc and less AEs than the DN-group. Based on previous work, we predicted the functional change of the unreported variants—whether they cause HI or DN via altered functional domains—and stratified them as predicted HI (pHI)- or pDN-group. The pHI-group including non-missense variants exhibited milder phenotypes compared to the pDN-group. Multivariable Cox model showed that the functional change was an independent risk of AEs ($P = 0.005$).

Conclusion Stratification based on molecular biological studies enables us to better predict clinical outcomes in the patients with LQT2.

* Corresponding author. Tel: +1 615 343 5612; fax: +1 615 343 2325. *E-mail address*: yuko.wada@vumc.org

Graphical Abstract



AE, arrhythmia event; CI, confidence interval; CNBHD, cyclic nucleotide-binding homology domain; DN, dominant-negative; EAGD, *ether-à-go-go* domain; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; HR, hazard ratio; HI, haploinsufficiency; LOF, loss-of-function; LQT2, long QT syndrome type 2; mRNA, messenger RNA; NMD, nonsense mediated mRNA decay; PD, pore domain; pDN, predicted DN; pHI, predicted HI; PTC, premature termination codon; QTc, corrected QT interval; VSD, voltage sensor domain; WT, wild-type.

Keywords Long QT syndrome • *KCNH2* • Arrhythmia • Prognosis • Molecular mechanism

What's new?

- More than 30% of type 2 long QT syndrome (LQT2)-related *KCNH2* variants are non-missense variants, presumably causing haploinsufficiency that are not amenable to conventional functional assays; thus, the impact of functional consequences of non-missense variants on clinical outcomes has been unclear.
- We found that patients with a non-missense variant showed better arrhythmia outcomes than those with a rare LQT2-causing missense variant. Furthermore, we found that missense variants in certain functional domains caused similar phenotypes and outcomes as non-missense variants, suggesting that these missense variants may cause haploinsufficiency.
- Our results and approach will advance our knowledge about interpretation of *in vitro* studies and biological consequences of non-missense variants in LQT2.

Introduction

Congenital long QT syndrome (LQTS) is an inherited arrhythmia disease characterized by prolonged QT intervals on the electrocardiogram (ECG), leading to a bizarre form of ventricular tachycardia, Torsade de Pointes (TdP), and sudden death.[1,2] The human *ether-à-go-go*-related gene potassium channel (Kv11.1), encoded by *KCNH2*, assembles as functional tetramers and generates repolarizing current I_{Kr}. Loss-of-function variants of *KCNH2* cause type 2 LQTS (LQT2).[3,4]

More than one-third of patients with LQT2 carry a non-missense variant such as nonsense, frameshift, or splicing variants[3,5,6] that may undergo nonsense-mediated messenger RNA decay (NMD)[7,8] leading to haploinsufficiency (HI). However, as of yet, only a few studies have focused on the clinical outcome of the patients with non-missense variants.[9,10,11] The remaining two-thirds of LQT2-related variants are missense variants, scattering over known functional domains in Kv11.1 such as *ether-à-go-go* domain (EAGD), voltage sensor domain (VSD), pore domain (PD), and cyclic nucleotide-binding homology domain (CNBHD).[12–14] Among them, variants in the PD have been shown to exert dominant-negative (DN) effects and are thereby associated with worst arrhythmia outcomes.[10,15]

Recent *in vitro* studies have revealed that more than 80% of *KCNH2* missense variants cause mis-trafficking of Kv11.1 onto the cell surface, resulting in the reduction of I_{Kr}[16,17] and affecting channel abundance at a different stage during Kv11.1 translation via endoplasmic reticulum-associated degradation.[18,19] Most missense variants in the PD are degraded *after* assembling tetramers, thus resulting in the DN trait by degrading the wild-type (WT) channel. On the other hand, variants in the EAGD, C-linker, or CNBHD likely are degraded *before* channel assembly, therefore not affecting the multi-merization of healthy subunits. This may lead to trafficking of fewer functional WT channels, thereby producing HI effects.[17]

LQT2-related gene variants thus result in different levels of loss-of-function. In 429 patients with LQT2, we aimed to test our

hypothesis that potentially HI-causing non-missense and missense variants will exhibit better clinical outcomes than variants that can cause DN. This study will significantly help bridge an insight of molecular evidence with the interpretation of LQT2 patient clinical manifestations.

Methods

Study population

This study was comprised of 429 patients from 243 families with a single rare variant of *KCNH2*, who were referred to Shiga University of Medical Science or Kyoto University Graduate School of Medicine between 1996 and 2021 for examination of primary arrhythmia syndrome. The study was approved by the institutional review committees, and the patients or their guardians gave written informed consent.

Declaration of Helsinki

This study complies with the Declaration of Helsinki. We obtained written informed consent from all the patients and their guardians per the guidelines approved by the institutional review board of Shiga University of Medical Science and Kyoto University hospital.

Genotyping and variant interpretation

Genomic DNA was extracted from peripheral lymphocytes. Denaturing high-performance liquid chromatography (WAVE system Model 3500, Transgenomic, NE, USA) (between 1996 and 2014 for *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2*) or targeted panel sequencing (between 2015 and 2021) was used for initial screening. All identified variants were verified by Sanger sequencing.[20] *KCNH2* variants with minor allele frequency (MAF) greater than 0.00025 were excluded in consideration of the prevalence of LQTS in 1/2000.[21] Cases with compound *KCNH2* variants were also excluded; if a patient carried another variant in LQTS-related genes in addition to a *KCNH2* variant and the MAF of the concurrent variant were < 0.00025, it was excluded from the analysis. MAF of each variant was assessed using both Genome Aggregation Database (gnomAD: https://gnomad.broadinstitute.org) and the National Bioscience Database Center's integrated database of Japanese genomic variation (Togovar: https://togovar.biosciencedbc.jp).

Classification of *KCNH2* variants

KCNH2 variants were divided into two types; 'missense variant' included all missense variants regardless of variant domains and 'non-missense variant' included nonsense, frameshift, and canonical (± 2) splice site variants.[22] Kv11.1's domains were defined as follows: EAGD (p. 1–135), N-linker (p. 136–398), VSD (p. 399–545), PD (p. 546–670), C-linker (p. 671–736), CNBHD (p. 737–863), and C-tail domain (p. 864–1159).[23]

To assess the functional changes of each *KCNH2* missense variant in our cohort, we made an extensive survey of previous experimental reports. According to the functional assay, there were reports on 41 variants in our cohort, and we classified them as either HI or DN variant (see Supplementary material online, *Table S1*). Furthermore, to predict the functional changes of the remaining 61 variants which were not previously investigated, we examined functional changes of each domain by reviewing past literature and then predicted their loss-of-function mechanisms as HI or DN (see Supplementary material online, *Table S2*). Online packages (SIFT, https://sift.bii.a-star.edu.sg; PROVEAN, https://www.jcvi.org/research/provean; polyphen-2, http://genetics.bwh.harvard.edu/pph2) were used to predict functional changes of the missense variants.

For non-missense variants, we considered them all as HI due to the lack of evidence that conventional functional assays using exogenous complementary DNA are capable of incorporating NMD mechanism in the cell model. In this study, we did not consider whether they escape NMD depending on the locations where the premature termination codons occurred.[7,8]

Assessment of the clinical characteristics and outcomes

The clinical characteristics of patients were evaluated before initiation of drug treatment. QT intervals were measured in II or V5 lead by the threshold method[24] using Image J software (NIH) and were corrected for heart rate by the Bazett's formula [corrected QT (QTc)]. To plot QTc across domains, QTc was plotted at each amino acid residue for the corresponding variant. When a variant was identified in multiple patients, averaged QTc was plotted. Bradycardia was diagnosed using normal limits in pediatric patients[25] or below 50 bpm for patients at 4-year-old or older. When available, a Holter-monitoring was subject for diagnosis of bradycardia. Notched T wave was considered to be positive if a notch on the T wave was found in three or more leads.[2] LQTS risk score (or Schwartz score) was calculated based on the most recent updates.[26] Arrhythmia events (AEs) were assessed up to 40 years of age, combining syncope including unknown cause or suspected cardiac syncope, and lethal AEs including documented TdP or ventricular tachycardia/fibrillation, cardiopulmonary arrest, and sudden death with or without documentation of fatal arrhythmias.

Statistics

Data are shown as mean ± SD for continuous variables and number (percentage) for categorical variables. For continuous variables, unpaired Student's *t*-test or analysis of variance (ANOVA) was used followed by Tukey's test if an ANOVA found differences among groups. For categorical variables, the χ^2 test followed by the Bonferroni corrections was used. The probability of first AEs was estimated using Kaplan–Meier method with log-rank test up to 40 years. Patients were censored when drug treatment was started. The Cox proportional hazard model was used to predict the association between AEs and predictor variables. At first, we analysed the effect of each variable on probability of AEs over time and identified sex as a time-varying covariate, a confirmatory result consistent with previous studies.[10,27] Given this result, we used time-dependent Cox models, using an age of 12 as an optimal cut-point, as indicated by likelihood-based exhaustive search method, to create a step function of time to evaluate the time-dependent effect of sex.[28] Two-tailed $P < 0.05$ was considered as significant. All statistical analyses were performed using SPSS 28.0 (IBM, Armonk, NY, USA).

Results

Study population and identified *KCNH2* variants

We included 429 Japanese patients (59% female) with a single rare *KCNH2* variant for analysis. Among them, 147 patients (34%) experienced arrhythmia events (syncope and/or lethal AE). There were no sudden death cases. The trigger of arrhythmia events was drug or hypokalaemia in 12 patients (8% of patients with AEs) and sudden arousal or emotional stress in 33 (22%). The situation of arrhythmia events was at rest in 97 patients (66% of patients with AEs), exercise in 10 (7%), and both (rest and exercise) in 24 (16%).

We identified 178 unique variants (see Supplementary material online, *Table S1*): 102 missense variants ($n = 239$ patients, 56% of study cohort) and 76 non-missense variants ($n = 190$, 44%). Missense variants included 28 variants in EAGD ($n = 56$), 5 in *n*-linker ($n = 7$), 11 in VSD ($n = 15$), 35 in PD ($n = 114$), 3 in C-linker ($n = 5$), 11 in CNBHD ($n = 24$), and 9 in C-tail domains ($n = 18$). *Figure 1* shows the distribution of QTc at each corresponding amino acid residue with a missense (*Figure 1A*) and a non-missense (*Figure 1B*) variant, respectively, across functional domains of Kv11.1.

Comparison of variant types: non-missense vs. missense variants

Table 1 shows clinical characteristics and outcomes between non-missense and missense variants. QTc intervals in non-missense variant carriers were significantly shorter than those with missense variants (484 ± 40 vs. 495 ± 55 ms, $P = 0.019$). The incidence of AEs was lower in non-missense than missense variant carriers (29% vs. 39%, $P = 0.023$), and the Kaplan–Meier survival analysis revealed that the probability of AEs was lower in non-missense variant carriers than those of missense variant (log-rank $P = 0.006$, *Figure 2A*).

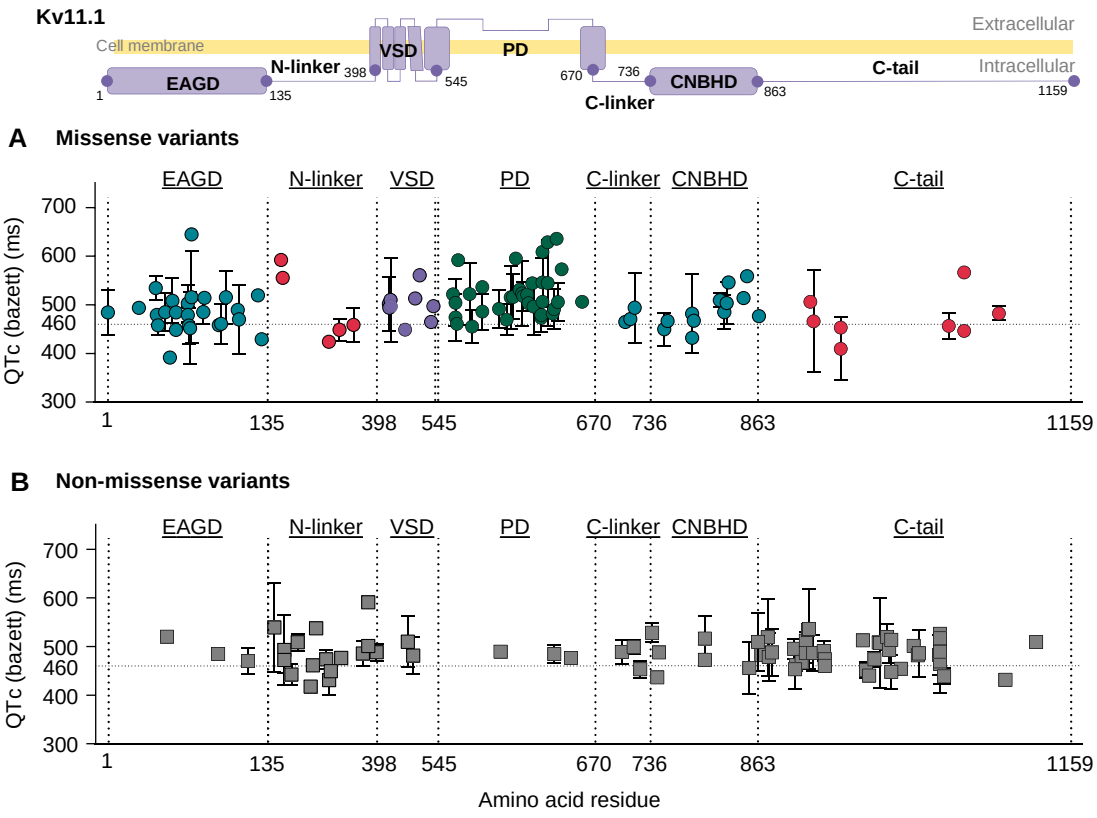


Figure 1 Qtc values across functional domains of Kv11.1. The topology of Kv11.1 and the mean QTc of respective variant carriers were plotted on the Y-axis, at each amino acid residue on the X-axis for all missense variants (*A*) and non-missense variants (*B*). CNBHD, cyclic nucleotide-binding homology domain; EAGD, *ether-à-go-go* domain; PD, pore domain; QTc, corrected QT interval; VSD, voltage sensor domain.

Missense variants: haploinsufficient vs. dominant-negative effects

We then focused on the functional outcome of missense variants. Many experimental studies have examined the functional change induced by previously reported variants. Among the missense variants in our cohort, 41 (40%) variants had been previously studied: 22 HI and 19 DN variants (see Supplementary material online, *Table S1*).

As shown in *Table 2*, non-missense variants showed nearly identical phenotypes with missense variants that had been reported as HI (HI-group), including QTc and incidence of AEs. In contrast, reported DN missense variants (DN-group) showed longer QTc and higher incidence of AEs compared to the non-missense variants or the HI-group. In Kaplan–Meier analysis (*Figure 2B*), the DN-group showed significantly higher probability of AEs than the other variant carriers (log-rank $P = 0.001$). HI-group showed an intermediate prognosis between non-missense and DN-groups.

Estimation of functional changes and arrhythmia outcome with HI and DN variants

The remaining 61 (60%) of missense variants in our cohort were not biophysically examined previously, and so we attempted to predict their functional outcomes based on their locations. We surveyed previous reports and classified a total of 224 missense variants as DN or not (see Supplementary material online, *Table S2*). *Figure 3* shows the details of known functional changes according to the variant domain among variants in our dataset ($n = 41$ previously studied) (*Figure 3A*). These functional changes correlate with previously reported functional changes of the variants in each domain ($n = 224$ previously studied) (*Figure 3B*). We thus determined the dominant functional changes in each domain. Following this finding, 61 functionally unknown variants were classified into either predicted DN (pDN)-group (VSD and PD) or predicted HI (pHI)-group (other domains) (see Supplementary material online, *Table S1*). Then, above mentioned functionally known DN and HI variants were included to pDN and pHI-group, respectively. Finally, we classified all non-missense variants as pHI-group since they likely exert HI effects through the NMD phenomenon.[7,8]

We then compared the clinical outcomes between pHI- and pDN-groups. *Table 3* summarizes the clinical characteristics in two groups. PHI-group showed mild phenotypes compared with pDN-group: shorter QTc, lower LQTS scores, lower presence of notched T wave, and lower incidence of AEs at older age. The incidence of both syncope and lethal AEs was lower in pHI-group than pDN-group. The Kaplan–Meier analysis (*Figure 2C*) revealed that the pHI-group was at a significantly lower risk of AEs compared to the pDN-group (log-rank $P < 0.001$).

Among patients with AEs (91 patients in pHI-group and 56 in pDN-group), there was no difference in medical therapy between groups. Recurrent arrhythmia event rate under medication (on-drug) was similar between groups (11% and 18%, respectively; $P = 0.24$) (see Supplementary material online, *Table S3*).

Table 1 Clinical characteristics of two variant types: non-missense vs. missense

	Non-missense variant	Missense variant	*P*-value
n	190	239	
Proband, *n* (%)	93 (49)	141 (59)	0.038
Female, *n* (%)	111 (58)	144 (60)	0.70
Age at genotyping, years	28 ± 19	27 ± 20	0.59
QT, ms	459 ± 62	462 ± 76	0.73
QTc (Bazett), ms	484 ± 40	495 ± 55	0.019
FH of SD (≤30 years), *n* (%)	16 (9)	33 (14)	0.12
Bradycardia, *n* (%)	34 (18)	47 (20)	0.64
Notched T wave, *n* (%)	106 (56)	139 (60)	0.46
LQTS score	4.1 ± 1.9	4.2 ± 1.9	0.48
AEs, *n* (%)	54 (29)	93 (39)	0.023
Age at onset, years	16 ± 7	16 ± 10	0.63
Syncope, *n* (%)	45 (24)	77 (32)	0.052
Age at syncope, years	17 ± 7	16 ± 9	0.84
Lethal AE, *n* (%)	25 (13)	45 (19)	0.11
Age at lethal AE, years	21 ± 8	20 ± 12	0.77

Data are expressed as mean ± SD or number (%) unless stated otherwise.
AE, arrhythmia event; FH, family history; LQTS, long QT syndrome; QT, QT interval; QTc, corrected QT interval; SD, sudden death.

Risk stratification based on predicted functional changes

Finally, we evaluated variables by the Cox regression hazard model analysis. As previously reported,[10,27,29] sex effect on AEs was not observed before age 12 but was clearly discernible after age 12 in our dataset (see Supplementary material online, *Figure S1*). We conducted the time-dependent Cox regression analysis by setting sex as a time-dependent covariate.

In *Table 4*, pDN-group showed significantly higher lifetime risk of AEs than pHI-group [hazard ratio (HR): 2.05, 95% confidence interval (CI): 1.25–3.38, $P = 0.005$], whereas the risk in variant types (missense vs. non-missense variants) was not significantly different (HR: 0.92, 95% CI: 0.55–1.55, $P = 0.75$). Proband status (HR: 7.02, 95% CI: 3.63–13.6, $P < 0.001$), females older than 12-year-old (HR: 2.33, 95% CI: 1.23–4.40, $P = 0.009$), severely prolonged QTc (≥480 ms) (HR: 1.68, 95% CI: 1.03–2.73, $P = 0.039$) and positive notched T wave (HR: 1.56, 95% CI: 1.02–2.41, $P = 0.043$) were also risk variables for lifetime AEs.

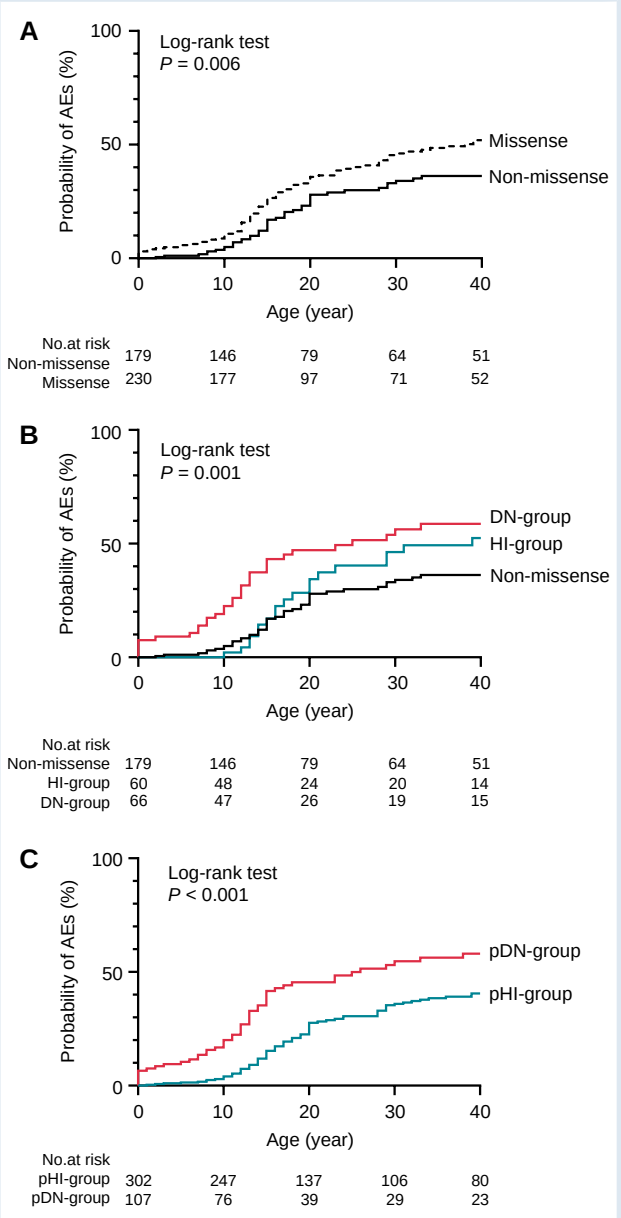


Figure 2 Kaplan–Meier survival analyses for the probability of AEs. (*A*) Probability of arrhythmia events (AEs) among the non-missense variant carriers and the missense variant carriers. (*B*) Probability of AEs among carriers with non-missense variant, HI-group, and DN-group. (*C*) Probability of AEs among carriers with pHI-group, and pDN-group. AEs, arrhythmia events; DN, dominant-negative; HI, haploinsufficiency; pDN, predicted DN; pHI, predicted HI.

Discussion

In 429 patients with LQT2, the present study demonstrated that *KCNH2* non-missense variant carriers showed shorter QTc and lower risk for cardiac events compared to those with missense variants which included various functional effects on Kv11.1 (*Figure 2A*). According to previously reported functional analyses, 40% of our missense variants could be classified either into HI- or DN-groups. Non-missense and HI variant groups showed a significantly better prognosis than the DN-group (*Figure 2B*). Finally, prediction of functional changes induced by remaining missense variants (60%) was made based on their location. Because of NMD, non-missense variants were classified as pHI-group. Such a prediction showed that pHI presented a significantly better prognosis than pDN (*Figure 2C*). The classification of variants according to the molecular mechanism, not variant types, contrasted the difference in phenotypes, thereby predicting the prognosis in LQT2 patients more clearly. Subsequent multivariable analyses revealed that significant risk factors are: proband status, female sex older than the age of 12 years, QTc ≥480 ms, positive notched T wave, and pDN-group.

Functional analyses are thus helpful for the understanding of LQTS mechanisms. However, functional assay of non-missense variants is

Table 2 Clinical characteristics of non-missense, HI and DN variants

	Non-missense variant	HI-group	DN-group	*P*-value
n	190	60	71	
Proband, *n* (%)	93 (49)	32 (53)	45 (63)	0.12
Female, *n* (%)	111 (58)	36 (60)	47 (66)	0.52
Age at genotyping, years	28 ± 19	25 ± 19	27 ± 21	0.64
QT, ms	459 ± 62	444 ± 77 †	476 ± 76	0.039
QTc (Bazett), ms	484 ± 40 ‡	484 ± 40 ‡	509 ± 52	< 0.001
FH of SD (≤30 years), *n* (%)	16 (9) *	4 (7)	14 (21)	0.017
Bradycardia, *n* (%)	34 (18)	12 (20)	11 (15)	0.80
Notched T wave, *n* (%)	106 (56)	32 (56)	46 (67)	0.29
LQTS score	4.1 ± 1.9	4.0 ± 1.8	4.6 ± 1.9	0.096
AEs, *n* (%)	54 (29) **	19 (32)	36 (51)	0.002
Age at onset, years	16 ± 7 †	19 ± 8 ‡	12 ± 9	0.004
Syncope, *n* (%)	45 (24) *	18 (30)	29 (41)	0.021
Age at syncope, years	17 ± 7	19 ± 8 †	13 ± 7	0.023
Lethal AE, *n* (%)	25 (13)	9 (14)	14 (22)	0.26
Age at lethal AE, years	21 ± 8 †	23 ± 6 †	13 ± 12	0.018

Data are expressed as mean ± SD or number (%) unless stated otherwise.
AE, arrhythmia event; DN, dominant-negative; FH, family history; HI, haploinsufficiency; LQTS, long QT syndrome; QT, QT interval; QTc, corrected QT interval; SD, sudden death.
†$P < 0.05$, ‡$P < 0.01$ vs. DN-group by analysis of variance followed by Tukey post-hoc test.
*$P < 0.05$, **$P < 0.01$ vs. DN-group by the χ^2 test followed by the Bonferroni corrections.

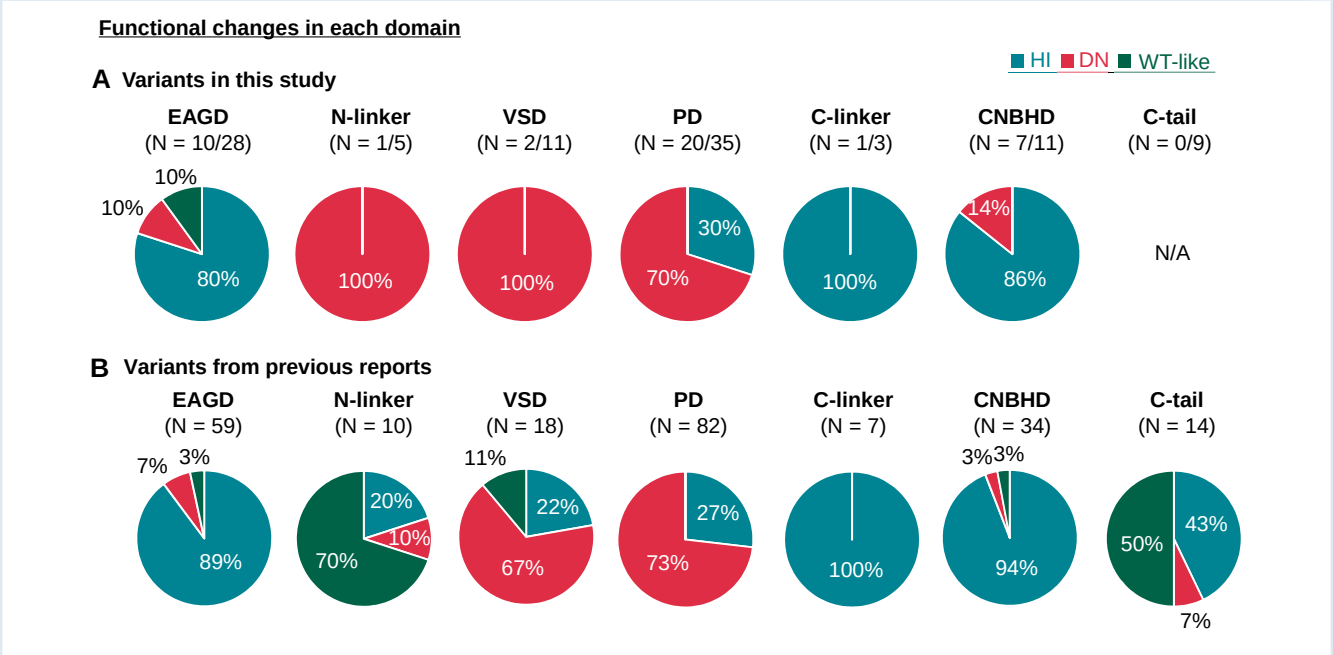


Figure 3 Functional changes in each domain. Proportion of known functional changes in each domain in our cohort (*A*) and in the literature (*B*). Numbers in the pie chart indicate the percentage of each functional change among variants previously studied *in vitro*. References of the literature are in Supplementary material online, *Table S2*. CNBHD, cyclic nucleotide-binding homology domain; DN, dominant-negative; EAGD, ether-à-go-go domain; HI, haploinsufficiency; PD, pore domain; VSD, voltage sensor domain; WT, wild-type.

Table 3 Two groups of variant carriers based on functional prediction

	pHI-group	pDN-group	*P*-value
n	314	115	
Proband, *n* (%)	162 (52)	72 (63)	0.042
Female, *n* (%)	182 (58)	73 (64)	0.30
Age at genotyping, years	27 ± 19	27 ± 21	0.99
QT, ms	455 ± 67	475 ± 76	0.013
QTc (Bazett), ms	483 ± 45	511 ± 54	< 0.001
FH of SD (≤30 years), *n* (%)	28 (9)	21 (19)	0.008
Bradycardia, *n* (%)	60 (19)	21 (18)	0.84
Notched T wave, *n* (%)	168 (54)	77 (68)	0.011
LQTS score	4.0 ± 1.9	4.7 ± 1.9	< 0.001
AEs, *n* (%)	91 (29)	56 (49)	< 0.001
Age at onset, years	18 ± 8	12 ± 9	< 0.001
Syncope, *n* (%)	77 (25)	45 (39)	0.003
Age at syncope, years	18 ± 8	13 ± 7	0.001
Lethal AE, *n* (%)	43 (14)	27 (23)	0.015
Age at lethal AE, years	22 ± 8	16 ± 13	0.024

Data are expressed as mean ± SD or number (%) unless stated otherwise.
AE, arrhythmia event; FH, family history; LQTS, long QT syndrome; pDN, predicted dominant-negative; pHI, predicted haploinsufficiency; QT, QT interval; QTc, corrected QT interval; SD, sudden death.

technically difficult because they are postulated to undergo NMD.[7,8] Although these variants are thought to be relatively benign compared to DN variants, there were no systematic analyses covering this. Indeed, using the international LQTS registry, Moss *et al.*[15] (2002) examined 44 *KCNH2* variants according to the location, irrespective of variants types. They did not analyse non-missense and missense variants separately. Using the same and expanded registry, Shimizu *et al.*[10] (2009) showed that there was no significant difference in event rates between missense and non-missense variants, which contrasts with our finding (*Figure 2A*) that missense patients showed a worse prognosis. This difference may be driven by the different study protocol by which patients were censored at the time when pharmacologic therapy was initiated in our study whereas the previous study did not. This difference may partially result from the inclusion of more patients with milder phenotypes in our cohort. Specifically, our cohort included school-age children who took routine ECG check-ups in Japan.[30] The school-based ECG check-up can identify various variants including non-missense variants ($n = 190$, 44%) and therefore made a sharp contrast with overall missense carriers that contained both HI and DN variants.

The loss-of-function type of *KCNH2* variants may cause LQT2 by four different mechanisms: abnormal transcription or translation of Kv11.1 (Class 1); mis-trafficking (Class 2); disrupting channel gating or kinetics (Class 3); or altering ion selectivity or permeability (Class 4).[7,31] PD variants reportedly induce DN effects by interfering with WT proteins through endoplasmic reticulum-associated degradation, leading to worse outcomes.[16,32,33,34] An *in-vitro* study revealed that 88% of LQT2-associated missense variants showed loss-of-function by a Class 2 mechanism.[17] In their study, among mis-trafficking variants, 76% of PD variants exerted DN effects, while all (100%) of EAGD, C-linker, and CNBHD variants produced HI effects, suggesting that they were degraded with

Table 4 Univariable and multivariable cox regression analysis for AEs

	Univariable		Multivariable	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Status				
Proband vs. family	11.2 (6.47–19.3)	< 0.001	7.02 (3.63–13.6)	< 0.001
Sex (< 12 years)				
Male vs. female	1.69 (0.88–3.23)	0.11	1.93 (0.95–3.92)	0.069
Sex (≥ 12 years)				
Female vs. male	3.84 (2.17–6.78)	< 0.001	2.33 (1.23–4.40)	0.009
QTc				
≥ 480 vs. < 480 ms	4.16 (2.66–6.52)	< 0.001	1.68 (1.03–2.73)	0.039
FH of SD				
Yes vs. no	1.15 (0.70–1.89)	0.59	0.73 (0.40–1.31)	0.29
Bradycardia				
Yes vs. no	1.49 (1.01–2.20)	0.044	1.19 (0.75–1.88)	0.46
Notched T wave				
Yes vs. no	1.79 (1.24–2.58)	0.002	1.56 (1.02–2.41)	0.043
Variant type				
Missense vs. non-missense	1.65 (1.15–2.37)	0.007	0.92 (0.55–1.55)	0.75
Predicted functional change				
pDN-group vs. pHI-group	2.09 (1.46–2.98)	< 0.001	2.05 (1.25–3.38)	0.005

Multivariable model includes all variables simultaneously.
AEs, arrhythmia events; CI, confidence interval; FH, family history; HR, hazard ratio; pDN, predicted dominant-negative; pHI, predicted haploinsufficiency; QTc, corrected QT interval; SD, sudden death.

or without WT subunits, revealing that the PD-mediated DN was caused through Class 2 rather than Class 3 or 4 mechanisms. In contrast, other missense variants may cause HI by Class 2 mechanism without affecting the transport of WT subunit.

In this study with many LQT2 patients, the prediction of loss-of-function mechanisms by functional domains (*Figure 2C*) was found to be helpful for assessing the prognosis. These dominant functional effects in each domain (*Figure 3*) showed accordance with their severity of clinical characteristics (see Supplementary material online, *Table S4*). Furthermore, our data support for the first time that transmembrane domains including not only PD but VSD presented poorer clinical outcomes than others. In the literature, 12 of 18 previously studied variants in the VSD exerted a DN phenotype (*Figure 3*), and in our cohort, 15 carriers with variants in the VSD showed severe phenotypes (see Supplementary material online, *Table S4*).

Study limitations

The functional consequences of identified variants in this study were limited in the literature and were not fully evaluated, including non-missense variants that may escape NMD. This may result in discordance between predicted functional changes and *in vitro* findings. Our binary classification of HI vs. DN status may fail to fully reflect the underlying complex properties of genetic variants. Further validation of this new approach to verify our findings in different cohorts is necessary.

Conclusion

We identified, for the first time, that the clinical phenotypes of both non-missense variants and known HI missense variants of *KCNH2* exhibited better outcomes than those with variants that are known to cause DN effects. Risk stratification supplemented by prediction of functional consequence revealed that HI-causing variants have better arrhythmia outcomes than DN-causing variants. Our study highlights the advantage of combining molecular biology and clinical phenotypes to bridge the knowledge gap between biological consequence of the non-missense variants and clinical outcomes in LQT2 patients.

Supplementary material

Supplementary material is available at *Europace* online.

Acknowledgements

We thank Ms. Arisa Ikeda, Ms. Kazu Toyooka, and Ms. Madoka Tanimoto for their contributions to genetic testing.

Funding

This work was supported by a grant from the Ministry of Health, Labor and Welfare of Japan for Clinical Research on Intractable Disease (H27-032 and H29-055 to M.H., T.M., S.O.), MEXT KAKENHI (grant numbers: 15H04818 to M.H., 19K08538 to T.M., 18K07875 to S.O.), Heart Rhythm Society (Clinical Research Award in Honor of Mark Josephson and Hein Wellens to Y.W.), and American Heart Association postdoctoral fellowship (grant no. 830951 to Y.W.).

Conflict of interest: None declared.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Mizusawa Y, Horie M, Wilde AA. Genetic and clinical advances in congenital long QT syndrome. *Circ J* 2014;**78**:2827–33.
2. Schwartz PJ, Crotti L, Insolia R. Long-QT syndrome: from genetics to management. *Circ Arrhythm Electrophysiol* 2012;**5**:868–77.
3. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Effect of clinical phenotype on yield of long QT syndrome genetic testing. *J Am Coll Cardiol* 2006;**47**:764–8.
4. Schwartz PJ, Ackerman MJ, George AL Jr, Wilde AAM. Impact of genetics on the clinical management of channelopathies. *J Am Coll Cardiol* 2013;**62**:169–80.
5. Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL *et al.* Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. *Circulation* 2000;**102**:1178–85.
6. Ono M, Burgess DE, Schroder EA, Elayi CS, Anderson CL, January CT *et al.* Long QT syndrome type 2: emerging strategies for correcting class 2 KCNH2 (hERG) mutations and identifying new patients. *Biomolecules* 2020;**10**:1144.
7. Gong Q, Zhang L, Vincent GM, Horne BD, Zhou Z. Nonsense mutations in hERG cause a decrease in mutant mRNA transcripts by nonsense-mediated mRNA decay in human long-QT syndrome. *Circulation* 2007;**116**:17–24.
8. Gong Q, Stump MR, Zhou Z. Position of premature termination codons determines susceptibility of hERG mutations to nonsense-mediated mRNA decay in long QT syndrome. *Gene* 2014;**539**:190–7.
9. Bhuiyan ZA, Momenah TS, Gong Q, Amin AS, Ghamdi SA, Carvalho JS *et al.* Recurrent intrauterine fetal loss due to near absence of HERG: clinical and functional characterization of a homozygous nonsense HERG Q1070X mutation. *Heart Rhythm* 2008;**5**:553–61.
10. Shimizu W, Moss AJ, Wilde AA, Towbin JA, Ackerman MJ, January CT *et al.* Genotype-phenotype aspects of type 2 long QT syndrome. *J Am Coll Cardiol* 2009;**54**:2052–62.
11. Zarraga IG, Zhang L, Stump MR, Gong Q, Vincent GM, Zhou Z. Nonsense-mediated mRNA decay caused by a frameshift mutation in a large kindred of type 2 long QT syndrome. *Heart Rhythm* 2011;**8**:1200–6.
12. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL *et al.* The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science* 1998;**280**:69–77.
13. Morais Cabral JH, Lee A, Cohen SL, Chait BT, Li M, Mackinnon R. Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. *Cell* 1998;**95**:649–55.
14. Brelidze TI, Gianulis EC, DiMaio F, Trudeau MC, Zagotta WN. Structure of the C-terminal region of an ERG channel and functional implications. *Proc Natl Acad Sci U S A* 2013;**110**:11648–53.
15. Moss AJ, Zareba W, Kaufman ES, Gartman E, Peterson DR, Benhorin J *et al.* Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go-related gene potassium channel. *Circulation* 2002;**105**:794–9.
16. Anderson CL, Delisle BP, Anson BD, Kilby JA, Will ML, Tester DJ *et al.* Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation* 2006;**113**:365–73.
17. Anderson CL, Kuzmicki CE, Childs RR, Hintz CJ, Delisle BP, January CT. Large-scale mutational analysis of Kv11.1 reveals molecular insights into type 2 long QT syndrome. *Nat Commun* 2014;**5**:5535.
18. Gong Q, Keeney DR, Molinari M, Zhou Z. Degradation of trafficking-defective long QT syndrome type II mutant channels by the ubiquitin-proteasome pathway. *J Biol Chem* 2005;**280**:19419–25.
19. Foo B, Barbier C, Guo K, Vasantharuban J, Lukacs GL, Shrier A. Mutation-specific peripheral and ER quality control of hERG channel cell-surface expression. *Sci Rep* 2019;**9**:6066.
20. Ohno S, Ozawa J, Fukuyama M, Makiyama T, Horie M. An NGS-based genotyping in LQTS; minor genes are no longer minor. *J Hum Genet* 2020;**65**:1083–91.
21. Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G *et al.* Prevalence of the congenital long-QT syndrome. *Circulation* 2009;**120**:1761–7.
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics And Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–24.
23. Wang W, MacKinnon R. Cryo-EM structure of the open human ether-à-go-go-related K+ channel hERG. *Cell* 2017;**169**:422–430.e10.
24. Vink AS, Neumann B, Lieve KVV, Sinner MF, Hofman N, El Kadi S *et al.* Determination and interpretation of the QT interval. *Circulation* 2018;**138**:2345–58.
25. Rijnbeek PR, Witsenburg M, Schrama E, Hess J, Kors JA. New normal limits for the paediatric electrocardiogram. *Eur Heart J* 2001;**22**:702–11.
26. Schwartz PJ, Ackerman MJ, Antzelevitch C, Bezzina CR, Borggrefe M, Cuneo BF *et al.* Inherited cardiac arrhythmias. *Nat Rev Dis Primers* 2020;**6**:58.
27. Ozawa J, Ohno S, Hisamatsu T, Itoh H, Makiyama T, Suzuki H *et al.* Pediatric cohort with long QT syndrome- KCNH2 mutation carriers present late onset but severe symptoms. *Circ J* 2016;**80**:696–702.

28. Therneau TM, Grambsch PM. The cox model. In: Therneau TM and Grambsch PM (eds.), *Modeling Survival Data: Extending the Cox Model. Statistics for Biology and Health*. 1st ed. Heidelberg, Germany: Springer; 2000. p39–79.
29. Moss AJ, Shimizu W, Wilde AA, Towbin JA, Zareba W, Robinson JL *et al.* Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation* 2007;**115**:2481–9.
30. Fukuyama M, Horie M, Aoki H, Ozawa J, Kato K, Sawayama Y *et al.* School-based routine screenings of electrocardiograms for the diagnosis of long QT syndrome. *Europace* 2022;**24**:1496–1503.
31. Delisle BP, Anson BD, Rajamani S, January CT. Biology of cardiac arrhythmias: ion channel protein trafficking. *Circ Res* 2004;**94**:1418–28.
32. Ficker E, Dennis AT, Obejero-Paz CA, Castaldo P, Taglialatela M, Brown AM. Retention in the endoplasmic reticulum as a mechanism of dominant-negative current suppression in human long QT syndrome. *J Mol Cell Cardiol* 2000;**32**:2327–37.
33. Huo J, Zhang Y, Huang N, Liu P, Huang C, Guo X *et al.* The G604S-hERG mutation alters the biophysical properties and exerts a dominant-negative effect on expression of hERG channels in HEK293 cells. *Pflugers Arch* 2008;**456**:917–28.
34. Zhao JT, Hill AP, Varghese A, Cooper AA, Swan H, Laitinen-Forsblom PJ *et al.* Not all hERG pore domain mutations have a severe phenotype: G584S has an inactivation gating defect with mild phenotype compared to G572S, which has a dominant negative trafficking defect and a severe phenotype. *J Cardiovasc Electrophysiol* 2009;**20**:923–30.