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CLINICAL RESEARCH
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SCN5A mutations in 442 neonates and children: genotype–phenotype correlation and identification of higher-risk subgroups

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Aims To clarify the clinical characteristics and outcomes of children with *SCN5A*-mediated disease and to improve their risk stratification.

Methods and results A multicentre, international, retrospective cohort study was conducted in 25 tertiary hospitals in 13 countries between 1990 and 2015. All patients ≤16 years of age diagnosed with a genetically confirmed *SCN5A* mutation were included in the analysis. There was no restriction made based on their clinical diagnosis. A total of 442 children {55.7% boys, 40.3% probands, median age: 8.0 [interquartile range (IQR) 9.5] years} from 350 families were included; 67.9% were asymptomatic at diagnosis. Four main phenotypes were identified: isolated progressive cardiac conduction disorders (25.6%), overlap phenotype (15.6%), isolated long QT syndrome type 3 (10.6%), and isolated Brugada syndrome type 1 (1.8%); 44.3% had a negative electrocardiogram phenotype. During a median follow-up of 5.9 (IQR 5.9) years, 272 cardiac events (CEs) occurred in 139 (31.5%) patients. Patients whose mutation localized in the C-terminus had a lower risk. Compound genotype, both gain- and loss-of-function *SCN5A* mutation, age ≤1 year at diagnosis in probands and age ≤1 year at diagnosis in non-probands were independent predictors of CE.

Conclusion In this large paediatric cohort of *SCN5A* mutation-positive subjects, cardiac conduction disorders were the most prevalent phenotype; CEs occurred in about one-third of genotype-positive children, and several independent risk factors were identified, including age ≤1 year at diagnosis, compound mutation, and mutation with both gain- and loss-of-function.

Keywords Brugada syndrome • Genotype–phenotype correlation • Long QT syndrome • Progressive cardiac conduction disorders • SCN5A • Sodium channelopathy

Introduction

Mutations in the gene (*SCN5A*) encoding the alpha subunit of the cardiac sodium channel (NaV1.5) cause type 3 long QT syndrome (LQT3),[1] type 1 Brugada syndrome (BrS-1),[2,3] progressive cardiac conduction disorders (PCCD),[3,4] atrial standstill and sick sinus syndrome (SSS),[5] familial atrial fibrillation (AF),[6] multifocal ectopic Purkinje-related premature contractions (MEPPC), [7] dilated cardiomyopathy (DCM),[8] and sudden infant death syndrome (SIDS).[9,10] Some patients with *SCN5A* mutations are predisposed to sudden cardiac death (SCD), independently of age. A cardiac sodium channelopathy comprises a substantial proportion of aborted cardiac arrest (ACA) in children and adolescents.[11] Cardiac sodium channelopathies are diagnosed in infancy and early childhood following symptoms, sudden death, or family screening.[12,13] Due to cascade genetic screening, the number of detected asymptomatic children with a *SCN5A* mutation is increasing. There is a significant variation in management of these asymptomatic *SCN5A* mutation-positive children amongst paediatric electrophysiologists.[14] This is due to their relative rarity in the paediatric population. Therefore, challenging questions in clinical practice remain unanswered, and risk stratification is inadequate. This study aimed to assess the genotype–phenotype relationship and the risk analysis of cardiac sodium channelopathies in a large cohort of infants and children in order to improve their management.

Methods

Study design

A multicentre, international, retrospective cohort study was conducted in 25 tertiary hospitals in 13 different countries from January 1990 to December 2015. Institutional review board approval was obtained from all participating institutions. All deceased and living patients ≤16 years of age diagnosed with a genetically confirmed *SCN5A* mutation were eligible for the study. There were no restrictions to the clinical diagnoses. Patients without a baseline electrocardiogram (ECG) were excluded from the analysis.

Clinical investigations

In all patients, demographic data, personal and family history (FH), mode of presentation, ECGs, echocardiography, treatment, and major cardiac events (MCEs) throughout follow-up were ascertained. Electrolyte and metabolic disturbances were excluded through laboratory tests. Study physicians gave their patients information about lifestyle modifications, such as aggressive antipyretic measures, the need for ECG monitoring during fever episodes, and avoidance of appropriate proarrhythmic drugs. Therapeutic management of the patients was based on the clinical judgement of the referring cardiologist. In case of device implantation, pacemaker (PM) type and mode of pacing, or implantable cardioverter-defibrillator (ICD) type and number of appropriate/inappropriate shocks were noted, as well as other device-related complications.

Genetic analysis

Mutation analysis of the *SCN5A* gene followed standard accepted protocols for genetic testing. Amino acid numbering was made according to transcription variant 1 of *SCN5A* (http://www.ncbi.nlm.nih.gov/; NM_198056) and the predicted structure reported by Wang *et al.*,[15] according to which the NaV1.5 alpha subunit protein consists of four transmembrane domains, each composed of six segments. The biophysical properties, type, and topological location of *SCN5A* mutations were determined on the basis of previously published data.[16,17] All variants were reclassified by a group of authors (A.E.B., F.K., E.R.B., V.P.) at the time of this analysis according to the recommendation of the American College of Medical Genetics.[18] *SCN5A* variants with minor allele frequency >0.1% in ExAC database (Exome Aggregation Consortium, Cambridge, MA, USA) and neutral synonymous variants were excluded.

Variants were then classified into three groups: missense pathogenic; non-missense pathogenic including truncating variants (non-sense, splice acceptor, splice donor, and frameshift mutations) and in-frame indels; and variants of uncertain significance (VUS). Missense variants were classified as pathogenic/likely pathogenic or VUS using generally accepted criteria[18]: disease-causative mutation databases, localization to highly conserved amino acid residues/key functional domains, co-segregation of the variant with the disease phenotype, evidence of perturbed ion channel function through *in vitro* functional studies. In case of double *SCN5A* mutation, patients were considered for risk analysis according to mutation location only if both mutations had the same location.

Statistical analysis

Continuous data were presented as mean (±standard deviation) or median [interquartile range (IQR)] based on the distribution. Categorical variables were presented as counts (proportions). The Mann–Whitney *U* and Kruskal–Wallis tests were performed to test for statistical differences in continuous parameters between two or more groups, respectively. The χ^2 or the Fisher exact test (based on expected frequency) were used to compare categorical variables between groups. Bonferroni method was used for *post hoc* tests. We adjusted *P*-value level on number of hypothesis tested. The Kaplan–Meier method estimator was used to assess the time to a first MCE. A Cox proportional-hazards regression analysis with random effect on family [with hazard ratios (HR) and confidence intervals (CI)] was used to evaluate the independent risk of clinical- and genetic-factors of interest for first MCE. From univariate analysis, we selected variables with *P*-value <0.10 (statistical criterion) and looked at multicollinearity between variables. For the multivariate model, we kept the following variables: proband, age <1 year at diagnosis, phenotype at baseline, genotype, location, HR, atrioventricular block (AVB), RBBB, and supraventricular arrhythmia. Variables were eliminated from highest to lowest *P*-values but remained in the final model if the *P*-value was less than 0.05 or seem to be confounders (more than 10% change in estimate). Final multivariable Cox model was stratified by phenotype (LQT3, PCCD, overlap phenotype, and ECG phenotype-negative) at baseline to relax the assumption of proportional hazards. All two-way interactions between pairs of predictors in the model were tested, one at a time. The mean event rate per year was evaluated by the number of events occurring during the follow-up divided by the number of patients multiplied by the average duration of follow-up. A *P*-value <0.05 was considered statistically significant when no Bonferroni correction was made. All *P*-values are two-sided. Due to the small number of patients in BrS-1, DCM and SSS phenotypes, these were not included in all the analysis. Data were analysed with the SAS packages (SAS Institute Inc. version 9, 4, Cary, NC, USA).

Results

A total of 442 children [246 boys (56%), 178 probands (40%), and median age at diagnosis of 8.0 (IQR 9.5) years] from 350 distinct families were eligible for the study.

Baseline clinical characteristics

Most of the patients (68%) were asymptomatic at diagnosis (Supplementary material online, *Figure S1*). The four 'major' ECG phenotypes at baseline were isolated PCCD (26%), overlap phenotype (16%), isolated LQT3 (11%), and isolated BrS1 (2%); 196 (44%) patients had a negative ECG phenotype at baseline (*Figure 1*). Clinical characteristics of each patients' group are detailed in Supplementary material online. All groups had similar gender distribution ($P = 0.13$) and median age at diagnosis ($P = 0.32$). The proportion of probands differed among groups ($P = 0.02$). The mode of presentation also differed ($P < 0.001$), an initial cardiac arrest being more frequent in overlap phenotype patients [16/69 (23%), $P = 0.0001$], isolated PCCD patients [20/113 (18%), $P = 0.002$], and isolated LQT3 patients [11/47 (23%), $P = 0.0005$] compared to negative ECG phenotype patients [13/196 (7%)] (Supplementary material online, *Table S1*).

Clinical outcomes

Overall there were 272 MCEs in 139 (31%) patients during a median follow-up period of 5.9 years (IQR 5.9). Fifty (11%) patients had recurrent MCEs on treatment. Of the 77 (17%) ICD-implanted patients, 100 appropriate shocks were delivered in 28 (36%) patients during a median follow-up period of 3.3 years (Supplementary material online, *Table S2*). Inappropriate ICD shocks occurred in nine patients (12%; T wave oversensing in seven patients, AF in one, and lead fracture in one). The four 'major' ECG phenotypes at baseline developed as follows:

Isolated progressive cardiac conduction disorder patients

At a median follow-up of 5.7 (0.0–35.7) years, 26/113 (23%) patients kept an isolated PCCD phenotype; 13/113 (11%) had received PM implantation at a median age of 5.42 (0.06–15.58) years; 85% of PCCD patients had their first PM insertion by the age of 11; permanent PM were implanted for symptomatic bradycardia in 7/13 patients (syncope in 5, exercise-induced dyspnoea in 2), whilst the indications were prophylactic in 6/13 patients, including a mean daytime heart rate <50 b.p.m. in 4 children >1 year of age and ventricular pauses longer than 3 RR intervals in 2; 38/113 (34%) experienced ≥1 MCE, the first of which being cardiac arrest [18% including 3 documented ventricular tachycardia (VT), 1 polymorphic VT with torsades de pointes (TdP), and 1 ventricular fibrillation (VF)], SIDS (2%), or syncope (14%). At the time of their event, PCCD patients presented with the association of an AVB and right bundle branch block (RBBB) (17/38, 45%), an isolated first-degree AVB (13/38, 34%), an isolated complete RBBB (4/38, 10.5%) or a trifascicular block (4/38, 10.5%).

Two patients died (one during infancy, one SCD) and one required heart transplantation for intractable arrhythmias; although none of them underwent a sodium-channel blocker challenge, all three patients maintained an isolated cardiac conduction disorders phenotype throughout follow-up.

Overlap phenotype patients

After 5.7 (0.0–45.7) years, 34/69 (50%) patients had pharmacological treatment (beta-blocker: 39%, sodium channel blocker: 22% according to the combination of phenotypes, see Supplementary material online, *Table S3*); PM or ICD had been implanted in 10/69 (14%) and 17/69 (25%) respectively. At least one MCE occurred in 31/69 patients (45%; 1 recurrence in 6 patients, 2 recurrences in 1 patient, and ≥2 recurrences in 5 patients). Three patients died from SCD and one required extracorporeal membrane oxygenation support and was then transplanted for intractable arrhythmias.

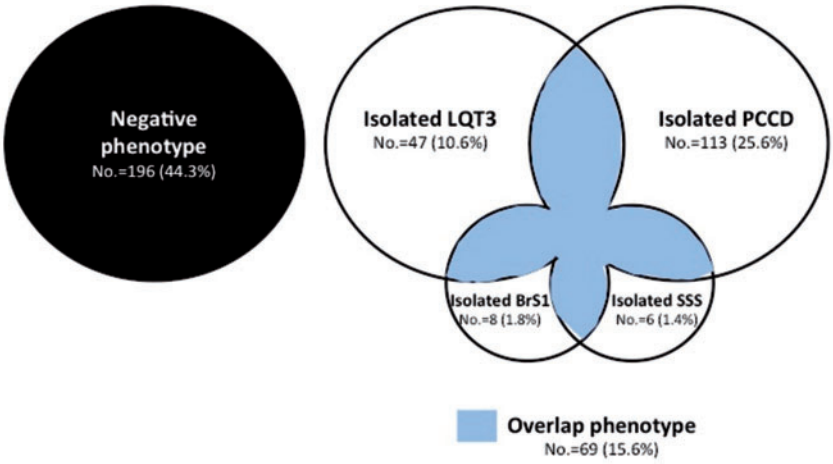


Figure 1 Venn diagram of baseline electrocardiogram phenotypes.

Isolated type 3 long QT syndrome patients

At a median follow-up of 5.9 (0.0–26.5) years, 32/47 (68%) patients received a beta-blocker, coprescribed with a sodium channel blocker in 10 (21%), 3 (6%) had undergone left cardiac sympathetic denervation and PM and ICD implantation occurred in 3 (6%) and 11 (23%), respectively.

Major cardiac event occurred in 25 patients [53%, 5/25 (11%) ≤1 year of age, 1/25 (4%) on beta-blocker at the time of the event] (Supplementary material online, *Table S4*). The first MCE was a SCD (2/47: 4%, including 1 during infancy), an ACA (19%) or a syncope (30%). Nine patients experienced more than one MCE. At the time of the first recurrent event, 7/9 patients were receiving beta-blocker therapy (Supplementary material online, *Table S5*); three patients experienced several recurrences under a coprescription of beta-blocker and mexiletine. Seven ICD shocks (6 appropriate, 1 inappropriate) were delivered in 3/11 (27%) implanted patients. Six (13%) patients died throughout follow-up, three of them had experienced a MCE in the first year of life.

Isolated type 1 Brugada syndrome patients

After 8.1 (1.8–15.7) years, 3/8 (37%) symptomatic BrS1 patients had an ICD (2.8, 11.5, and 18.8 years at implantation). They had presented with syncope (2 patients) or documented VT. One of them experienced a fever-associated VF-induced appropriate ICD shock at 13 years whilst under treatment. No death occurred. The five remaining patients were asymptomatic and left untreated.

Negative electrocardiogram phenotype patients

One hundred and ninety-six patients [44%, 52% boys, 33% probands, median age at diagnosis: 8.8 (IQR 8.7) years] had a normal ECG at baseline and underwent genetic screening because of cardiac arrest (7%), syncope (13%), or because of familial screening in asymptomatic patients (80%). A FH of either SCD/ICD implantation or PCCD/PM implantation was noted in 55% and 15%, respectively.

Of the 196 phenotype-negative patients, 27% developed an ECG phenotype throughout follow-up [5.9 (0.4–26.5) years], represented by an isolated PCCD phenotype (13%), an isolated LQT3 (5%), an isolated BrS1 (5%), or an overlap phenotype (4%), whereas 73% remained phenotype-negative. At least one MCE occurred in 40 (20%).

Of the 39 (20%) symptomatic, negative ECG phenotype patients, 26 received a beta-blocker. All but one negative ECG phenotype patients who experienced MCEs during follow-up were already symptomatic at diagnosis. Twelve experienced at least one recurrent MCE at a median delay of 3.9 (9.6) years since the diagnosis [median age of recurrent event: 3.0 (4.3) years]. All but one were treated by beta-blocker therapy at the time of the recurrent MCE; Of these 12 children, 8 kept a negative ECG phenotype at last visit, whereas 4 were further diagnosed with an isolated LQT3 phenotype and, despite additional treatment with mexiletine, experienced further recurrent MCEs leading to left cardiac sympathetic denervation (LCSD) and ICD implantation.

The vast majority (156/157, 99%) of the asymptomatic, negative ECG phenotype children remained asymptomatic throughout follow-up; one patient (0.6%) however became later symptomatic: this was a 5 year-old female patient with a normal ECG at familial screening; she was further diagnosed with an isolated LQT3 on follow-up ECGs at age 13 (QTc 491 ms) and received mexiletine; at age 18, she presented with an electrical storm whilst receiving mexiletine (500 mg morning, 250 mg afternoon, and 500 mg evening), leading to ICD implantation.

Genetic characteristics

The 442 *SCN5A* genotype-positive children had 185 independent *SCN5A* variants (Supplementary material online, *Table S5*). Three (0.7%) patients harboured a double heterozygous *SCN5A* mutation; 9 (2%) had a compound genotype with an additional disease-causing mutation in another gene: *KCNQ1* (3 patients), *KCNH2* (4 patients), *RYR2* (1 patient), or *CACNA1C* (1 patient). A loss-of-function mutation was found in 178 (40%) patients whereas, 87 (20%) had a gain-of-function mutation, 85 (19%) a both gain- and loss-of-function

Table 1 Risk analysis for major cardiac event ($n = 442$)

	No MCE ($n = 303$)	MCE ($n = 139$)	Analysis	HR (95% CI)	*P*-value
Clinical characteristics					
Male, *n* (%)	169 (55.8)	77 (55.4)	Yes vs. No	1 (0.7–1.5)	0.87
Proband, *n* (%)	75 (24.8)	103 (74.1)	Yes vs. No	7.8 (5.1–12.1)	**<0.0001**
Age ≤1 year at diagnosis, *n* (%)	34 (11.2)	41 (29.5)	Yes vs. No	11.3 (6.7–18.9)	**<0.0001**
Baseline ECG phenotype					
Isolated LQT3, *n* (%)	22 (7.3)	25 (18.0)	Yes vs. No	1.9 (1.1–3.1)	**0.01**
Isolated BrS-1, *n* (%)	5 (2.0)	3 (2.2)	Yes vs. No	1.2 (0.3–4.4)	0.69
Isolated PCCD, *n* (%)	75 (24.7)	38 (27.3)	Yes vs. No	1.2 (0.8–1.8)	0.29
Isolated DCM, *n* (%)	3 (0.9)	0 (0.0)	Yes vs. No	Not applicable	0.32[a]
Isolated SSS, *n* (%)	4 (1.3)	2 (1.4)	Yes vs. No	0.9 (0.2–4.3)	0.84
Overlap phenotype, *n* (%)	38 (12.5)	31 (22.3)	Yes vs. No	1.9 (1.2–3.1)	**0.004**
Negative ECG phenotype, *n* (%)	156 (51.5)	40 (28.8)	Yes vs. No	0.4 (0.3–0.6)	**<0.001**
First available ECG characteristics[a]					
Median age at ECG (years) (IQR)	8.2 (8.4)	7.6 (12.8)	Unit = 2	0.8 (0.7–0.9)	**<0.0001**
Heart rate, b.p.m. (IQR)	79 (26.7)	77 (47.1)	Unit = 20	1.1 (1.0–1.3)	**0.005**
PR interval, ms (IQR)	160 (42)	160 (41)	Unit = 20	1.0 (0.9–1.1)	0.52
QRS complex, ms (IQR)	80 (24)	80 (40)	Unit = 20	1.0 (0.8–1.2)	0.97
QT interval, ms (IQR)	360 (100)	380 (110)	Unit = 20	1.0 (0.9–1.1)	0.17
QTc interval, ms (IQR)	430 (68)	452 (88)	Unit = 20	1.1 (1.1–1.2)	**<0.0001**
QTc ≥500 ms	37 (12.7)	41 (30.8)	Yes vs. No	2.2 (1.4–3.4)	**0.0002**
Diagnosis of LQT3, *n* (%)	70 (23.1)	57 (41.0)	Yes vs. No	1.8 (1.2–2.7)	**0.001**
Diagnosis of sinus node dysfunction, *n* (%)	12 (4.0)	11 (7.9)	Yes vs. No	1.5 (0.7–3.1)	0.18
Diagnosis of AV block (any grade), *n* (%)	93 (30.8)	59 (42.4)	Yes vs. No	1.7 (1.2–2.6)	**0.003**
Diagnosis of RBBB (any grade), *n* (%)	122 (40.4)	66 (47.5)	Yes vs. No	1.5 (1.0–2.1)	**0.03**
Diagnosis of LBBB (any grade), *n* (%)	9 (3.0)	8 (5.8)	Yes vs. No	2.2 (0.9–4.9)	0.05
Diagnosis of SVT, *n* (%)	4 (1.3)	11 (7.9)	Yes vs. No	4 (1.9–8.9)	**0.0002**
Diagnosis of spontaneous BrS1, *n* (%)	24 (7.9)	14 (10.1)	Yes vs. No	1.2 (0.7–2.3)	0.42
Genetic characteristics					
Genotype					**0.004**
Single *SCN5A* mutation, *n* (%)	299 (98.7)	131 (94.2)	Reference	1	
Double *SCN5A* mutation, *n* (%)	1 (0.3)	2 (1.4)	vs. single	10.3 (1.8–58.7)	
Compound mutation, *n* (%)	3 (1.0)	6 (4.3)	vs. single	2.2 (0.8–6.2)	
Mutation type					0.52
Non-missense pathogenic mutation, *n* (%)	74 (24.4)	39 (28.1)	Reference	1	
Missense pathogenic mutation, *n* (%)	200 (66.0)	83 (59.7)	vs. non-missense	0.84 (0.54–1.31)	
Unknown functional effect, *n* (%)	29 (9.6)	17 (12.2)	vs. non-missense	1.03 (0.53–2.00)	
Mutation location (domains)					**<0.0001**
N-terminus location, *n* (%)	4 (1.3)	3 (2.2)	vs. DI domain	1.3 (0.3–5.6)	
DI domain, *n* (%)	37 (12.2)	27 (19.4)	Reference	1	
DI/DII interdomain linker, *n* (%)	18 (5.9)	8 (5.8)	vs. DI domain	0.7 (0.3–1.9)	
DII domain, *n* (%)	29 (9.6)	9 (6.5)	vs. DI domain	0.5 (0.2–1.1)	
DII/DIII interdomain linker, *n* (%)	22 (7.3)	8 (5.8)	vs. DI domain	0.5 (0.2–1.2)	
DIII domain, *n* (%)	49 (16.2)	19 (13.7)	vs. DI domain	0.5 (0.2–1.0)	
DIII/DIV interdomain linker, *n* (%)	15 (5.0)	13 (9.4)	vs. DI domain	1.3 (0.5–3.2)	
DIV domain, *n* (%)	40 (13.2)	31 (22.3)	vs. DI domain	1.4 (0.7–2.8)	
C-terminus, *n* (%)	89 (29.4)	21 (15.1)	vs. DI domain	0.3 (0.1–0.5)	
Mutation location (segments, $n = 241$)					0.52
S1–S4, *n* (%)	51 (32.9)	29 (33.7)	Reference	1	
S5–S6, *n* (%)	104 (67.1)	57 (66.3)	vs. S1–S4	1.1 (0.7–1.9)	
Mutation functional effect					**<0.0001**
Loss of function, *n* (%)	126 (41.6)	52 (37.4)	Reference	1	
Gain of function, *n* (%)	46 (15.2)	41 (29.5)	vs. loss-of-function	2.3 (1.4–3.9)	

Continued

Table 1 *Continued*

	No MCE ($n = 303$)	MCE ($n = 139$)	Analysis	HR (95% CI)	*P*-value
Gain and loss, *n* (%)	71 (23.4)	14 (10.1)	vs. loss-of-function	0.4 (0.2–0.8)	
Unknown functional effect, *n* (%)	60 (19.8)	32 (23.0)	vs. loss-of-function	1.2 (0.7–2.1)	

AV block, atrioventricular block; BrS-1, Brugada syndrome type 1; CE, cardiac event; DCM, dilated cardiomyopathy; FH, family history; FU, follow-up; ICD, implantable cardioverter-defibrillator; LBBB, left bundle branch block; LQT3, long QT syndrome type 3; PCCD, progressive cardiac conduction defect; PM, pacemaker; QTc, corrected QT value; RBBB, right bundle branch block; SCD, sudden cardiac death; SSS, sick sinus syndrome; SVT, supraventricular tachycardia.
[a]Cox model is not applicable when subgroups contain no event. In this later case, we presented log-rank test.
Bold values are statistically significant *P*-values.

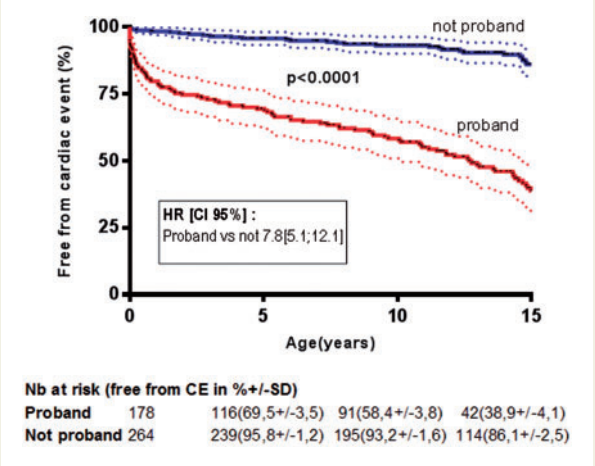
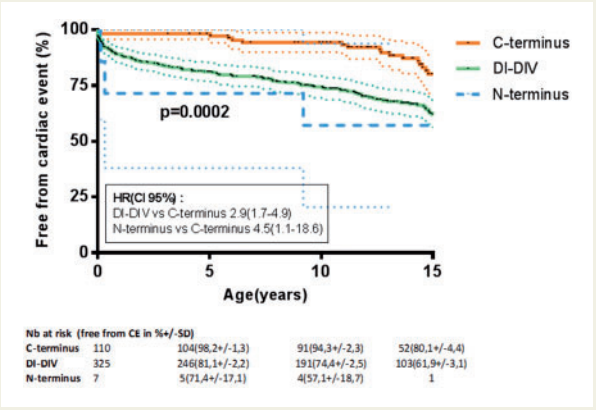


Figure 2 Freedom from major cardiac event in probands and non-probands.



Take home figure. Freedom from major cardiac event according to *SCN5A* mutation location (domains).

mutation, and 92 (21%) had a VUS. Although VUS patients were more frequently probands ($P = 0.003$), their clinical characteristics did not differ from those of patients with a variant of known functional effect (Supplementary material online, *Table S6*). Most variants were missense pathogenic mutations (64%), whereas 25% were non-missense pathogenic mutations (truncation mutations: 18%, in-frame mutations: 7%). Topological location of mutations is shown in *Supplementary material online, Figure S2*.

Genotype–phenotype correlations

Mutation topological location

Patients with a mutation in the C-terminus domain ($n = 110$) were less frequently probands ($P = 0.03$), were diagnosed later in life ($P = 0.01$), were less frequently symptomatic at diagnosis ($P = 0.001$), had less MCEs ($P = 0.0002$) and less appropriate ICD shocks ($P = 0.03$) during follow-up (Supplementary material online, *Table S7* and *Take home Figure*). No significant difference was found when comparing variants localized in S1–S4 to those localized in S5–S6 in the relevant 241 patients (Supplementary material online, *Table S8*).

Mutation functional effect

Children with a gain-of-function *SCN5A* mutation mainly presented with a baseline negative ECG phenotype (45%) or isolated LQT3 (26%); those with a loss-of-function mutation presented mainly with isolated PCCD (38%), negative ECG phenotype (27%), or overlap phenotype (19%) at baseline; and those with a both gain- and loss-of-function mainly had negative ECG phenotype (35%), isolated PCCD (22%), isolated LQT3 (12%), or overlap phenotype (14%) (Supplementary material online, *Table S9*). Comparison between groups by looking at the functional effect of the mutation (gain of function, loss of function, or both) demonstrated that gain-of-function mutation carriers were more likely to have a cardiac arrest as first presentation ($P < 0.001$) and a greater rate of both MCEs during follow-up ($P < 0.001$) and ICD implantation ($P < 0.001$).

Mutation type

Non-missense mutation were more frequently identified in case of isolated PCCD ($P < 0.006$) but less frequently found in case of negative ECG phenotype ($P < 0.007$) (Supplementary material online, *Table S10*). The following clinical parameters differed according to mutation type: age at diagnosis ($P = 0.02$), proportion of diagnosis ≤1 year ($P = 0.02$), FH of SCD/ICD ($P = 0.03$), and FH of PCCD/PM ($P = 0.001$), as did the following baseline phenotypes: isolated PCCD ($P = 0.006$) and negative ECG phenotype ($P = 0.007$) (Supplementary material online, *Table S10*). However, the type of mutation did not change the risk of MCE during follow-up.

Univariate risk analysis

The risk of MCE during follow-up was related to phenotype (*Table 1*). Age ≤1 year at diagnosis [HR (95% CI) 11.3 (6.7–18.9), $P < 0.0001$],

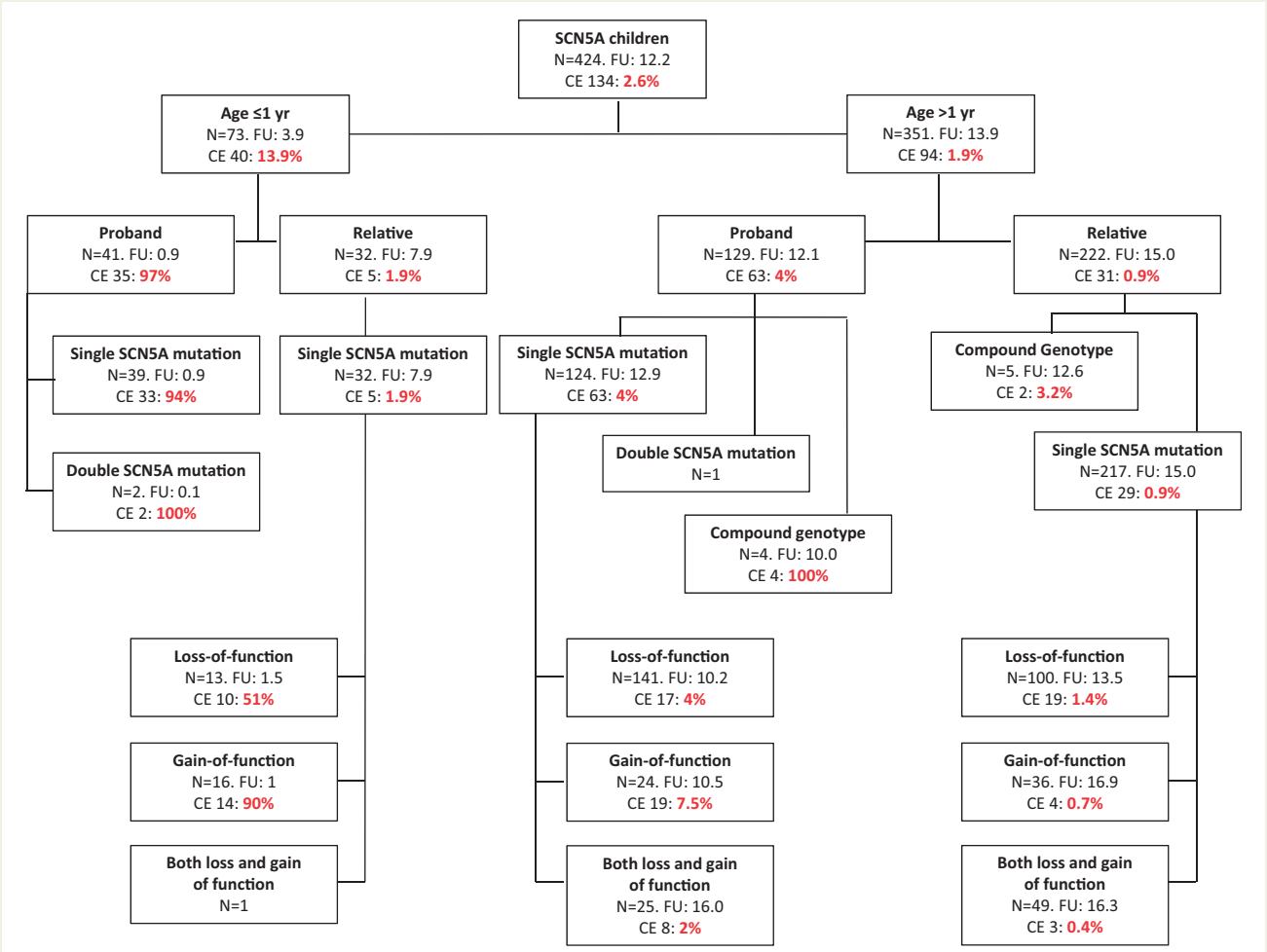


Figure 3 Mean event rate per year according to risk factors identified on multivariate analysis. FU, follow-up, %, mean event rate per year.

proband status [HR (95% CI) 7.8 (5.1–12.1), $P < 0.0001$] (*Figure 2*), supraventricular tachycardia [HR (95% CI) 4.0(1.9–8.9), $P = 0.0002$], baseline QTc ≥500 ms [HR (95% CI) 2.2(1.4–3.4), $P = 0.0002$], and AVB of any type [HR (95% CI) 1.7(1.2–2.6), $P = 0.003$] were predictors of MCEs. The effect of baseline ECG phenotype on the occurrence of MCE varied with age and the assumption of proportional hazards was not respected.

Occurrence of MCE also differed according to genotype ($P = 0.004$) [double vs single mutation: HR (95% CI) 10.3 (1.8–58.7); compound vs. single mutation: HR (95% CI) 2.2 (0.8–6.2)] (*Table 1*), gain-of-function mutation [HR (95% CI) 2.3 (1.4–3.9), $P < 0.0001$] and C-terminus mutation location [HR (95% CI) 0.3 (0.1–0.5), $P < 0.0001$] (Supplementary material online, *Figure S3*). Mutation type did not associate with outcomes ($P = 0.52$) (Supplementary material online, *Figure S4*).

Five *SCN5A* mutations correlated with specific clinical characteristics (Supplementary material online, *Table S11*). For instance, p.Glu1784Lys was associated with a lower risk of CE [$P = 0.0002$, HR (95% CI) 3.7 (1.8–7.6)], whereas the presence of p.Val411Met or p.Val1763Met was associated with a higher risk of CE [$P < 0.0001$, HR (95% CI) 5.1 (2.3–11.4) and $P < 0.0001$, HR (95% CI): 15.4 (5.4–43.4), respectively].

Multivariable analysis

A multivariable analysis stratified by baseline phenotype and adjusted on age ≤1 year at diagnosis and proband status (interaction, $P = 0.0002$), genotype ($P = 0.03$), and mutation functional effect ($P = 0.001$), showed that age ≤1 year at diagnosis in probands [$P < 0.0001$; HR (95% CI) 35.4 (16.2–77.6)], compound mutation [$P = 0.03$; HR (95% CI) 3.7 (1.2–12.0)], age ≤1 year at diagnosis in non-probands [$P = 0.03$; HR (95% CI) 3.2 (1.1–9.1)] and mutation with both gain- and loss-of-function [$P = 0.04$; HR (95% CI) 0.5 (0.2–0.9)] were independent risk factors for first CE (Supplementary material online, *Table S12*). Quantifiable indication of risk of events in an *SCN5A* mutation-positive child is presented in Supplementary material online, *Figure S5*.

Discussion

This study reports the clinical evaluation and follow-up of the largest paediatric population of *SCN5A*-mutation-positive individuals reported to date. We presented a highly symptomatic cohort with

SCD and ACA in 14%, syncope in 16%, and events during follow-up in 31%. Cardiac conduction disorder was the most prevalent phenotype. Age ≤1 year at diagnosis in probands, compound genotype, age ≤1 year at diagnosis in non-probands, and both gain- and loss-of-function *SCN5A* mutation were independent predictors of MCE. We also found that asymptomatic negative ECG phenotype children have a good prognosis, whereas previously symptomatic children with a negative ECG phenotype may undergo recurrent events even under treatment.

Clinical characteristics

The risk for life-threatening arrhythmias was higher in previously symptomatic patients, as previously shown in young BrS[19,20] and LQT3 patients.[21,22] We found no gender difference, in phenotype or in the risk for a MCE. Unlike previous adult studies where BrS was predominant in male subjects[23] and life-threatening events were higher among LQT3 men,[24] our results are concordant with previous smaller paediatric reports[19,25,26] and the contradiction might be explained by similarities in sex hormones between prepubertal boys and girls. However, the underlying molecular mechanisms are still poorly understood.[27]

In our series, more than one-third of isolated PCCD patients experienced MCE, the first of which being cardiac arrest in a high proportion of cases. Phenotypic expression of *SCN5A* mutations may vary from individual to individual and has an age-dependent onset.[28] Although there is no genotype-based risk stratification for PCCD patients, the occurrence of tachyarrhythmia and SCD was expected to be more frequent in case of loss-of-function *SCN5A* mutation, as per *SCN5A*-associated BrS that is a similar disease entity.[29] This was also suggested by familial reports of overlapping phenotypes of BrS1, LQTS, and PCCD[3,12] and the observation that BrS patients with *SCN5A* mutations exhibit more conduction abnormalities and have a higher risk for MCEs.[30] Our results demonstrate that some isolated PCCD patients are at increased risk of SCD indeed, even at an early age and even if an isolated PCCD phenotype is maintained throughout follow-up, an AVB of any type being an univariate risk factor for CE. Children diagnosed with an AVB of any type should therefore be offered genetic screening; when a *SCN5A* mutation is diagnosed, ICD therapy should be discussed in this high-risk group in case of additional risk factors that are age ≤1 year at diagnosis in probands, compound mutation, age ≤1 year at diagnosis in non-probands and *SCN5A* mutation with both gain- and loss-of-function.

There is also limited data on *SCN5A* genotype positive children with a negative ECG phenotype.[12,14] We found that the vast majority of those who are asymptomatic at diagnosis have a good long-term prognosis; however, they need to be followed, as negative ECG phenotype patients may develop a phenotype over time. Negative ECG phenotype children can also present with symptoms; close follow-up and ICD implantation should be considered in symptomatic *SCN5A* mutation-positive children, even if displaying a negative ECG phenotype, because a substantial proportion of them will experience further recurrent events, even under appropriate treatment.

Correlation between genotype and phenotype

Unlike a previous small report of loss-of-function cardiac sodium channelopathies that indicated that missense pathogenic variants were more common,[25] non-missense pathogenic variants were over-represented in isolated PCCD in our much larger sample. This is concordant with the role of haploinsufficiency in causing greater impairment of I_{Na} and more severe phenotype leading to PCCD. Phenotype correlation of *SCN5A* mutation-positive subjects, based on variant location has not been possible before due to small numbers.[31] We found that the N-terminus domain, the DI–DIV region and the C-terminus domain were not over-represented amongst the five main ECG phenotypes. No difference appeared when considering the six segments of the transmembrane domains. However, in a recent case/control study, Kapplinger *et al.*[17] were able to identify regions of Nav1.5 associated with a high probability of pathogenicity in both BrS and LQT3. In their study, the transmembrane region yielded an overrepresentation of BrS-associated variants, whereas the DIII/DIV interdomain linker and the S3–S5 + 6 segment of all transmembrane domains hosted an over-representation of LQT3-associated variants.[17] These differences are likely due to ascertainment biases inherent to each study design.

Clinical severity: clinical and genetic predictors

The high incidence of MCEs in our cohort was concordant with a previous small LQT3 paediatric multicentre international study[21] and a recent multicentre series of 391 adult and paediatric LQT3 patients.[22] However, the burden of events was higher than reported by other LQT3 or BrS series in the past.[26,32,33] The rate of SCD or ACA in our cohort was 14%, similar to other recent reports on LQT3 patients[21,22] but significantly higher than that reported in BrS children.[19,26,34] This may reflect an over-representation of LQT3 phenotypes in our cohort, as LQT3 patients who experience MCE during the first year of life are at high risk for subsequent MCEs.[32,35,36] Indeed, we found that ACA was the first symptom in 23% of the 47 isolated LQT3 children who exhibited a 7% annual rate of CE per year throughout follow-up, although only 1 (4%) was on beta-blocker at the time of the first MCE. Moreover, the two *SCN5A* mutations associated with an increased risk of MCEs in our series, namely p.Val411Met and p.Val1763Met were both gain-of-function mutations.

SCN5A mutations localizing to the transmembrane regions or the N-terminus were associated with a higher risk for CE compared to the C-terminus. This is an important finding that may help geneticists and physicians counselling young affected individuals and their families.

It is recognized that double *SCN5A* mutation carriers have a more severe phenotype with longer QTc intervals, a younger age at diagnosis, and more CEs despite therapy.[33]

Schwartz *et al.*[36] first raised the issue of different response of LQT3 patients to beta-blockers and/or LCSD between infants with MCEs in the first year of life and those presenting later. This concept was then confirmed by data from the International LQTS Registry showing that patients with an ACA during their first year of life had a very high risk for subsequent ACA or SCD during their next 10 years

of life and that beta-blockers might not be effective in preventing fatal MCEs in this high-risk subset.[37] Our results extend this observation to all paediatric *SCN5A* genotype positive subjects, whatever their ECG phenotype, as we found that both age ≤1 year at diagnosis in probands and age ≤1 year at diagnosis in non-probands were independent risk factors for first CE. A significant subset of these patients might represent *de novo* mutations, which are usually associated with greater physico-chemical difference and are more likely to be more severe in effect than inherited mutations.[38] This is in keeping with the observation of *de novo* mutations in the *SCN5A* gene associated with early onset of sudden infant death.[9,10,39] Our observation may therefore be due to a clustering of *de novo* mutations[40] and *SCN5A* mutation-positive patients with no FH constitute a subgroup at high-risk of ACA and arrhythmic events and should be treated accordingly.

Conclusions

In this large paediatric cohort of *SCN5A* genotype positive patients, cardiac conduction disorders were the most prevalent phenotype. Symptomatic individuals and LQT3 patients had the worst prognosis. Age ≤1 year at diagnosis in probands was associated with the highest risk. However, both negative ECG phenotype children and isolated PCCD children can also present with symptoms and these patients need to be accurately treated and followed. Compound genotype with associated mutation in another gene and for the first time variant topological location were independent risk factors for CEs. These findings offer therapeutic opportunity for determining risk in these vulnerable young patients.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: M.J.A. is consultant for Audentes Therapeutics, Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical. M.J.A. and Mayo Clinic have an equity/royalty relationship with AliveCor, Blue Ox Health Corporation, and StemoniX. However, none of these entities were involved in this study in any manner. None of the authors has any financial relationships relevant to this article to disclose.

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