ESC European Society of Cardiology

Europace (2024) **26**, euae136
https://doi.org/10.1093/europace/euae136

CLINICAL RESEARCH

Genetic characterization of *KCNQ1* variants improves risk stratification in type 1 long QT syndrome patients

Charles Morgat [1]*, Véronique Fressart [2], Alessandra Pia Porretta [1,3], Nathalie Neyroud[4], Anne Messali[1], Yassine Temmar[5], Vincent Algalarrondo [1], Elodie Surget [1], Adrien Bloch[2], Antoine Leenhardt [1], Isabelle Denjoy [1], and Fabrice Extramiana [1]

[1]CNMR Maladies Cardiaques Héréditaires Rares, APHP, Hôpital Bichat, 46 rue Henri Huchard, 75018 Paris, France; [2]AP-HP, Service de Biochimie Métabolique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France; [3]Service of Cardiology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [4]Sorbonne Université, Inserm, Research Unit on Cardiovascular and Metabolic Diseases, UMRS-1166, Paris, France; and [5]AP-HP, Unité Rythmologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

Received 5 March 2024; accepted after revision 21 May 2024; online publish-ahead-of-print 3 June 2024

Aims

KCNQ1 mutations cause QTc prolongation increasing life-threatening arrhythmias risks. Heterozygous mutations [type 1 long QT syndrome (LQT1)] are common. Homozygous *KCNQ1* mutations cause type 1 Jervell and Lange–Nielsen syndrome (JLNS) with deafness and higher sudden cardiac death risk. *KCNQ1* variants causing JLNS or LQT1 might have distinct phenotypic expressions in heterozygous patients. The aim of this study is to evaluate QTc duration and incidence of long QT syndrome–related cardiac events according to genetic presentation.

Methods and results

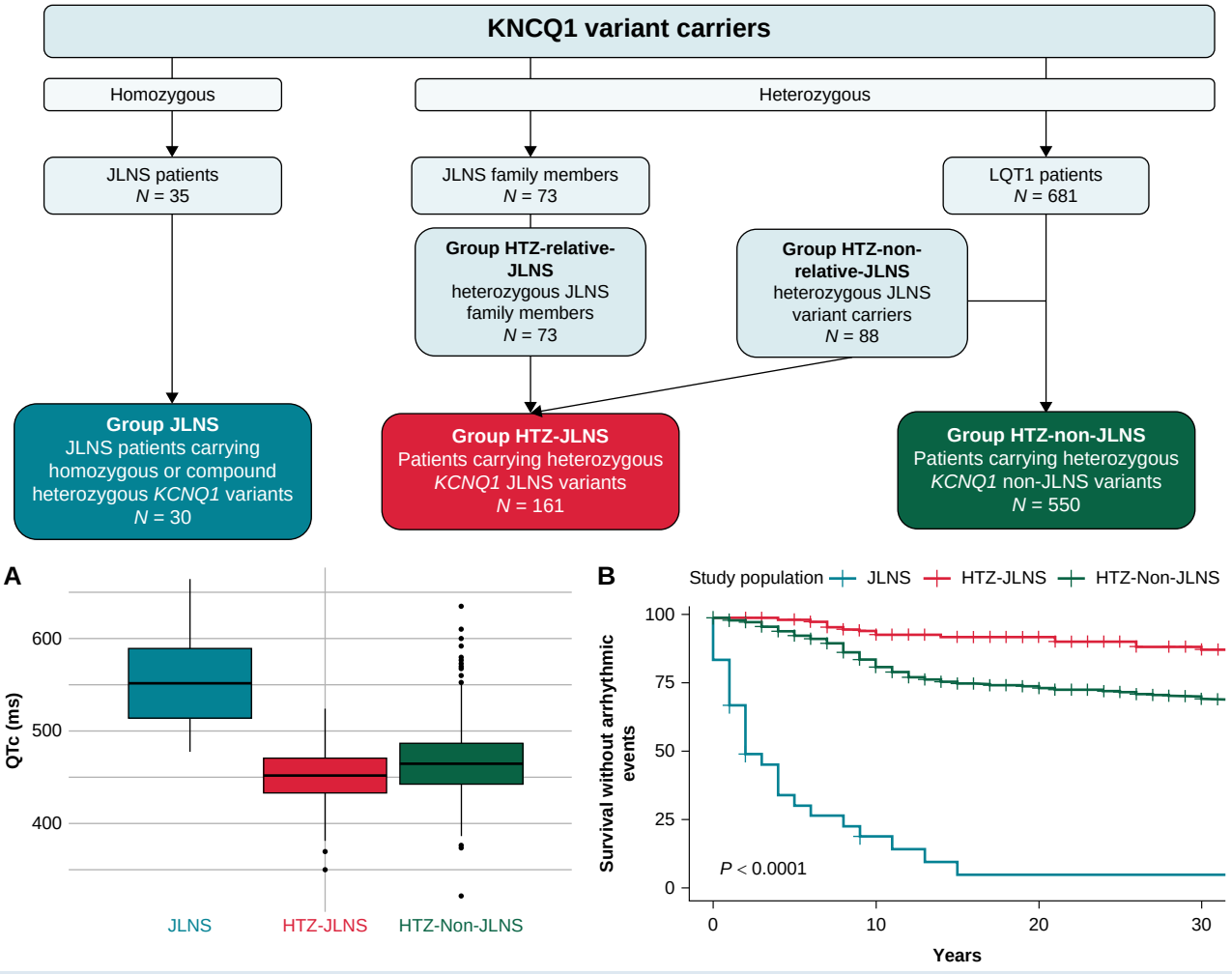
We enrolled LQT1 or JLNS patients with class IV/V *KCNQ1* variants from our inherited arrhythmia clinic (September 1993 to January 2023). Medical history, ECG, and follow-up were collected. Additionally, we conducted a thorough literature review for JLNS variants. Survival curves were compared between groups, and multivariate Cox regression models identified genetic and clinical risk factors. Among the 789 *KCNQ1* variant carriers, 3 groups were identified: 30 JLNS, 161 heterozygous carriers of JLNS variants (HTZ-JLNS), and 550 LQT1 heterozygous carriers of non-JLNS variants (HTZ-Non-JLNS). At diagnosis, mean age was 3.4 ± 4.7 years for JLNS, 26.7 ± 21 years for HTZ-JLNS, and 26 ± 21 years for HTZ-non-JLNS; 55.3% were female; and the mean QTc was 551 ± 54 ms for JLNS, 441 ± 32 ms for HTZ-JLNS, and 467 ± 36 ms for HTZ-Non-JLNS. Patients with heterozygous JLNS mutations (HTZ-JLNS) represented 22% of heterozygous *KCNQ1* variant carriers and had a lower risk of cardiac events than heterozygous non-JLNS variant carriers (HTZ-Non-JLNS) [hazard ratio (HR) = 0.34 (0.22–0.54); $P < 0.01$]. After multivariate analysis, four genetic parameters were independently associated with events: haploinsufficiency [HR = 0.60 (0.37–0.97); $P = 0.04$], pore localization [HR = 1.61 (1.14–1.2.26); $P < 0.01$], C-terminal localization [HR = 0.67 (0.46–0.98); $P = 0.04$], and group [HR = 0.43 (0.27–0.69); $P < 0.01$].

Conclusion

Heterozygous carriers of JLNS variants have a lower risk of cardiac arrhythmic events than other LQT1 patients.

* Corresponding author. Tel: +33140257126. *E-mail address*: charles.morgat@aphp.fr

Graphical Abstract



Keywords Type 1 long QT syndrome • Jervell and Lange–Nielsen syndrome • *KCNQ1* • Heterozygous carriers • Risk stratification

What's new?

- Key question
  What is the prevalence of heterozygous Jervell and Lange–Nielsen syndrome variants in type 1 long QT syndrome patients, are the clinical courses different compared with other type 1 long QT syndrome variants, and can phenotypic and genotypic predictors of arrhythmic events be identified in heterozygous KCNQ1 variant carriers?
- Key finding
  Heterozygous Jervell and Lange–Nielsen syndrome variant carriers represented 22% of heterozygous KCNQ1 variant carriers and had a lower risk of cardiac events than other type 1 long QT syndrome (hazard ratio = 0.34 [0.22–0.54]; $P < 0.01$). Four genetic parameters were independently associated with events: QTc, Jervell and Lange–Nielsen syndrome-related variants, haploinsufficiency, and pore/C-terminal localization.
- Take-home message
  Clinicians should be aware that about one-fourth to one-third of type 1 long QT syndrome patients may indeed carry a heterozygous Jervell and Lange–Nielsen syndrome-related variant and that such genotype is associated to a risk reduction ranging from 30 to 70%, as compared with other type 1 long QT syndrome patients.

Introduction

In the last decades, significant advancements in molecular biology have paved the way for an increasingly solid understanding of the puzzling relation between genetic findings and related clinical expression.[1] However, unravelling the complex interaction between genotype and phenotype remains particularly difficult for genes encoding proteins which act in multicomponent systems and have cell-specific functions, such as the potassium (K^+) channel genes.[2–4] Among them, the gene *KCNQ1*, located at 11p15.5, encodes the alpha subunit of the Kv7.1 channel, conducting the slow component of the delayed rectifier K^+ current (I_{Ks}). The main human channel isoform is a tetrameric protein of 676 amino acids, resulting from the co-assembly of 4 alpha subunits, each 1 containing 6 transmembrane segments (S1 to S6) with an amino (*n*)- and carboxyl (C)-terminus.[2–6] Kv7.1 is expressed in multiple tissues including, among others, the heart and the stria vascularis of the cochlear duct.[7,8] In the heart, I_{Ks} plays a key role in the repolarization process of myocardial cells. In the inner ear, I_{Ks} is crucial to ensure endolymph K^+ homeostasis.

In this context, loss-of-function mutations in *KCNQ1* have been shown to result in I_{Ks} reduction, prolonged cardiac repolarization, and QT lengthening–related ventricular arrhythmias. Different clinical

phenotypes may however arise according to the degree of I_{Ks} impairment.[9] Homozygous or compound heterozygous *KCNQ1* mutations, leading to the complete or nearly complete suppression of I_{Ks}, are associated to type 1 Jervell and Lange–Nielsen syndrome (JLNS).[10] Jervell and Lange–Nielsen syndrome has a prevalence ranging from one to six cases per million births and is characterized by congenital bilateral neuro-sensorial deafness and a high cumulative risk of sudden cardiac death (estimated to be between 20 and 30%).[11,12]

Heterozygous *KCNQ1* pathogenic variants are instead associated to type 1 long QT syndrome (LQT1). Type 1 1ong QT syndrome represents the most common form (45%) of genotyped long QT syndrome (LQTS) cases, following an autosomal dominant transmission pattern. The risk of sudden cardiac death is much lower, ranging from 1.3 to 0.05% per year.[13]

Due to the extremely rare nature of the disease, only limited studies or anecdotal case series have explored the phenotypic expression and the clinical course of heterozygous carriers of JLNS pathogenic variants as compared with other LQT1 variant carriers.[12,14,15] Previous evidences seem to support the hypothesis that JLNS variants, at a heterozygous state, are usually associated to a non-penetrant phenotype (patients are asymptomatic and with normal or borderline QTc).[16,17] Few case reports, however, have recently pointed out that some *KCNQ1* variants associated to JLNS at the homozygous state can cause LQT1 at the heterozygous state.[6,18] Nevertheless, the prevalence and severity of pathogenic variants causing both JLNS at the homozygous state and LQT1 at the heterozygous state still remain unknown and require additional studies.

On this basis, the aim of our study was to characterize and compare patients carrying *KCNQ1* pathogenic variants in heterozygous and homozygous state. We had three specific objectives: (1) to evaluate the prevalence of JLNS variants causing LQT1 at the heterozygous state, (2) to assess the clinical course of heterozygous carriers of JLNS when compared with other LQT1 variant carriers,[12–14] and (3) to evaluate phenotypic and genotypic predictors of arrhythmic events (AE) in *KCNQ1* variant carriers.

Methods

Study population

We enrolled *KCNQ1* variant carriers (in homozygous, compound heterozygous, or heterozygous state) from a series of consecutive patients referred, from September 1993 till January 2023 to the Reference Center for Inherited Arrhythmia Syndromes of the Bichat-Claude-Bernard University Hospitals in Paris, France. Enrolled patients came from proband-identified families diagnosed with LQTS or JLNS. The diagnosis of JLNS and LQTS complied with the established diagnostic criteria for LQTS, associated with congenital bilateral deafness for JLNS.[19–22]

All patients or the authorized family members gave their written informed consent for the genetic testing and the use of their personal medical data for research purposes. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki and its subsequent amendments. In line with the French legislation on studies of routine clinical practice, the study protocol was approved by a hospital committee competent for studies not requiring approval by an institutional review board. Furthermore, the study database was anonymized and registered with the French National Data Protection [*Commission Nationale de l'Informatique et des Libertés* (CNIL), Paris, France; reference: PI2022_843_0059].

Data collection

At the time of diagnosis, patients underwent a specialized consultation with retrospective collection of demographic information and personal/family medical history, as well as genetic testing. Prospective follow-up was initiated in our centre, involving annual consultations with clinical data collection addressing personal medical history, medical treatment, and occurrence of events. Severe arrhythmic events (SAE) occurring before diagnosis or during follow-up included sudden cardiac death, resuscitated cardiac arrest, or appropriate shock from an implantable cardioverter defibrillator for ventricular fibrillation. Arrhythmic events included both SAE and syncope episodes deemed of cardiac cause. During follow-up, patients underwent periodic 12-lead ECG recording and Holter monitoring. Beta-blocker therapy was initiated or continued at treating physician discretion. Data on ICD therapy and on left cardiac sympathetic denervation were also retrieved.

Genotype evaluation

Genetic analyses were conducted via Sanger sequencing prior to 2014, followed by the adoption of high-throughput sequencing techniques, in accordance with the standardized protocols established by our laboratory. Each identified variant was systematically confirmed on a second sample, using capillary sequencing or another appropriate technique based on variant nature. Once a genetic variant was identified within a family, cascade screening was performed using Sanger capillary sequencing.

The pathogenicity of identified genetic variants was evaluated according to the established criteria at the time of molecular diagnosis. Only patients with mutations considered pathogenic at the time of diagnosis (class IV or V) were selected. Of note, to ensure data update, all mutations listed in the study were re-evaluated based on our recent knowledge (June 2023) and graded according to the criteria from the 2015 consensus of the American College of Medical Genetics and Genomics (class I benign/class II likely benign/class III uncertain significance/class IV probably pathogenic/class V pathogenic).[23]

According to genotype, the study population was divided into three groups:

- Group JLNS: JLNS patients carrying *KCNQ1* homozygous or compound heterozygous variants and congenital bilateral neuro-sensorial deafness
- Group HTZ-JLNS: heterozygous JLNS variant carriers, including either heterozygous relatives of JLNS index cases (group HTZ-Relative-JLNS) or patients diagnosed with LQT1 (HTZ-Non-Relative-JLNS) carrying, at the heterozygous state, *JLNS* variants detected in group JLNS
- Group HTZ-Non-JLNS: LQT1 patients carrying *KCNQ1* variants not observed in the JLNS population

To facilitate result interpretation, patients in groups HTZ-JLNS and HTZ-Non-JLNS carrying mutations in both alleles (compound heterozygous variants) were excluded from the analyses because, although they do not meet the criteria for JLN syndrome, they appear to have a higher rhythmic risk.[24]

For all variants, we determined the type of mutation and the affected protein region. The transmembrane domain of the *KCNQ1*-encoded channel was defined as the coding sequence spanning amino acid residues 120 to 355 (pore region 283 to 327), with the *n*-terminus region preceding residue 120 and the C-terminus region following residue 355.

Then, we classified variants according to the biophysical functional effect expected on the mutated protein. In particular, according to literature,[6] we assumed haploinsufficiency (HI), with a ≤50% impairment of the channel function, for non-missense mutations including truncating mutations, frameshift mutations, and splicing mutations. Conversely, missense mutations and in-frame deletions were a priori considered as inducing a dominant negative (DN) effect characterized by a >50% impairment of the channel function.

Literature-based analysis

After a first analysis, we performed a detailed examination of the literature available for the totality of variants identified in our population. Since some variants, carried by patients included in our original group of LQT1 carriers of non-JLNS variants (group HTZ-Non-JLNS), were associated in literature to a JLNS phenotype, we performed a second analysis on new *literature-based groups*. We switched indeed the patients with literature-based JLNS variants from group HTZ-Non-JLNS, adding them to our original group of LQT1 patients carrying a heterozygous variant identified in the JLNS population (group HTZ-Non-Relative-JLNS), resulting in a new *literature-based group HTZ-Non-Relative-JLNS* and consequently in a new *literature-based group HTZ-JLNS*.

Similarly, since missense mutation effect may sometimes be unexpected, leading to HI instead of a DN effect, we performed a detailed examination of the literature for each missense mutation found in our study population.

As available in online supplementary materials, functional studies demonstrated HI for several of our missense mutations. On this basis, we then repeated our analyses after shifting the new missense haploinsufficient literature-based variants from the group of likely DN mutations and adding them to HI variants (see online supplementary materials).

Statistical analysis

The distribution of continuous variables was evaluated with the Shapiro–Wilk test. In case of normal distribution, continuous variables were presented as means ± standard deviation while as p50 median value and interquartile ranges (p25; p75) in case of non-normal distribution. Categorical variables were expressed as frequency and percentage. The χ^2 test or the Fisher's exact test was used, as appropriate, to assess differences between categorical data. In case of inter-group χ^2 tests, Bonferroni correction was applied. According to distribution type (normal or not), the one-way analysis of variance or the Kruskal–Wallis test was used to evaluate the differences between groups, supplemented with *post hoc* analysis when necessary. A *P*-value < 0.05 was considered statistically significant, and all tests were two-sided.

Event-free survival curves were generated using the Kaplan–Meier method and compared using the log-rank test based on lifelong follow-up. Of note, in the supplementary analyses, event collection was censored when the first event occurred before 41 years old and upon beta-blocker introduction, to avoid potential interference from other cardiovascular diseases (e.g. coronary artery diseases) on the predefined cardiac endpoints. Similarly, we analysed time to first event for SAE and for AE. Moreover, we evaluated the prognostic contribution of clinical (gender and QTc) and genetic factors (location of the mutation, genetic group, and functional effect) to the occurrence of cardiac events by performing a univariate Cox modelling. We then included factors with a *P*-value < 0.1 in a multivariate Cox regression model, while extrapolating hazard ratios (HRs) with the related 95% confidence intervals (CIs). Furthermore, we created an algorithm based on the 15-year event rate estimated from the Kaplan–Meier curves, considering different clinical and genetic factors.

All statistical analyses were conducted using R software (version 1.3.1093, 2009–20 RStudio©, PBC).

Results

Study population

Between September 1993 and January 2023, a total of 789 patients underwent diagnostic evaluation and genetic testing for JLNS or LQT1. Patients were classified into three groups based on their diagnostic and genetic findings (*Figure 1*).

- Group JLNS (*KCNQ1* homozygous or compound heterozygous variant carriers)

  Out of the 789 patients, 35 (4%) were diagnosed with JLNS. All of them were type 1 JLNS. Among these patients, 30 individuals (20 variants in 19 families) had genetic results that met the inclusion criteria, while 5 were excluded due to missing or incomplete genetic data. Homozygous pathogenic variants were observed in 28 patients, while 2 patients had composite heterozygous variants on different *KCNQ1* alleles (arranged in *trans*). The most common pathogenic variant identified in our cohort was a guanine to cytosine substitution at position 914 of exon 6, present in 23.3% of cases. Supplementary material online, *Table S1*, provides a detailed overview of all JLNS variants found in our cohort.
- Group HTZ-JLNS (heterozygous JLNS variant carriers)

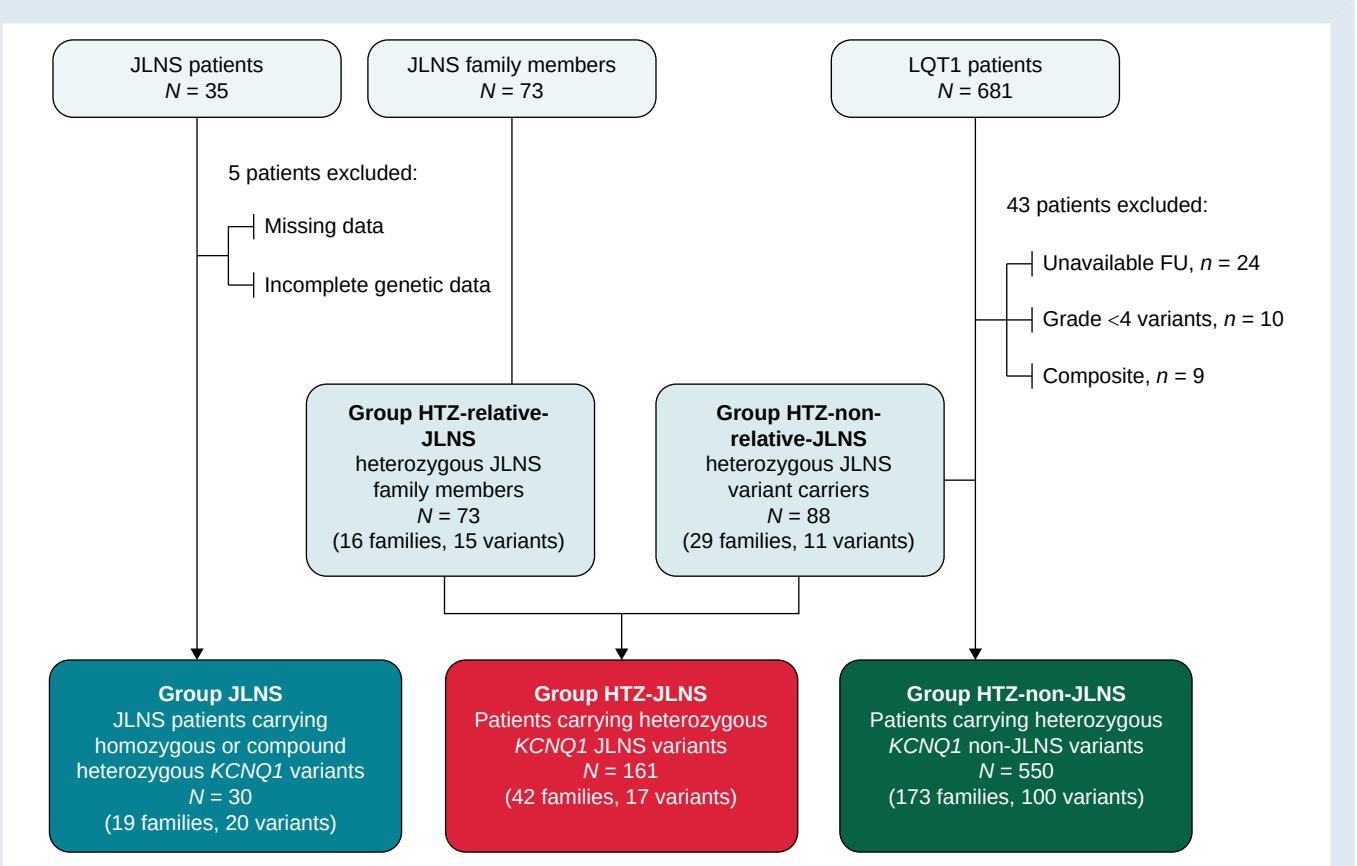


Figure 1 Flow chart. JLNS, Jervell and Lange–Nielsen syndrome; LQT1, long QT syndrome type 1; HTZ-JLNS, heterozygous carriers of variants found in the JLNS population; HTZ-Non-JLNS, heterozygous carriers of variants not found in the JLNS population.

Table 1 Study population characteristics

	Group JLNS ($n = 30$)	Group HTZ-JLNS ($n = 161$)	Group HTZ-Non-JLNS ($n = 550$)	*P*
Females, *n* (%)	17 (56.7%)	86 (53%)	310 (55.6%)	0.9
Age at diagnosis (years)	3.4 ± 4.7	26.7 ± 21[a]	26 ± 21[a]	<0.01
	2 (3.75)	27 (36)	25 (33)	
	0 ; 21	0 ; 83	0 ; 88	
Proband (%)	21 (70%)	19 (12%)[b]	146 (26%)	<0.01
Diagnosis pathway				
Secondary to symptoms	15 (50%)	13 (8%)[b]	107 (19%)	<0.01
Family assessment	10 (30%)	139 (86%)[b]	395 (72%)	
Fortuitous/non-cardiac symptoms	5 (16.6%)	9 (6%)[b]	48 (9%)	
Schwartz score	6 ± 1.6	2.4 ± 1.2[a,c]	3.2 ± 1.5[a]	<0.01
	6 (2)	2.1 (2)	3 (2)	
	3 ; 9.5	1 ; 6	1 ; 7	
QTc Bazett (ms)	551 ± 54	451 ± 32[a,c]	467 ± 36[a]	0.01
	551 (75)	452 (37)	466 (43)	
	477 ; 665	350 ; 525	320 ; 635	
Pore location, *n* (%)	7 (23%)	13 (8%)[b]	118 (21%)	<0.01
HI, *n* (%)	15 (50%)	77 (48%)[b]	67 (12%)	<0.01
DN effect, *n* (%)	15 (50%)	84 (52%)[b]	479 (87%)	<0.01
Follow-up duration (years)	18 ± 14	33 ± 22	32 ± 20	0.04
	14 (17)	33 (35)	30 (32)	
	0 ; 51	0 ; 93	0 ; 91	
Arrhythmic events, *n* (%)	26 (87%)	21 (13%)[b]	183 (33%)	<0.01
Severe arrhythmic events, *n* (%)	9 (30%)	1 (0.6%)	22 (4%)	<0.01
Family history of SD < 35 years	6 (20%)	49 (30%)	154 (28%)	0.5
ß-blockers	24 (80%)	82 (51%)[b]	372 (68%)	<0.01

Continuous variables are presented by their mean ± standard deviation followed by their median (IQR) and range (min/max). For the quantitative variables, comparison of the three groups by analysis of variance with "[a]" in case of significance of the post-test compared with JLNS group and "[c]" compared with HTZ-JLNS group. For qualitative variables, χ^2 test with "[b]" in case of significant test compared with HTZ-JLNS group. HI, haploinsufficiency; DN, dominant negative; SD, sudden death.

We identified 161 patients who were heterozygous carriers of JLNS mutations (17 JLNS variants in 42 families). Group HTZ-JLNS included the following:

- Seventy-three relatives of JLNS-affected patients, carrying a heterozygous variant (*group HTZ-Relative-JLNS*)
- Eighty-eight LQT1 patients, carrying a heterozygous variant identified in the JLNS population (*group HTZ-Relative-Non-JLNS*)

- Group HTZ-Non-JLNS (LQT1 carriers of non-JLNS variants)

A total of 681 patients were diagnosed with LQT1 and followed up in our centre. Among them, 657 patients had available follow-up data and localization information. Finally, we focused the analysis on a global population of 638 LQT1 patients, while excluding from the study group 9 patients with either composite heterozygous or homozygous mutations without neuro-sensorial deafness as well as 10 patients carrying variants of uncertain significance. After variant analysis, we found that 550 heterozygous patients (100 variants in 173 families) did not carry variants detected within the JLNS population of our study.

Groups are described and compared in *Table 1*.

The proportion of women did not differ between groups. Jervell and Lange–Nielsen syndrome patients had a younger age at diagnosis (group JLNS 3.4 ± 4.7 years vs. group HTZ-JLNS 26.7 ± 21 vs. HTZ-Non-JLNS 26 ± 21, $P < 0.01$). Jervell and Lange–Nielsen syndrome patients had a significantly higher Schwartz score (6 ± 1.6 vs. 2.4 ± 1.2 vs. 3.2 ± 1.5, $P < 0.01$) and longer QTc interval (551 ± 54 ms vs. 451± 31 vs. 467 ± 36; $P = 0.01$) compared with heterozygous patients (*Figure 2*). Jervell and Lange–Nielsen syndrome patients were more frequently probands, and the diagnosis was more often established due to symptoms when compared with heterozygous patients, more frequently diagnosed in the context of a pre-symptomatic family screening. Finally, beta-blocker treatment was less frequently prescribed among group HTZ-JLNS.

Focusing on heterozygous JLNS variant carriers, patients initially diagnosed with LQT1 (group HTZ-Non-Relative-JLNS) had higher Schwartz scores, longer QTc intervals, and a lower rate of HI variants (*Table 2*) when compared with heterozygous relatives of JLNS-affected patients (group HTZ-Relative-JNLS). Supplementary material online, *Table S2*, provides a detailed overview of all Non_JLNS variants found in our cohort.

Genetic characteristics

A total of 120 class IV or V variants (20 JLNS variants + 100 non-JLNS variants) were identified. Among them, 39 variants (5 CNV, 14 splice site, and 20 frameshift) were determined to likely result in HI, while 81 variants were considered as potentially causing a DN effect (76 missense and 5 in-frame). Regarding missense and in-frame variants, 51 (63%) were located in the transmembrane region, with 16 specifically

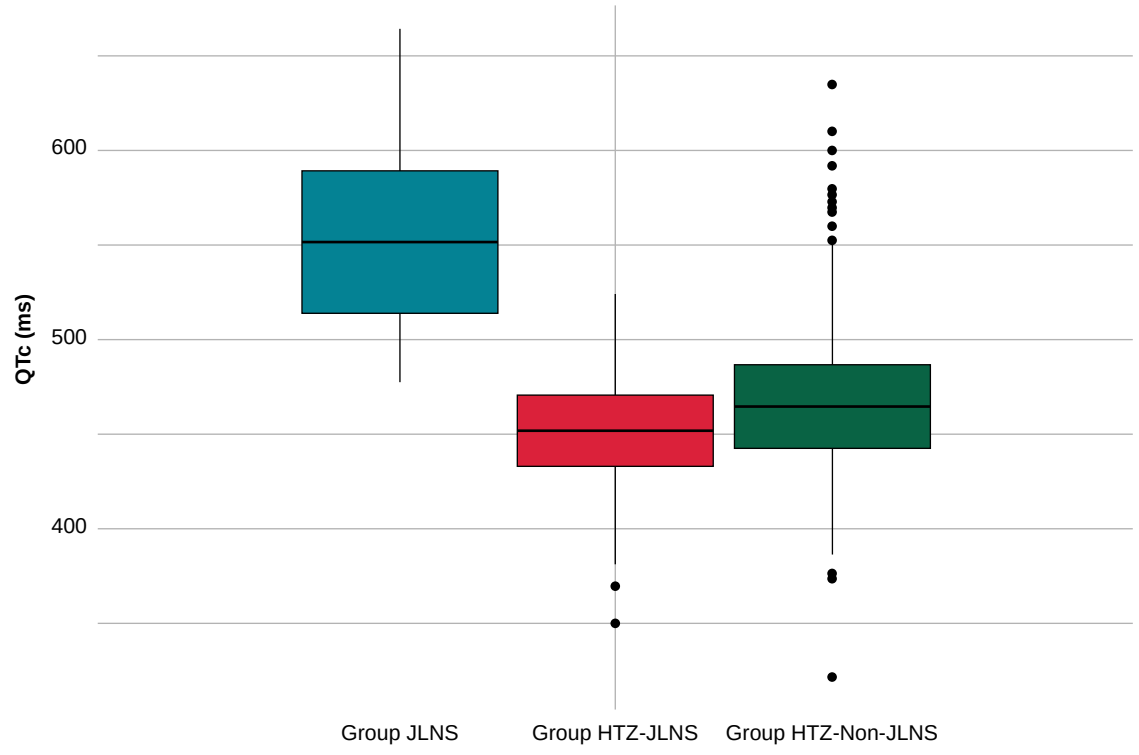


Figure 2 Box plot of the distribution of QTc according to the groups at diagnosis.

positioned inside the pore. In contrast, 30 (37%) DN variants were non-transmembrane, out of which 24 were situated in the C-terminal region.

Focusing on variant localization and on biophysical effect, HTZ-JLNS variant carriers had a lower rate of variants localized in the pore region in comparison with JLNS and HTZ-Non-JLNS (8% vs. 23% vs. 21%, $P < 0.01$). Moreover, the proportion of variants potentially causing HI was lower among HTZ-Non-JLNS patients [12% vs. 48% vs. 50% ($P < 0.01$) among HTZ-Non-JLNS, HTZ-JLNS, and JLNS groups, respectively].

Arrhythmic outcomes

Patients were followed up, until a mean age of 31.6 ± 21 years, with a median of 29 years [interquartile range (IQR) 33].

For JLNS patients, 87% ($n = 24$) experienced at least one AE, all of them occurring before the age of 15, and 30% ($n = 9$) suffered from SAE during follow-up. These latter included one recovered cardiac arrest, seven sudden deaths, and one ICD therapy for ventricular fibrillation. Of note, 6 (66.7%) out of the 9 patients experienced a SAE despite beta-blocker treatment. The median time before AE onset was 2 years (IQR 3.75), while the median time of survival before SAE was 14 years (IQR 19).

Conversely, concerning heterozygous patients, the rate of AE was significantly lower among HTZ-JLNS variant carriers when compared with HTZ-Non-JLNS variant carriers (13% vs. 33%, $P < 0.01$), with a median time of survival without AE of 10 (IQR 19 and 18.5, respectively) years for both groups. Similarly, we observed a trend toward lower SAE among HTZ-JLNS variant carriers as compared with HTZ-Non-HLNS variant carriers (0.6% vs. 4%, $P = 0.06$) (*Table 1*).

The HTZ-JLNS patients had a lower risk of cardiac events compared with HTZ-Non-JLNS patients [HR 0.34 (0.22–0.54); $P < 0.01$] (*Figure 3*). Moreover, HTZ-Non-Relative-JLNS patients had significantly fewer events compared with HTZ-Non-JLNS patients [HR 0.68 (0.52–0.88); $P < 0.01$]. Conversely, there was no significant difference observed between the subgroups HTZ-Relative-JLNS vs. HTZ-Non-Relative-JLNS [HR 0.46 (0.18–1.19); $P = 0.11$] (*Figure 4*).

Risk factors for arrhythmic events among heterozygous patients

Table 3 shows the results of univariate and multivariate Cox model.

After multivariate analysis, 5 factors were independently associated with the occurrence of AE: pore-located variant and QTc interval > 500 ms were associated with an increased risk of events while the presence of a JLNS variant, of a mutation leading to HI or located at the C-terminal region, was associated with a reduced risk of AE.

Figure 5 shows the 15-year event rates estimated from the Kaplan–Meier curves according to different QTc and genetic data (HI and pore involvement). We observe a 15-year event rates ranging from 11.8% for patients with a QTc < 500 ms and a variant inducing HI to 46.9% for patients with a QTc > 500 ms and a mutation located in the pore. Supplementary material online, *Figure S1*, shows another algorithm that takes groups into account.

After a detailed examination of the literature available for the 120 variants identified in our population, 29 were previously reported as associated with a JLNS phenotype. Among them, 13 variants were carried by patients included in our JLNS group, accounting for 65% of the JLNS variants in our cohort. The remaining 16 variants were conversely detected in 89 patients included in our original group of HTZ-Non-JLNS (see Supplementary material online, *Table S3*).

On this basis, we moved these 89 patients from HTZ-Non-JLNS group, adding them to our original group of 88 LQT1 patients carrying a heterozygous variant identified in the JLN population (HTZ-Non-Relative-JLNS), resulting in a new *literature-based group HTZ-Non-Relative-JLNS*, of 177 individuals.

Table 2 Characteristics of heterozygous JLNS variant carriers

	Group HTZ-Relative-JLNS (*n* = 73)	Group HTZ-Non-Relative-JLNS (*n* = 88)	*P*
Females, *n* (%)	36 (49%)	50 (57%)	0.4
Age at diagnosis (years)	24 ± 21	28 ± 21	0.2
	24 (34)	30 (35)	
	0 ; 72	0 ; 83	
Proband	0	19 (22%)	<0.01
Diagnosis pathway			
Secondary to symptoms	2 (3%)	11 (13%)	<0.01
Family assessment	71 (97%)	68 (77%)	
Fortuitous/non-cardiac symptoms	0	9 (10%)	
Schwartz score	2.1 ± 1	2.6 ± 1.3	0.02
	2 (1.8)	3 (2)	
	1 ; 6	1 ; 6	
QTc Bazett (ms)	444 (30)	456 (32)	0.03
	446 (40)	460 (41)	
	370 ; 518	355 ; 555	
Pore location, *n* (%)	7 (10%)	6 (7%)	1
HI, *n* (%)	48 (66%)	29 (33%)	<0.01
DN effect, *n* (%)	25 (34%)	59 (67%)	<0.01
Follow-up time (years)	34 ± 23	32 ± 20	0.6
	35 (34)	32 (34)	
	0 ; 93	0 ; 83	
Arrhythmic events, *n* (%)	5 (7%)	15 (17%)	0.05
Severe arrhythmic events, *n* (%)	0	1 (1.1)	1
Family history of SD < 35 years	35 (48%)	14 (16%)	<0.01
ß-blockers	18 (25)	64 (73%)	<0.01

Continuous variables are presented as mean ± standard deviation followed by their median (IQR) and range (min/max). HI, haploinsufficiency; DN, dominant negative; SD, sudden death.

We then re-calculated a new *literature-based HTZ-JLNS* (group HTZ-Relative-JLNS + HTZ-Non-Relative-JLNS) of 250 heterozygous JLNS variant carriers and a new *literature-based group HTZ-Non-JLNS* (original group HTZ-Non-JLNS—89) of 461 LQT1 carriers of non-JLNS variants (see Supplementary material online, *Figure S2*).

The new uni- and multivariate analyses on these literature-based groups showed a persistent lower risk of AE [HR 0.51 (0.37–0.70); $P < 0.01$] among patients carrying a heterozygous JLNS variant compared with other LQT1 cases (HTZ-Non-JLNS) (see Supplementary material online, *Table S4*).

Discussion

The present study is a systematic analysis of *KCNQ1* loss-of-function variants, based on a large cohort with a 30-year follow-up, which provides new insights concerning both homozygous and heterozygous JLNS variant carriers. We showed that between one-third and one-fourth of the LQT1 patients carry JLNS variants. These patients have less severe phenotypes, and this clinical difference could be partly explained by genetic characterization.

Epidemiological insight

Jervell and Lange–Nielsen syndrome is a rare disease, and determining its precise prevalence is challenging due to variations in calculation methods across studies, with estimates ranging from 1.6/million to 1/200 000 children.[25–27] Given the rarity of this pathology, our cohort of 30 patients seems therefore to represent a significant proportion of JLNS subjects in France.

Moreover, out of the 20 pathogenic variants detected in our JLNS population, 35% of them have never been described in the literature, thus making our study a major contribution to the attempt to repertory JLNS-related variants.

In addition, 22% of our population was represented by heterozygous carriers of JLNS-related variants, rising to 34% if we add additional JLNS-related variants described in literature and retrieved in our LQT1 population. This striking result may suggest that about one-fourth to one-third of patients diagnosed with LQT1 carry a JLNS-related variant in a heterozygous state. To our knowledge, this study provides for the first time the evidence of the relatively frequent detection of a heterozygous JLNS-related variant among patients diagnosed with LQT1.

Clinical insight

Corroborating the results of the previous studies,[12,13] we clearly confirmed the severe natural history of JLNS. Indeed, 87% of patients became symptomatic, experiencing at least one AE, and 30% experienced SAE during follow-up. Breakthrough events while on medical therapy were common: 67% of patients suffered from a SAE despite being

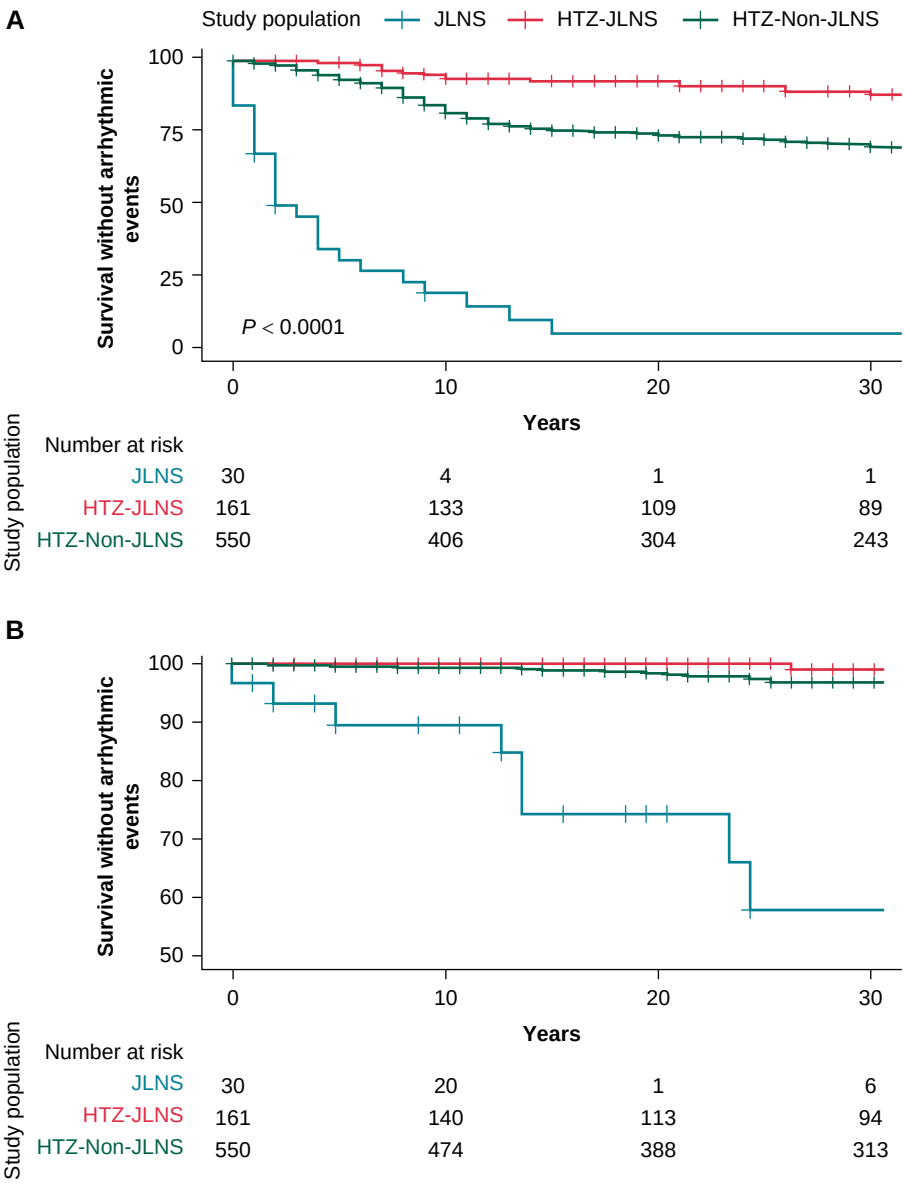


Figure 3 Survival curves without arrhythmic events (*A*) and severe arrhythmic events (*B*) according to the groups. JLNS, Jervell and Lange–Nielsen syndrome; HTZ-JLNS, heterozygous carriers of variants found in the JLNS population; HTZ-Non-JLNS, heterozygous carriers of variants not found in the JLNS population.

under beta-blocker treatment (nadolol in most cases). The failure of beta-blocker treatment was similarly described with 51% of patients remaining or becoming symptomatic despite beta-blockers treatment.[13]

The major and novel contribution of our study, however, is to shed light on the phenotype characterizing HTZ-JLNS variant carriers, in comparison with other LQT1 patients (HTZ-Non-JLNS). Our work pointed out that the rate of AE was significantly lower among HTZ-JLNS patients (13%) when compared with HTZ-Non-JLNS patients (33%) with a comparable average follow-up of 33 and 32 years respectively.

It is worth to note that we have corroborated the strength of this result with several additional analyses. Firstly, we confirmed our results after censoring follow-up upon beta-blocker introduction as well as at 41 years of age [HR 0.37 (0.23–0.59); $P < 0.01$], to avoid interferences from other cardiovascular diseases (e.g. coronary artery diseases) which may potentially impact on the predefined cardiac endpoints (see Supplementary material online, *Figure S3*). Secondly, we showed that not only heterozygous JLNS family members but also unrelated heterozygous JLNS variant carriers initially diagnosed with LQT1 had significantly fewer events as compared with other LQT1 cases. Thirdly, we performed a detailed examination of the literature, allowing us to detect 16 additional JLNS-associated variants, according to literature, among the 89 patients previously included in our original HTZ-Non-JLNS group. On this basis, the comparison between the new literature-based groups HTZ-JLNS and HTZ-Non-JLNS demonstrates a persistent lower risk of AE [HR 0.51 (0.37–0.70)] among patients carrying a heterozygous JLNS variant compared with other LQT1 cases.

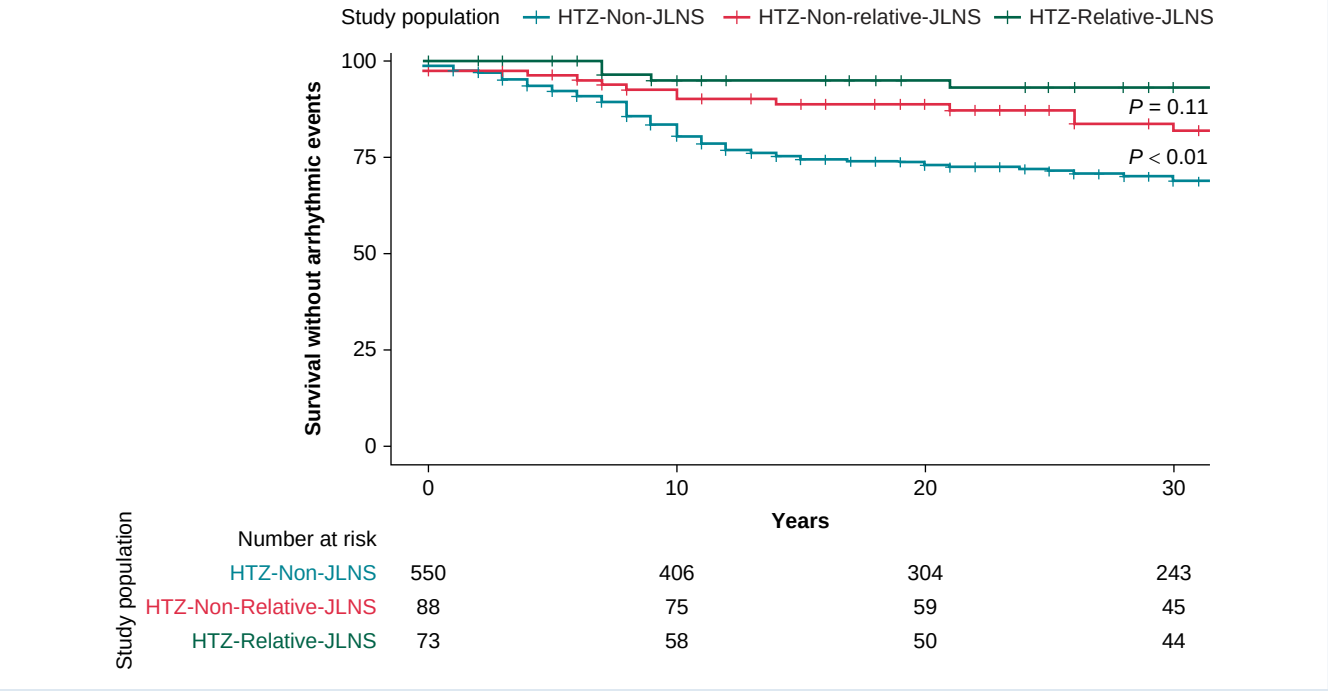


Figure 4 Free-event survival curves related to LQT1 JLNS variant carriers (HTZ-Non-Relative-JLNS), relatives of JLNS-affected patients (HTZ-Relative-JLNS) and others LQT1 patients (HTZ-Non-JLNS).

Table 3 Uni- and multivariate Cox model

	Univariate Cox model		Multivariate Cox model	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Female	1.10 (0.83–1.46)	0.5		
Group HTZ-JLNS vs. HTZ-Non-JLNS	0.34 (0.22–0.54)	<0.01	0.43 (0.27–0.69)	<0.01
Haploinsufficiency	0.46 (0.30–0.71)	<0.01	0.60 (0.37–0.97)	0.04
Pore location	2.26 (1.66–3.07)	<0.01	1.61 (1.14–2.26)	0.01
C-terminal	0.72 (0.51–1.02)	0.06	0.67 (0.46–0.98)	0.04
QTc Bazett > 500 ms	1.98 (1.41–2.78)	<0.01	1.71 (1.21–1.41)	<0.01

HTZ-JLNS, heterozygous carriers of variants found in the JLNS population; HTZ-Non-JLNS, heterozygous carriers of variants not found in the JLNS population.

Thus, our results support the hypothesis of a true class effect associating to any heterozygous JLNS variant carrier a better prognosis, burdened by a lower risk of AE. Considering both our epidemiological and clinical insights, we do provide hereby a clinically relevant prognostic information for a significant subset of LQT1 population.

Mechanistic insight: toward refining risk stratification

Our analyses found four independent modulators of the arrhythmic risk among *KCNQ1* heterozygous patients: QTc interval > 500 ms, HI, variant localization (C-terminal region vs. pore location), and JLNS-related variants.

To understand this genotype–phenotype correlation, a pathophysiological reading of our results is necessary.

As expected, our work confirms the importance of QTc prolongation in arrhythmic risk stratification, as demonstrated by the team of Priori *et al.*[28–30] doubling the risk of AE in the case of QTc > 500 ms. However, QT interval is an insufficient marker, as torsades de pointes can occur in patients with normal or slightly prolonged QTc interval.[31]

As previously described,[6,9,17,32] we found a significant higher rate of non-missense mutations and thus of likely HI variants among JLNS variant carriers (homozygous and heterozygous) when compared with other LQT1 patients (50, 48, and, 12%, respectively). Considering the tetrameric structure of the functional I_{Ks} channel, such mutations lead indeed either to truncated proteins or RNA degradation, avoiding their co-assembly to form functional channels. This phenomenon explains why, at a homozygous state, the complete or near complete (<10%) absence of I_{Ks} is associated to a more severe phenotype, burdened of bilateral neuro-sensorial deafness and high life-threatening

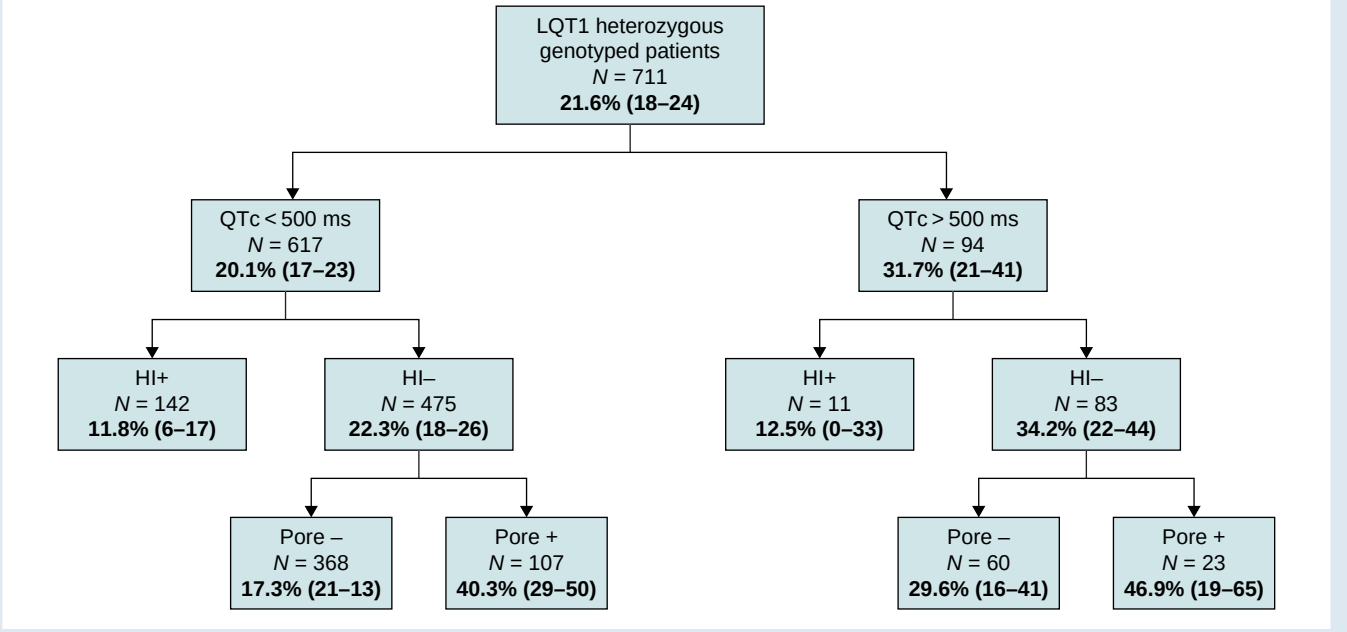


Figure 5 Algorithm for the 15-year AE rate based on QTc, HI, and pore location. HI, haploinsufficiency.

arrhythmic risk. Heterozygous JLNS patients instead express a milder phenotype since I_{Ks} is ensured by the co-assembly of normal proteins, encoded by the wild-type allele. The more severe phenotype of HTZ-Non-JLNS variant carriers may thus be explained by the co-assembly of wild-type and mutated proteins, which produces non-functional channels and harshly alters I_{Ks} properties by capturing wild-type subunits. This aspect also contributes to explain why, in these cases, patients express an isolated cardiac phenotype without earing defects. As elegantly demonstrated by Bhuiyan *et al*,[11] 'the ear can do with less than the heart', since an auditory phenotype seems to arise only in case of *KCNQ1*-encoded protein level < 10%. Paradoxically, however, if the complete absence of I_{Ks} proteins is not tolerated by the ear, as exemplified by the onset of bilateral deafness, it is borne by the heart and still compatible with life. Indeed, the ventricular repolarization process involves several repolarizing currents, and according to the concept of the cardiac repolarization reserve,[33] defects of single currents may be cushioned if other repolarization currents are intact.

In our work, similarly to the study from Moss *et al*,[6] we assumed that HI (≤50% impairment of the channel function) was the functional effect resulting from non-missense mutations including truncating mutations, frameshift mutations, and splicing mutations. Since missense mutation effect may sometimes be unexpected, leading to HI instead of a DN effect, we also performed a detailed examination of the literature for each missense mutation retrieved in our study population and performed new analyses based on literature-based variant classification. This approach confirmed the higher rate of likely HI variants among JLNS variant carriers (homozygous and heterozygous) as compared with other LQT1 patients (63% vs. 64% vs. 30%, $P < 0.005$). We also confirmed the reduced risk of AE associated to HI (see Supplementary material online, *Table S5* and *Figure S4*).

Our results also corroborate the few previous studies which have explored the relation between variant location and clinical phenotype.[34] Conversely, a pore-located variant (residues 283–327) was a significant predictor of a more severe phenotype at both univariate and multivariate analyses. Our results slightly differ from the study by Moss *et al*.[6,35] which demonstrated a higher risk associated to transmembrane mutations (residues 120–355), without any significant difference in event rate between patients with pore region variants (residues 285–355) vs. non-pore mutations. We should however mention that such difference may be in part explained by a different definition of pore variant between the two studies, with the inclusion in the pore region of the S6 segment (residues 328–355) in the study by Moss *et al*.

In addition to the above discussed parameters, the presence of a JLNS-related variant remained independently and negatively associated with the occurrence of events. Hence, this factor could probably not be explained by QTc duration, HI, or variant localization.

Since the *in vitro* functional effects of the variant do not always reflect the *in vivo* effect, it is possible that functional studies, most of which date back to the early 2000s, have underestimated the HI effect of JLNS variants. The localization of the variants was carried out on a linear protein model that could be held back by the complexities of 3D modelling[36] of the channel structure, making it difficult to know the precise location of the impact of a mutation on the channel.

As recently described, patients carrying variants on their both *KCNQ1* alleles (homozygous or composite heterozygous), whether deaf or not, appear to have a poor prognosis.[24] It is conceivable that the non-JLNS variants in the homozygous state are too severe and are not life-sustaining.

Beyond mechanistic consideration, evaluating a 15-year event according to QTc, HI, and variant location allows for refined more personalized risk–stratification scheme.

Limitations

Firstly, the current work is mainly based on retrospectively collected patient data. As all analyses are performed on retrospective registries, such approach might make difficult to ensure the rigorous collection of cardiac events, which might have been underestimated. Secondly, even if our homozygous and heterozygous JLNS population was quantitatively remarkable, considering the rarity of the disease, it could still have been underpowered to detect further significant differences, due to the small sample size. Thirdly, we could not exclude having embedded in our group of LQT1 non-JLNS variant carriers, patients with heterozygous JLNS variants, which have not yet been known/described

in the literature. However, patient misclassification (for symptomatic status and/or variant classification) would have potentially biased the results toward decreased differences between groups. Fourth, concerning variant biophysical effect, functional studies were available in literature only for a limited number of missense variants. We could not therefore exclude having incorporated any missense HI variants among mutations with DN effect. Our results underline the need for improving characterization of biophysical variant effects. High output patch clamp biophysical characterization and artificial intelligence (AI)-based 3D structure–function studies are needed to improved risk stratification.

To conclude, our study provides, for the first time to our knowledge, the evidence that JLNS variants are far more frequent than previously estimated and that up to one-third of patients diagnosed with LQT1 carry a JLNS-related variant at the heterozygous state. We confirm the severe natural history of JLNS in our cohort, and we demonstrated that heterozygous JLNS genotype is associated to a better prognosis when compared with other LQT1 non-JLNS variant carriers. Finally, beyond the classical and unanimous clinical predictor (QTc), we showed that the presence of JLNS-related variants, HI, and variant topography (C-terminal region vs. pore location) represent independent modulators of arrhythmic risk among *KCNQ1* heterozygous patients. Our study highlights the importance of integrating to clinical phenotyping, a detailed genetic variant characterization, encompassing their functional effect and location, to better refine risk stratification.

Supplementary material

Supplementary material is available at *Europace* online.

Funding

None declared.

Conflict of interest: none declared.

Data availability

The data underlying this article are available in the article and in its online Supplementary material.

References

1. Crotti L, Brugada P, Calkins H, Chevalier P, Conte G, Finocchiaro G *et al.* From gene-discovery to gene-tailored clinical management: 25 years of research in channelopathies and cardiomyopathies. *Europace* 2023;**25**:euad180.
2. Mousavi Nik A, Gharaie S, Jeong Kim H. Cellular mechanisms of mutations in Kv7.1: auditory functions in Jervell and Lange-Nielsen syndrome vs. Romano Ward syndrome. *Front Cell Neurosci* 2015;**9**:32, Available from: http://journal.frontiersin.org/Article/10.3389/fncel.2015.00032/abstract.
3. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C *et al.* Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;**103**:89–95.
4. Moore JP, Gallotti RG, Shannon KM, Bos JM, Sadeghi E, Strasburger JF *et al.* Genotype predicts outcomes in fetuses and neonates with severe congenital long QT syndrome. *JACC Clin Electrophysiol* 2020;**6**:1561–70.
5. Yang WP, Levesque PC, Little WA, Conder ML, Shalaby FY, Blanar MA. KvLQT1, a voltage-gated potassium channel responsible for human cardiac arrhythmias. *Proc Natl Acad Sci USA* 1997;**94**:4017–21.
6. Moss AJ, Shimizu W, Wilde AAM, 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.
7. Nishimura M, Ueda M, Ebata R, Utsuno E, Ishii T, Matsushita K *et al.* A novel KCNQ1 nonsense variant in the isoform-specific first exon causes both Jervell and Lange-Nielsen syndrome 1 and long QT syndrome 1: a case report. *BMC Med Genet* 2017;**18**:66.
8. Oertli A, Rinné S, Moss R, Kääb S, Seemann G, Beckmann BM *et al.* Molecular mechanism of autosomal recessive long QT-syndrome 1 without deafness. *IJMS* 2021;**22**:1112.
9. Neyroud N, Tesson F, Denjoy I, Leibovici M, Donger C, Barhanin J *et al.* A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nat Genet* 1997;**15**:186–9.
10. Jervell A, Lange-Nielsen F. Congenital deaf-mutism, functional heart disease with prolongation of the Q-T interval and sudden death. *Am Heart J* 1957;**54**:59–68.
11. Bhuiyan ZA, Wilde AAM. I_{ks} in heart and hearing, the ear can do with less than the heart. *Circ Cardiovasc Genet* 2013;**6**:141–3.
12. Schwartz PJ, Spazzolini C, Crotti L, Bathen J, Amlie JP, Timothy K *et al.* The Jervell and Lange-Nielsen syndrome: natural history, molecular basis, and clinical outcome. *Circulation* 2006;**113**:783–90.
13. Goldenberg I, Moss AJ, Zareba W, Mcnitt S, Robinson JL, Qi M *et al.* Clinical course and risk stratification of patients affected with the Jervell and Lange-Nielsen syndrome. *J Cardiovasc Electrophysiol* 2006;**17**:1161–8.
14. Rohatgi RK, Sugrue A, Bos JM, Cannon BC, Asirvatham SJ, Moir C *et al.* Contemporary outcomes in patients with long QT syndrome. *J Am Coll Cardiol* 2017;**70**:453–62.
15. Schwartz PJ. The congenital long QT syndromes from genotype to phenotype: clinical implications. *J Intern Med* 2006;**259**:39–47.
16. Sieliwonczyk E, Alaerts M, Simons E, Snyders D, Nijak A, Vandendriessche B *et al.* Clinical and functional characterisation of a recurrent KCNQ1 variant in the Belgian population. *Orphanet J Rare Dis* 2023;**18**:23.
17. Zehelein J, Kathoefer S, Khalil M, Alter M, Thomas D, Brockmeier K *et al.* Skipping of exon 1 in the KCNQ1 gene causes Jervell and Lange-Nielsen syndrome. *J Biol Chem* 2006;**281**:35397–403.
18. Gouas L, Bellocq C, Berthet M, Potet F, Demolombe S, Forhan A *et al.* New KCNQ1 mutations leading to haploinsufficiency in a general population: defective trafficking of a KvLQT1 mutant. *Cardiovasc Res* 2004;**63**:60–8.
19. Splawski I, Timothy KW, Vincent GM, Atkinson DL, Keating MT. Molecular basis of the long-QT syndrome associated with deafness. *N Engl J Med* 1997;**336**:1562–7.
20. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA *et al.* 2022 ESC guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J* 2022;**43**:3997–4126.
21. Wilde AAM, Semsarian C, Márquez MF, Shamloo AS, Ackerman MJ, Ashley EA *et al.* European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) expert consensus statement on the state of genetic testing for cardiac diseases. *Europace* 2022;**24**:1307–67.
22. Aktaa S, Tzeis S, Gale CP, Ackerman MJ, Arbelo E, Behr ER *et al.* European Society of Cardiology quality indicators for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Europace* 2023;**25**:199–210.
23. 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.
24. Righi D, Porco L, Di Mambro C, Gnazzo M, Baban A, Paglia S *et al.* Autosomal recessive long QT syndrome: clinical aspects and therapy. *Pediatr Cardiol* 2023;**44**:1736–40.
25. Fraser GR, Froggatt P, Murphy T. Genetical aspects of the cardio-auditory syndrome of Jervell and Lange-Nielsen (congenital deafness and electrocardiographic abnormalities). *Ann Hum Genet* 1964;**28**:133–57.
26. Winbo A, Stattin EL, Diamant UB, Persson J, Jensen SM, Rydberg A. Prevalence, mutation spectrum, and cardiac phenotype of the Jervell and Lange-Nielsen syndrome in Sweden. *Europace* 2012;**14**:1799–806.
27. Giudicessi JR, Ackerman MJ. Prevalence and potential genetic determinants of sensorineural deafness in *KCNQ1* homozygosity and compound heterozygosity. *Circ Cardiovasc Genet* 2013;**6**:193–200.
28. Mazzanti A, Maragna R, Vacanti G, Monteforte N, Bloise R, Marino M *et al.* Interplay between genetic substrate, QTc duration, and arrhythmia risk in patients with long QT syndrome. *J Am Coll Cardiol* 2018;**71**:1663–71.
29. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M *et al.* Risk stratification in the long-QT syndrome. *N Engl J Med* 2003;**348**:1866–74.
30. Moss AJ, Schwartz PJ, Crampton RS, Tzivoni D, Locati EH, MacCluer J *et al.* The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation* 1991;**84**:1136–44.
31. Goldenberg I, Horr S, Moss AJ, Lopes CM, Barsheshet A, McNitt S *et al.* Risk for life-threatening cardiac events in patients with genotype-confirmed long-QT syndrome and normal-range corrected QT intervals. *J Am Coll Cardiol* 2011;**57**:51–9.
32. Bellin M, Greber B. Human iPS cell models of Jervell and Lange-Nielsen syndrome. *Rare Diseases* 2015;**3**:e1012978.
33. Roden DM. Taking the 'idio' out of 'idiosyncratic': predicting torsades de pointes. *Pacing Clin Electro* 1998;**21**:1029–34.
34. Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M *et al. KVLQT1* C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997;**96**:2778–81.
35. Schwartz PJ, Moreno C, Kotta MC, Pedrazzini M, Crotti L, Dagradi F *et al.* Mutation location and I_{ks} regulation in the arrhythmic risk of long QT syndrome type 1: the importance of the KCNQ1 S6 region. *Eur Heart J* 2021;**42**:4743–55.
36. Xu Y, Wang Y, Meng XY, Zhang M, Jiang M, Cui M *et al.* Building KCNQ1/KCNE1 channel models and probing their interactions by molecular-dynamics simulations. *Biophys J* 2013;**105**:2461–73.