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Title: *SCN5A* Mutations in 442 Neonates and Children: Genotype-Phenotype Correlation and Identification of Higher-Risk Subgroups

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ABSTRACT

248 words

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

Methods and Results: A multicenter, international, retrospective cohort study was conducted in 25 tertiary hospitals in 13 countries between 1990-2015. 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 (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 ECG phenotype. During a median follow-up of 5.9 (IQR: 5.9) years, 272 cardiac events 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 cardiac event.

Conclusion: In this large pediatric cohort of *SCN5A* mutation-positive subjects, cardiac conduction disorders were the most prevalent phenotype; cardiac events 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 pediatric electrophysiologists.[14] This is due to their relative rarity in the pediatric 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 multicenter, 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 judgment 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.,[20] according to which the NaV1.5 alpha subunit protein consists of 4 transmembrane domains, each composed of 6 segments. The biophysical properties, type and topological location of *SCN5A* mutations were determined on the basis of previously published data.[21,22] All variants were reclassified by a group of authors (AEB, FK, ERB, VP) at the time of this analysis according to the recommendation of the American College of Medical Genetics.[23] *SCN5A* variants with minor allele frequency >0.1% in ExAC database (Exome Aggregation Consortium, Cambridge, MA) and neutral synonymous variants were excluded. Variants were then classified into three groups: missense pathogenic; non-missense pathogenic including truncating variants (nonsense, splice acceptor, splice donor and frameshift mutations)

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1 and in frame indels; and variants of uncertain significance (VUS). Missense Variants were

2 classified as pathogenic/likely pathogenic or VUS using generally accepted criteria:[23] disease-

3 causative mutation databases, localization to highly conserved amino acid residues/key

4 functional domains, co-segregation of the variant with the disease phenotype, evidence of

5 perturbed ion channel function through in-vitro functional studies. In case of double *SCN5A*

6 mutation, patients were considered for risk analysis according to mutation location only if both

7 mutations had the same location.

8

9 **Statistical analysis.** Continuous data were presented as mean (± standard deviation) or median

10 (interquartile range, IQR) based on the distribution. Categorical variables were presented as

11 counts (proportions). The Mann-Whitney-U and Kruskal-Wallis tests were performed to test

12 for statistical differences in continuous parameters between two or more groups, respectively.

13 The $\chi 2$ or the Fisher exact test (based on expected frequency) were used to compare categorical

14 variables between groups. Bonferroni method was used for post-hoc tests. We adjusted p-value

15 level on number of hypothesis tested. The Kaplan-Meier method estimator was used to assess

16 the time to a first MCE. A Cox proportional-hazards regression analysis with random effect on

17 family [with hazard ratios (HR) and confidence intervals (CI)] was used to evaluate the

18 independent risk of clinical- and genetic- factors of interest for first MCE. From univariate

19 analysis, we selected variables with p-value <0.10 (statistical criterion) and looked at

20 multicollinearity between variables. For the multivariate model, we kept the following

21 variables: proband, age <1 year at diagnosis, phenotype at baseline, genotype, location, HR,

22 AV block, RBBB and SV arythmia. Variables were eliminated from highest to lowest p-values,

23 but remained in the final model if the p-value was less than 0.05 or seem to be confounders

24 (more than 10% change in estimate). Final multivariable Cox model was stratified by

25 phenotype (LQT3, PCCD, overlap phenotype, and ECG phenotype-negative) at baseline to

26 relax the assumption of proportional hazards. All two-way interactions between pairs of

3

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 analyzed with the SAS packages (SAS Institute Inc version 9,4, Cary, NC).

RESULTS

A total of 442 children [246 boys (56%), 178 probands (40%), 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 (Online Figure 1). The four 'major' ECG phenotypes at baseline were isolated PCCD (26%), overlap phenotype (16%), isolated LQT3 (11%) and isolated BrS1 (2%); 196 patients (44%) had a negative ECG phenotype at baseline (Figure 1). Clinical characteristics of each patients' group are detailed in Online Materials. 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%)] (Online Table 1).

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

4

1   treatment. Of the 77 (17%) ICD-implanted patients, 100 appropriate shocks were delivered in

2   28 (36%) patients during a median follow-up period of 3.3 years (Online Table 2).

3   Inappropriate ICD shocks occured in 9 patients (12%; T wave oversensing in 7 patients, atrial

4   fibrillation in 1, lead fracture in 1). The four 'major' ECG phenotypes at baseline developed as

5   follows:

6   Isolated PCCD patients: At a median follow-up of 5.7 (0.0-35.7) years, 26/113 (23%) patients

7   kept an isolated PCCD phenotype; 13/113 (11%) had received PM implantation at a median

8   age of 5.42 (0.06-15.58) years; 85% of PCCD patients had their first PM insertion by the age of

9   11; Permanent PM were implanted for symptomatic bradycardia in 7/13 patients (syncope in 5,

10   exercise-induced dyspnoea in 2), whilst the indications were prophylactic in 6/13 patients,

11   including a mean daytime heart rate <50bpm in 4 children >1 year of age and ventricular

12   pauses longer than 3 RR intervals in 2; 38/113 (34%) experienced ≥1 MCE, the first of which

13   being cardiac arrest (18% including 3 documented ventricular tachycardia [VT], 1 polymorphic

14   VT with torsades de pointes [TdP] and 1 ventricular fibrillation [VF]), SIDS (2%) or syncope

15   (14%). At the time of their event, PCCD patients presented with the association of an AVB and

16   right bundle branch block (RBBB) (17/38, 45%), an isolated first-degree AVB (13/38, 34%),

17   an isolated complete RBBB (4/38, 10.5%) or a trifascicular block (4/38, 10.5%).

18   Two patients died (one during infancy, one SCD) and one required heart transplantation for

19   intractable arrhythmias; although none of them underwent a sodium-chanel blocker challenge,

20   all three patients maintained an isolated CCD phenotype throughout follow-up.

21   Overlap phenotype patients: After 5.7 (0.0-45.7) years, 34/69 (50%) patients had

22   pharmacological treatment (beta-blocker: 39%, sodium channel blocker: 22% according to the

23   combination of phenotypes, see Online Table 3); PM or ICD had been implanted in 10/69

24   (14%) and 17/69 (25%) respectively. At least one MCE occurred in 31/69 patients (45%; 1

25   recurrence in 6 patients, 2 recurrences in 1 patient, ≥2 recurrences in 5 patients). Three patients

26   died from SCD and one required ECMO support and was then transplanted for intractable

5

1 arrhythmias.

2 Isolated LQT3 patients: At a median follow-up of 5.9 (0.0-26.5) years, 32/47 (68%) patients

3 received a beta-blocker, coprescribed with a sodium channel blocker in 10 (21%), 3 (6%) had

4 undergone left cardiac sympathetic denervation and PM and ICD implantation occurred in 3

5 (6%) and 11 (23%) respectively.

6 MCE occurred in 25 patients [53%, 5/25 (11%) ≤1 year of age, 1/25 (4%) on betablocker at the

7 time of the event] (Online Table 4). The first MCE was a SCD (2/47: 4%, including 1 during

8 infancy), an ACA (19%) or a syncope (30%). Nine patients experienced more than one MCE.

9 At the time of the first recurrent event, 7/9 patients were receiving betablocker therapy (Online

10 Table 5); three patients experienced several recurrences under a coprescription of betablocker

11 and mexiletine. Seven ICD shocks (6 appropriate, 1 inappropriate) were delivered in 3/11

12 (27%) implanted patients. Six patients (13%) died throughout follow-up, three of them had

13 experienced a MCE in the first year of life.

14 Isolated BrS1 patients: After 8.1 (1.8-15.7) years, 3/8 (37%) symptomatic BrS1 patients had an

15 ICD (2.8, 11.5 and 18.8 years at implantation). They had presented with syncope (2 patients) or

16 documented VT. One of them experienced a fever-associated VF-induced appropriate ICD

17 shock at 13 years whilst under treatment. No death occurred. The 5 remaining patients were

18 asymptomatic and left untreated.

19

20 **Negative ECG phenotype patients.** 196 patients [44%, 52% boys, 33% probands, median age

21 at diagnosis: 8.8 (IQR: 8.7) years] had a normal ECG at baseline and underwent genetic

22 screening because of cardiac arrest (7%), syncope (13%) or because of familial screening in

23 asymptomatic patients (80%). A family history of either SCD/ICD implantation or PCCD/PM

24 implantation was noted in 55% and 15% respectively.

25 Of the 196 phenotype-negative patients, 27% developed an ECG phenotype throughout follow-

26 up [5.9 (0.4-26.5) years], represented by an isolated PCCD phenotype (13%), an isolated LQT3

1 (5%), an isolated BrS1 (5%), or an overlap phenotype (4%), whereas 73% remained

2 phenotype-negative. At least one MCE occurred in 40 (20%).

3 Of the 39 (20%) symptomatic, negative ECG phenotype patients, 26 received a betablocker.

4 All but one negative ECG phenotype patients who experienced MCEs during follow-up were

5 already symptomatic at diagnosis. Twelve experienced at least one recurrent MCE at a median

6 delay of 3.9 (9.6) years since the diagnosis [median age of recurrent event: 3.0 (4.3) yrs]. All

7 but one were treated by betablocker therapy at the time of the recurrent MCE; Of these 12

8 children, 8 kept a negative ECG phenotype at last visit, whereas 4 were further diagnosed with

9 an isolated LQT3 phenotype and, despite additional treatment with mexiletine, experienced

10 further recurrent MCEs leading to LCSD and ICD implantation.

11 The vast majority (156/157, 99%) of the asymptomatic, negative ECG phenotype children

12 remained asymptomatic throughout follow-up; one patient (0.6%) however became later

13 symptomatic: this was a 5 year-old female with a normal ECG at familial screening; she was

14 further diagnosed with an isolated LQT3 on follow-up ECGs at age 13 (QTc: 491 ms) and

15 received mexiletine; at age 18 she presented with an electrical storm whilst receiving

16 mexiletine (500mg morning, 250mg afternoon, 500mg evening), leading to ICD implantation.

17

18 **Genetic characteristics.** The 442 *SCN5A* genotype-positive children had 185 independent

19 *SCN5A* variants (Online Table 5). Three (0.7%) patients harbored a double heterozygous

20 *SCN5A* mutation; 9 (2%) had a compound genotype with an additional disease-causing

21 mutation in another gene: *KCNQ1* (3 patients), *KCNH2* (4 patients), *RYR2* (1 patient) or

22 *CACNA1C* (1 patient). A loss-of-function mutation was found in 178 (40%) patients whereas,

23 87 (20%) had a gain-of-function mutation, 85 (19%) a both gain- and loss-of-function mutation

24 and 92 (21%) had a VUS. Although VUS patients were more frequently probands (p=0.003),

25 their clinical characteristics did not differ from those of patients with a variant of known

26 functional effect (Online Table 6). Most variants were missense pathogenic mutations (64%),

7

1 whereas 25% were non-missense pathogenic mutations (truncation mutations: 18%, in-frame

2 mutations: 7%). Topological location of mutations is shown in Online Figure 2.

3

Genotype- phenotype correlations.

5 Mutation topological location (Online Table 7, Figure 2). Patients with a mutation in the C-

6 terminus domain (N=110) were less frequently probands (p=0.03), were diagnosed later in life

7 (p=0.01), were less frequently symptomatic at diagnosis (p=0.001), had less MCEs (p=0.0002)

8 and less appropriate ICD shocks (p=0.03) during follow-up. No significant difference was

9 found when comparing variants localized in S1-S4 to those localized in S5-S6 in the relevant

10 241 patients (Online Table 8).

11 Mutation functional effect (Online Table 9). Children with a gain-of-function *SCN5A* mutation

12 mainly presented with a baseline negative ECG phenotype (45%) or isolated LQT3 (26%);

13 those with a loss-of-function mutation presented mainly with isolated PCCD (38%), negative

14 ECG phenotype (27%) or overlap phenotype (19 %) at baseline; and those with a both gain-

15 and loss-of-function mainly had negative ECG phenotype (35%), isolated PCCD (22%),

16 isolated LQT3 (12%) or overlap phenotype (14%). Comparison between groups by looking at

17 the functional effect of the mutation (gain of function, loss of function or both) demonstrated

18 that gain-of-function mutation carriers were more likely to have a cardiac arrest as first

19 presentation (p<0.001) and a greater rate of both MCEs during follow-up (p<0.001) and ICD

20 implantation (p<0.001).

21 Mutation type (Online Table 10). Non missense mutation were more frequently identified in

22 case of isolated PCCD (p<0.006) but less frequently found in case of negative ECG phenotype

23 (p<0.007). The following clinical parameters differed according to mutation type: age at

24 diagnosis (p=0.02), proportion of diagnosis ≤1 year (p=0.02), FH of SCD/ICD (p=0.03), FH of

25 PCCD/PM (p=0.001), as did the following baseline phenotypes: isolated PCCD (p=0.006) and

26 negative ECG phenotype (p=0.007) (Online Table 10). However, the type of mutation did not

8

1   change the risk of MCE during follow-up.

2

3   **Univariate risk analysis.** The risk of MCE during follow-up was related to phenotype (Table

4   1). Age ≤1 year at diagnosis [HR (95%CI): 11.3(6.7-18.9), p<0.0001], proband status [HR

5   (95%CI): 7.8(5.1-12.1), p<0.0001] (Figure 3), supraventricular tachycardia [HR (95%CI):

6   4.0(1.9-8.9), p=0.0002], baseline QTc ≥500ms [HR (95%CI): 2.2(1.4-3.4), p=0.0002], and

7   AVB of any type [HR (95%CI): 1.7(1.2-2.6), p=0.003] were predictors of MCEs. The effect of

8   baseline ECG phenotype on the occurrence of MCE varied with age and the assumption of

9   proportional hazards was not respected.

10   Occurrence of MCE also differed according to genotype (p=0.004) [double vs single mutation:

11   HR (95%CI): 10.3(1.8-58.7); compound vs single mutation: HR (95%CI): 2.2(0.8-6.2)] (Table

12   1), gain-of-function mutation [HR (95%CI): 2.3(1.4-3.9), p<0.0001] and C-terminus mutation

13   location [HR (95%CI): 0.3(0.1-0.5), p<0.0001] (Online Figure 3). Mutation type did not

14   associate with outcomes (p=0.52) (Online Figure 4).

15   Five *SCN5A* mutations correlated with specific clinical characteristics (Online Table 11). For

16   instance, p.Glu1784Lys was associated with a lower risk of CE [p=0.0002, HR (95%CI):

17   3.7(1.8-7.6)], whereas the presence of p.Val411Met or p.Val1763Met was associated with a

18   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

19   (5.4-43.4) respectively].

20

21   **Multivariable analysis.** A multivariable analysis stratified by baseline phenotype and adjusted

22   on age ≤1 year at diagnosis and proband status (interaction, p=0.0002), genotype (p=0.03), and

23   mutation functional effect (p=0.001), showed that age ≤1 year at diagnosis in probands

24   [p<0.0001; HR (95%CI): 35.4(16.2-77.6)], compound mutation [p=0.03; HR (95%CI): 3.7(1.2-

25   12.0)], age ≤1 year at diagnosis in non probands [p=0.03; HR (95%CI): 3.2(1.1-9.1)] and

9

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 (Online Table 12). Quantifiable indication of risk of events in an *SCN5A* mutation positive child is presented in Figure 5.

DISCUSSION

This study reports the clinical evaluation and follow-up of the largest pediatric 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[24,25] and LQT3 patients.[26,27] 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[28] and life-threatening events were higher among LQT3 men,[29] our results are concordant with previous smaller pediatric reports[24,30,31] 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.[32]

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.[33] Although

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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.[34] 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.[35] 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,[30] non-missense pathogenic variants were overrepresented in isolated PCCD in our much larger sample. This is concordant with the role of haploinsufficiency in causing

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1   greater impairment of I_{Na} and more severe phenotype leading to PCCD. Phenotype correlation

2   of *SCN5A* mutation-positive subjects, based on variant location has not been possible before

3   due to small numbers.[36] We found that the N-terminus domain, the DI-DIV region and the C-

4   terminus domain were not overrepresented amongst the five main ECG phenotypes. No

5   difference appeared when considering the 6 segments of the transmembrane domains.

6   However, in a recent case/control study, Kapplinger et al. were able to identify regions of

7   Nav1.5 associated with a high probability of pathogenicity in both BrS and LQT3.[22] In their

8   study, the transmembrane region yielded an overrepresentation of BrS-associated variants,

9   whereas the DIII/DIV interdomain linker and the S3-S5+6 segment of all transmembrane

10 domains hosted an overrepresentation of LQT3-associated variants.[22] These differences are

11 likely due to ascertainment biases inherent to each study design.

12

13 **Clinical severity: clinical and genetic predictors.** The high incidence of MCEs in our cohort

14 was concordant with a previous small LQT3 pediatric multicenter international study[26] and a

15 recent multicenter series of 391 adult and pediatric LQT3 patients.[27] However, the burden of

16 events was higher than reported by other LQT3 or BrS series in the past.[31,37,38] The rate of SCD

17 or ACA in our cohort was 14%, similar to other recent reports on LQT3 patients[26,27] but

18 significantly higher than that reported in BrS children.[24,31,39] This may reflect an

19 overrepresentation of LQT3 phenotypes in our cohort, as LQT3 patients who experience MCE

20 during the first year of life are at high risk for subsequent MCEs.[37,40,41] Indeed, we found that

21 ACA was the first symptom in 23% of the 47 isolated LQT3 children who exhibited a 7%

22 annual rate of CE per year throughout follow-up, although only 1 (4%) was on beta-blocker at

23 the time of the first MCE. Moreover, the two *SCN5A* mutations associated with an increased

24 risk of MCEs in our series, namely p.Val411Met and p.Val1763Met were both gain-of-function

25 mutations.

26 *SCN5A* mutations localizing to the transmembrane regions or the N-terminus were associated

12

1 with a higher risk for CE compared to the C-terminus. This is an important finding that may

2 help geneticists and physicians counseling young affected individuals and their families.

3 It is recognised that double *SCN5A* mutation carriers have a more severe phenotype with longer

4 QTc intervals, a younger age at diagnosis and more CEs despite therapy.[38]

5 Schwartz et al. first raised the issue of different response of LQT3 patients to beta-blockers

6 and/or LCSD between infants with MCEs in the first year of life and those presenting later.[41]

7 This concept was then confirmed by data from the International LQTS Registry showing that

8 patients with an ACA during their first year of life had a very high risk for subsequent ACA or

9 SCD during their next 10 years of life and that beta-blockers might not be effective in

10 preventing fatal MCEs in this high-risk subset.[42] Our results extend this observation to all

11 pediatric *SCN5A* genotype positive subjects, ==whatever their ECG phenotype, as we found that==

12 ==both age ≤1 year at diagnosis in probands and age ≤1 year at diagnosis in non probands were==

13 ==independent risk factors for first CE.== A significant subset of these patients might represent *de*

14 *novo* mutations, which are usually associated with greater physico-chemical difference and are

15 more likely to be more severe in effect than inherited mutations.[43] This is in keeping with the

16 observation of *de novo* mutations in the *SCN5A* gene associated with early onset of sudden

17 infant death.[9,10,44] Our observation may therefore be due to a clustering of de novo mutations[45]

18 and *SCN5A* mutation-positive patients with no family history constitute a subgroup at high-risk

19 of ACA and arrhythmic events and should be treated accordingly.

20

21

CONCLUSIONS

23 In this large pediatric cohort of *SCN5A* genotype positive patients, cardiac conduction disorders

24 were the most prevalent phenotype. Symptomatic individuals and LQT3 patients had the worst

25 prognosis. Age ≤1 year at diagnosis in probands was associated with the highest risk. However,

26 both negative ECG phenotype children and isolated PCCD children can also present with

13

1 symptoms and these patients need to be accurately treated and followed. Compound genotype

2 with associated mutation in another gene and for the first time variant topological location were

3 independent risk factors for CEs. These findings offer therapeutic opportunity for determining

4 risk in these vulnerable young patients.

14

TAKE-HOME FIGURE AND ONE-SENTENCE SUMMARY

2

<mark>**Take-home figure**</mark>
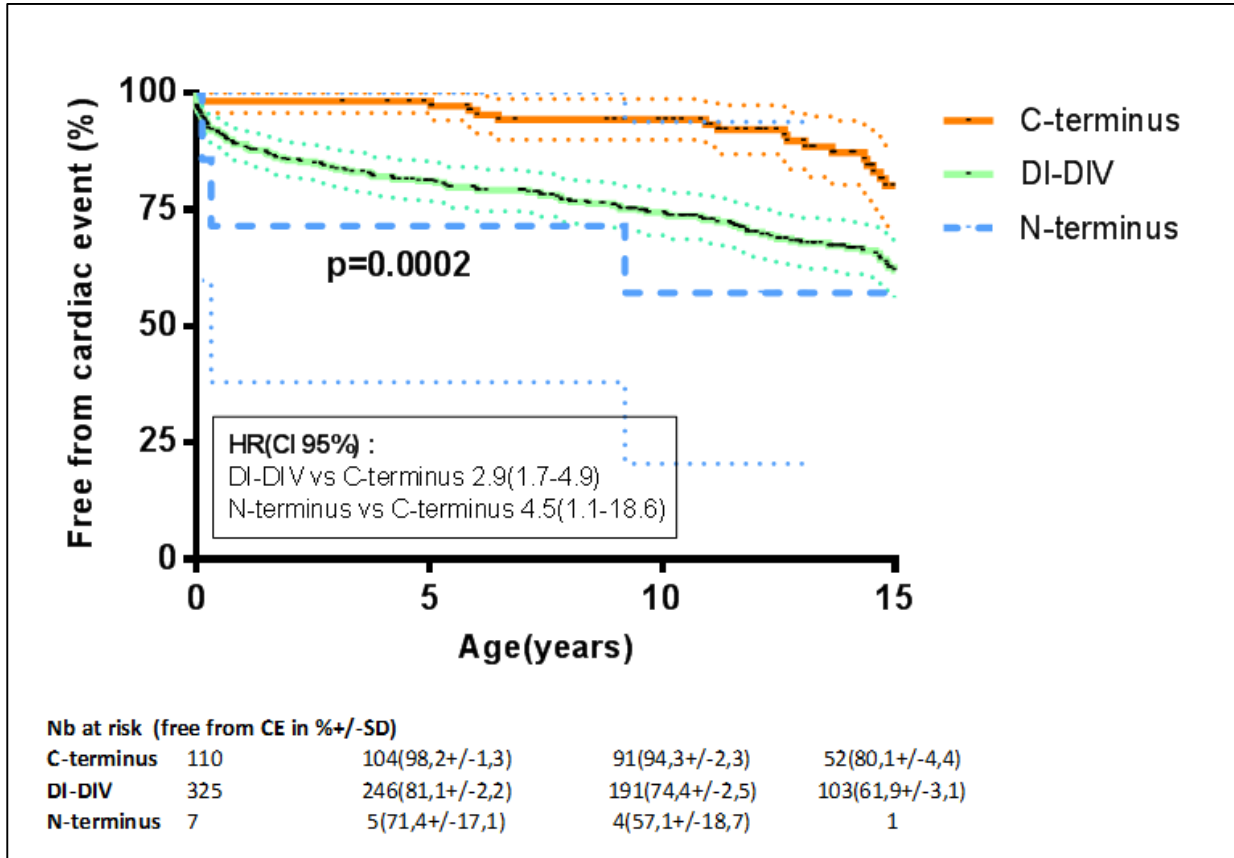
One-sentence summary

Analysing 442 *SCN5A* mutation-positive children, this multicenter, international retrospective cohort study provides a better understanding of clinical characteristics, clinical outcomes and risk factors for major cardiac events in *SCN5A*-associated diseases in the paediatric population.

DISCLOSURES

MJA is consultant for Audentes Therapeutics, Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical. MJA 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.

FIGURE LEGENDS

Figure 1   **Venn diagram of baseline ECG phenotypes**

Figure 2   **Freedom from major cardiac event according to *SCN5A* mutation location (domains)**

Figure 3   **Freedom from major cardiac event in probands and non-probands**

Figure 4   **Mean event rate per year according to risk factors identified on multivariate analysis**

FU: follow-up, %: mean event rate per year

TABLE

Table 1: Risk analysis for major cardiac event (N=442)

	no MCE (n=303)	MCE (n=139)	Analysis	HR (95%IC)	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*
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*					
Median age at ECG, yrs (IQR)	8.2 (8.4)	7.6 (12.8)	unit=2	0.8 (0.7-0.9)	**<0.0001**
Heart rate, bpm (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)	versus single	10.3 (1.8-58.7)	
Compound mutation, n (%)	3 (1.0)	6 (4.3)	versus single	2.2 (0.8-6.2)	
Mutation type					0.52
Non missense pathogenic	74 (24.4)	39 (28.1)	reference	1	

mutation, n (%)					
Missense pathogenic mutation, n (%)	200 (66.0)	83 (59.7)	versus non-missense	0.84 (0.54-1.31)	
Unknown functional effect, n (%)	29 (9.6)	17 (12.2)	versus non-missense	1.03 (0.53-2.00)	
Mutation location (domains)					<0.0001
N-terminus location, n (%)	4 (1.3)	3 (2.2)	versus 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)	versus DI domain	0.7 (0.3-1.9)	
DII domain, n (%)	29 (9.6)	9 (6.5)	versus DI domain	0.5 (0.2-1.1)	
DII/DIII interdomain linker, n (%)	22 (7.3)	8 (5.8)	versus DI domain	0.5 (0.2-1.2)	
DIII domain, n (%)	49 (16.2)	19 (13.7)	versus DI domain	0.5 (0.2-1.0)	
DIII/DIV interdomain linker, n (%)	15 (5.0)	13 (9.4)	versus DI domain	1.3 (0.5-3.2)	
DIV domain, n (%)	40 (13.2)	31 (22.3)	versus DI domain	1.4 (0.7-2.8)	
C-terminus, n (%)	89 (29.4)	21 (15.1)	versus 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)	versus 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)	versus loss-of-function	2.3(1.4-3.9)	
Gain and loss, n (%)	71(23.4)	14(10.1)	versus loss-of-function	0.4(0.2-0.8)	
Unknown functional effect, n (%)	60(19.8	32(23.0)	versus loss-of-function	1.2(0.7-2.1)	

CE: cardiac event; FH: family history; PCCD: progressive cardiac conduction defect; PM: pacemaker; SCD: sudden cardiac death; ICD: implantable cardioverter defibrillator; FU: follow-up; LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; QTc: corrected QT value; AV block: atrioventricular block; RBBB: right bundle branch block; LBBB: left bundle branch block; SVT: supraventricular tachycardia.

*Cox model is not applicable when subgroups contain no event. In this later case, we presented log-rank test.

Figure 1

Figure 2
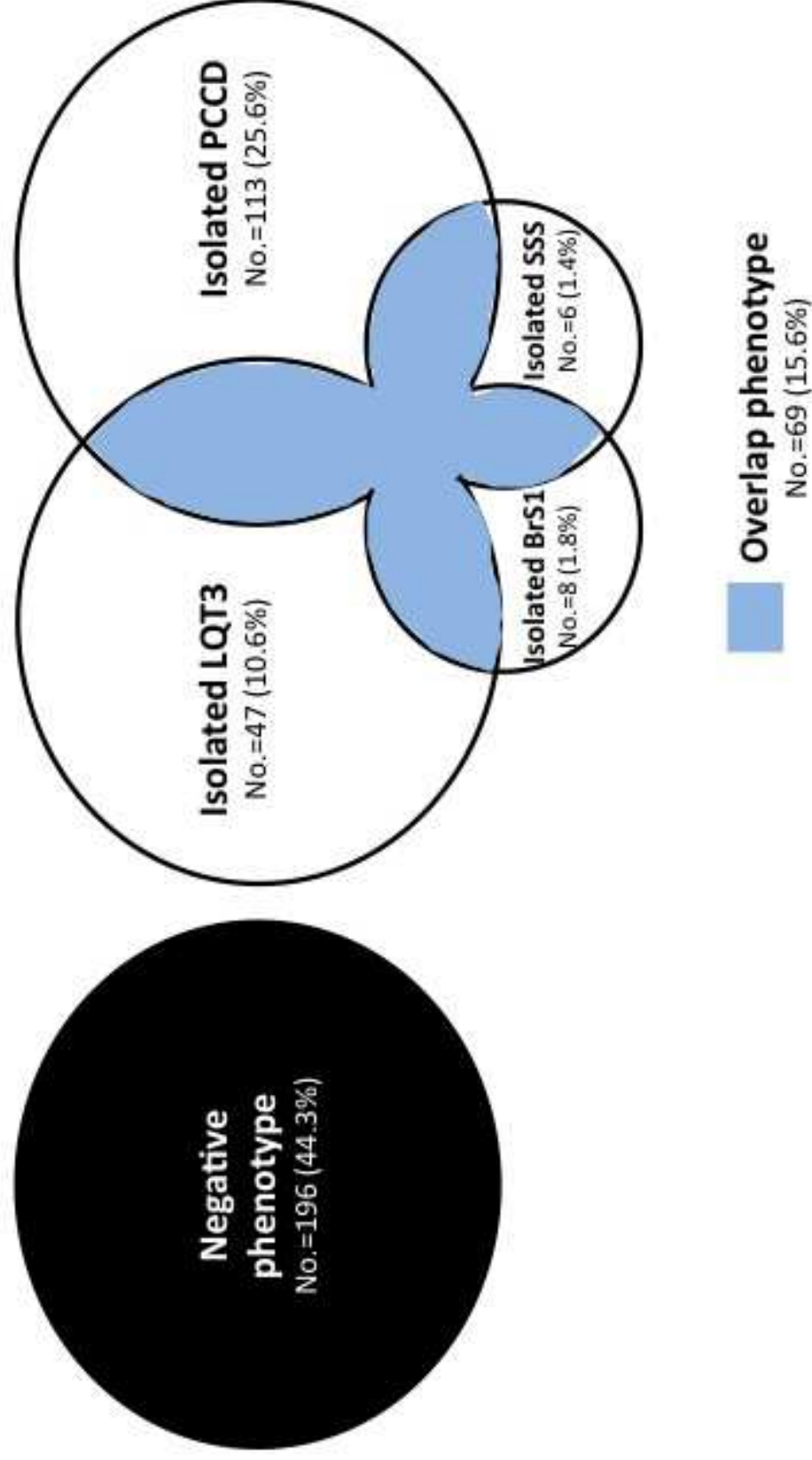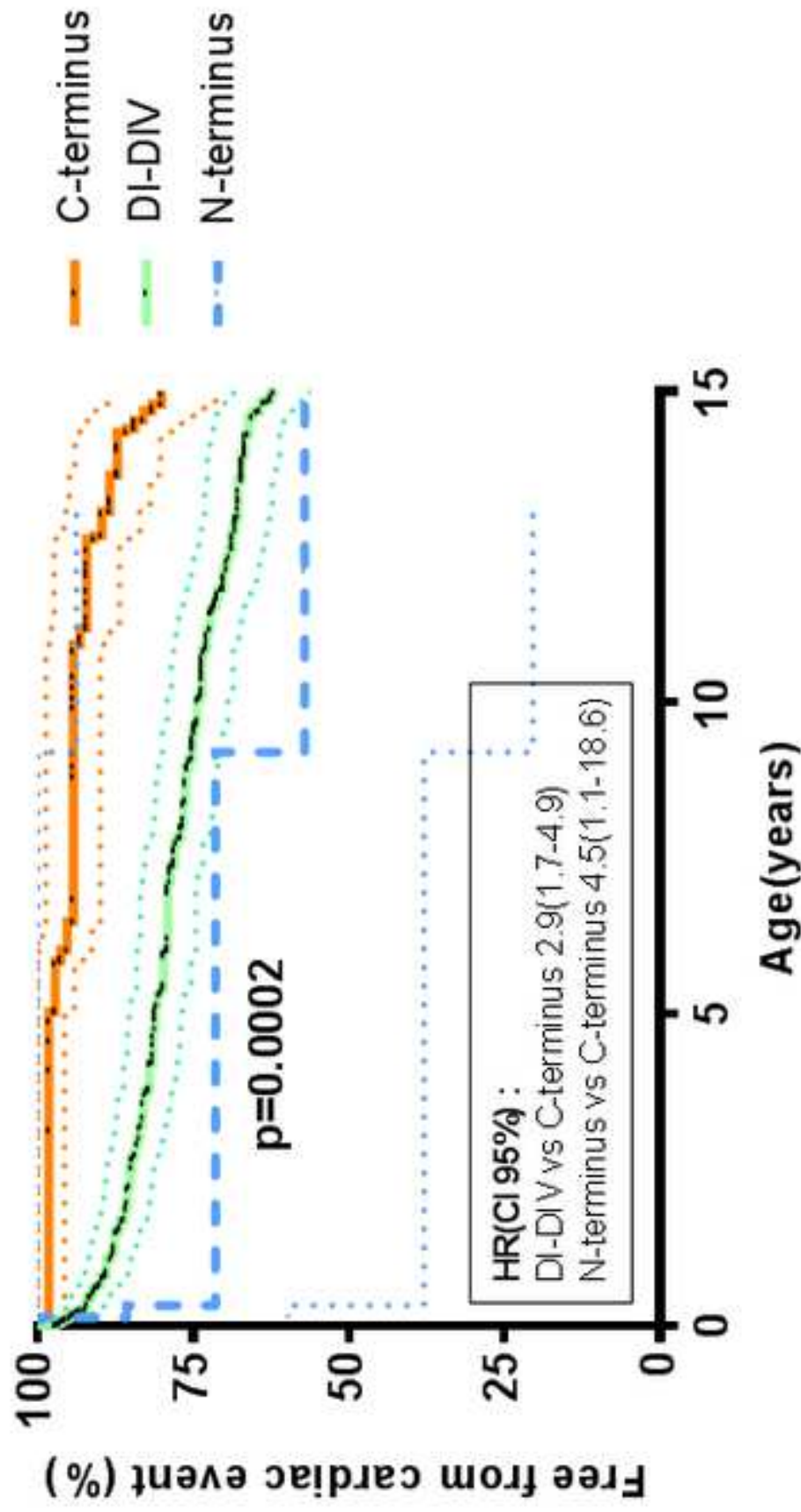
Nb at risk (free from CE in %+/-SD)

C-terminus	110	104(98,2+/-1,3)	91(94,3+/-2,3)	52(80,1+/-4,4)	
DI-DIV	325	246(81,1+/-2,2)	191(74,4+/-2,5)	103(61,9+/-3,1)	
N-terminus	7	5(71,4+/-17,1)	4(57,1+/-18,7)	1	
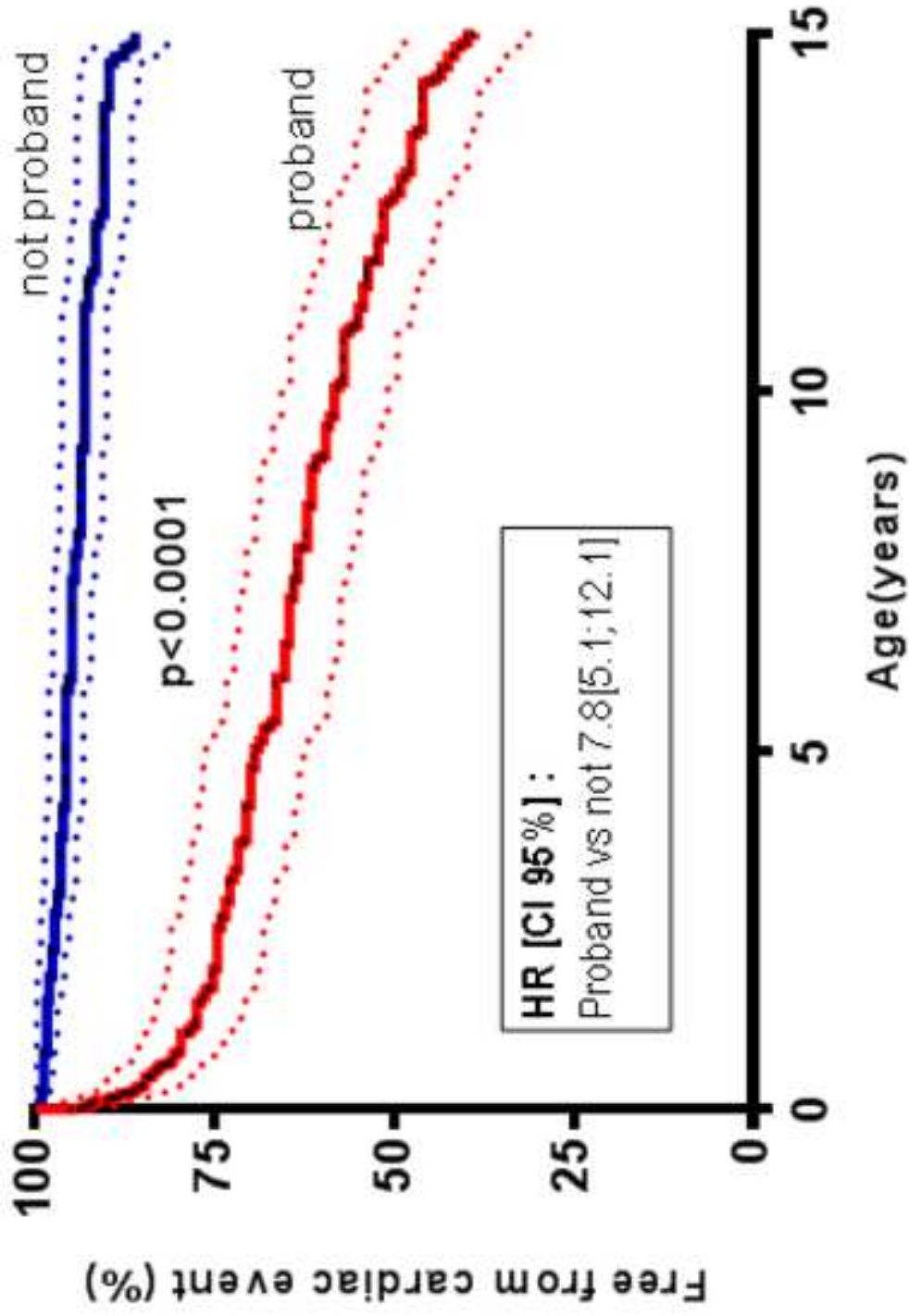
Figure 3

Nb at risk (free from CE in %+/-SD)

Proband	178	116(69,5+/-3,5)	91(58,4+/-3,8)	42(38,9+/-4,1)	
Not proband	264	239(95,8+/-1,2)	195(93,2+/-1,6)	114(86,1+/-2,5)	

HR [CI 95%] :
Proband vs not 7.8[5.1;12.1]

p<0.0001
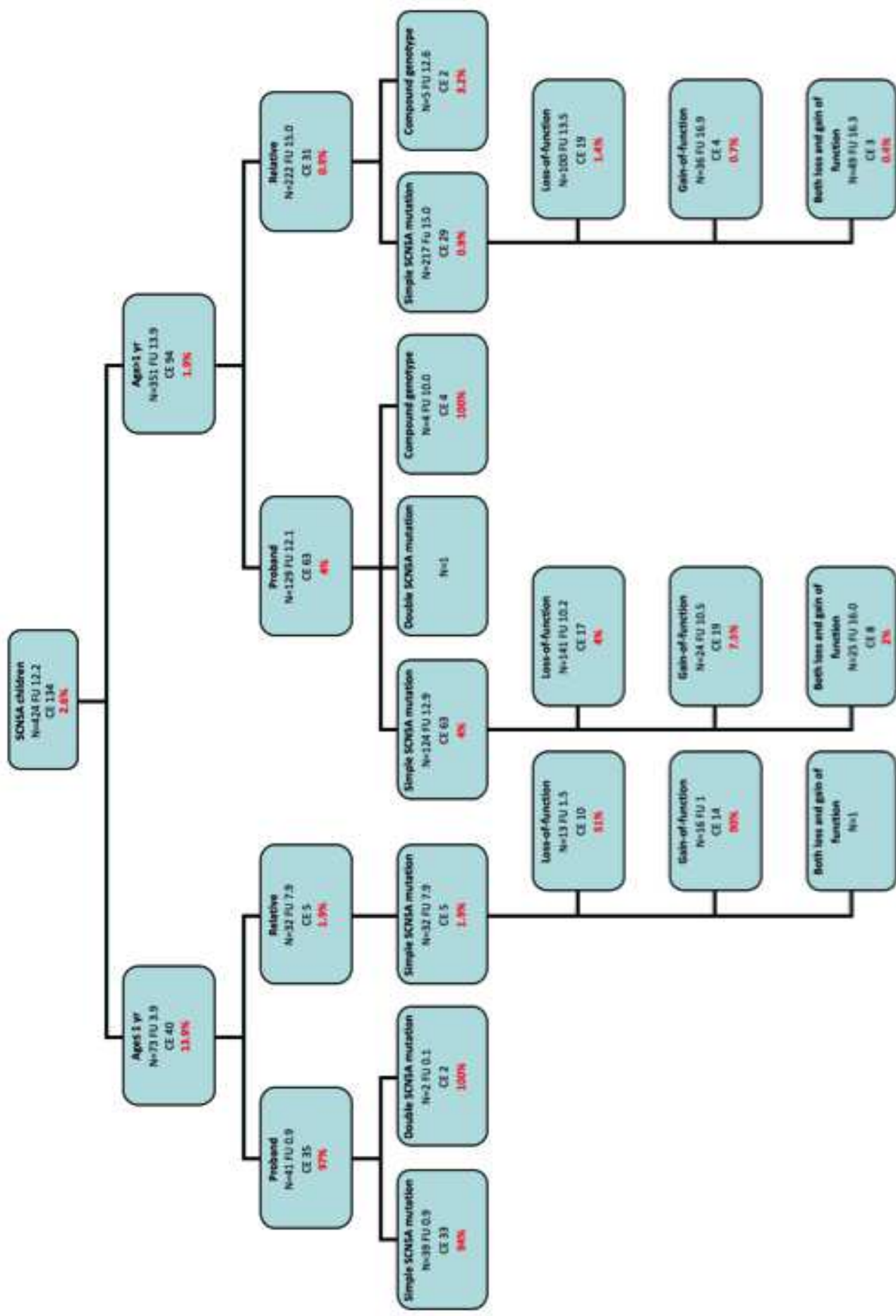
Figure 4

SUPPLEMENTAL MATERIALS

1- Supplemental Methods

2- Supplemental Results

3- Supplemental Tables

4- Supplemental Figures

5- Limitations

6- References

SUPPLEMENTAL METHODS

Definitions. The diagnoses of LQT3, BrS-1, PCCD and SSS were made according to the ESC/AEPC guidelines and HRS/EHRA/APHRS recommendations.[12,15] SIDS was defined as the sudden death of an infant under one year of age, that remained unexplained after a thorough case investigation, including performance of a complete autopsy, examination of the death scene and review of the clinical history.[12,16] Cardiac conduction abnormality was defined as PR interval prolongation and/or QRS complex prolongation and/or axis deviation according to age. Atrioventricular and intraventricular conduction disturbances were classified according to the age at the time of diagnosis using consensually agreed definitions and practice guidelines.[17,18] DCM was defined by left ventricular (LV) dilation (i.e., LV end-diastolic dimension ≥2 standard deviation [SD] above normal for body-surface area) and depressed LV systolic function (LV fractional shortening or LV ejection fraction ≥2 SD below normal for age).[19] Negative ECG phenotype was defined as patients with a confirmed pathogenic *SCN5A* mutation but a completely normal electrocardiogram and transthoracic echocardiography. A proband was defined as the first patient in a family diagnosed with a sodium channelopathy, non-probands were all other relatives. A major cardiac event (MCE) was defined as the occurrence of arrhythmic syncope, SCD at any age (including SIDS), ACA, ventricular fibrillation, monomorphic ventricular tachycardia, polymorphic VT with torsades de pointes characteristics, electrical storm or heart transplantation for intractable arrhythmias.

ECG analysis. Baseline 12-lead ECG and the ECG recorded at time of PM/ICD implantation or at last follow-up visit in non-paced patients were analyzed. Analysis of RR interval, PR

interval, QTc value, QRS axis and duration was done by four medical investigators (AEB, ML, AJ and VP) blinded to patient phenotype, cardiac events and genotype. All measurements were averaged. Atrioventricular and intraventricular conduction disturbances were classified according to the age at the time of diagnosis using accepted definitions and practice guidelines (Online Table 13).[17,18] The QT interval was corrected for heart rate using the Bazett's formula. Suggested QTc values for diagnosing QTc prolongation among our study population were QTc ≥480 ms in repeated ECGs or QTc ≥460 ms in case of a previous MCE.[15]

SUPPLEMENTAL RESULTS

Baseline clinical characteristics. Isolated PCCD, overlap phenotype, isolated LQT3 and isolated BrS1 were the four 'major' ECG phenotypes at baseline.

The initial resting ECG was already diagnostic in 276 (62%) patients. All patients had Holter monitoring, signal averaged ECG and ECG with high precordial leads. Pharmacological provocation test with sodium-channel blockers was used in 39 patients (9%; Ajmaline, N=24; Flecainide, N=7; Pilsicainide, N=5; Procainamide, N=5) at a median age of 12.3 (IQR: 5) years, leading to the diagnosis of drug-induced Brugada syndrome in 27 patients. An exercise treadmill test was performed in 127 (29%) patients at a median age of 12.8 (IQR: 5) years, unmasking LQTS in 11 patients with normal QTc at resting ECG.

Isolated PCCD patients: 113 patients [25.6%, 58.4% boys, 40.7% probands, median age at diagnosis: 6.8 (IQR: 11.6) years] had baseline PCCD; 29.2% were symptomatic at diagnosis presenting with cardiac arrest (17.7%) or syncope (11.5%). A family history for SCD/ICD implantation was present in 58.4% or PCCD/PM implantation in 25.7%. 6/18 (33%) ICD implanted, isolated PCCD patients had at least one appropriate shock.

Overlap phenotype patients: The 69 patients [15.6%, 65.2% boys, 43.5% probands, median age at diagnosis: 5.8 (IQR: 10.0) years] with overlap phenotype underwent genetic testing because of cardiac arrest (23.2%), syncope (20.3%) or because of familial screening (56.5%). Various associations were observed (Online Table 3). A family history of SCD/ICD implantation was present in 53.6% and of PCCD/PM implantation in 26.1%. 9/17 (53%) ICD implanted, overlap phenotype patients had at least one appropriate shock. In the 41 patients who had another baseline ECG phenotype, the median delay until the diagnosis of an overlap syndrome was established was 3.9 years (N= 41 patients; 2.7-10.4 years).

Isolated LQT3 patients: 47 patients [10.6%, 48.9% boys, 61.7% probands, median age at diagnosis: 10.1 (IQR: 9.4) years] displayed a baseline isolated LQT3 ECG phenotype; 42 of them (89.4%) demonstrated either late-onset, peaked and/or biphasic T-waves or asymmetrical peaked T waves, both described as typical LQT3 patterns. Although 46.8% were asymptomatic at diagnosis, 23.4% were diagnosed because of cardiac arrest and 29.8% because of syncope. A family history of either SCD/ICD implantation or PCCD/PM implantation was noted in 46.8% and 12.8% respectively. 2/11 (18%) ICD implanted, isolated LQT3 patients had at least one appropriate shock.

Isolated BrS1 patients: 8 patients [1.8%, 75.0% boys, 50.0% probands, median age at diagnosis: 8.9 (IQR: 9.2) years] had baseline BrS1, one of whom was drug-induced, the seven others being spontaneous; 37.5% were symptomatic at diagnosis presenting with cardiac arrest (12.5%) or syncope (25.0%). They presented with a family history of SCD/ICD implantation in 50.0% or PCCD/PM implantation in 50.0%. 1/3 (33%) ICD implanted, isolated BrS1 patients had at least one appropriate shock.

Clinical outcomes.

Overlap phenotype patients: 69 patients had a baseline diagnosis of an overlap syndrome. In the 41 patients who had another baseline ECG phenotype, the median delay until the diagnosis of an overlap syndrome was established was 3.9 years (N= 41 patients; 2.7-10.4 years).

ICD implanted patients: There was no uniform cut-off for VT in ICD programming. Cut-offs for VT and VF were 195bpm (150-240) and 222bpm (188-300), respectively. Sustained VT duration was programmed for 14/77 patients.

Genotype- phenotype correlations.

The most common SCN5A mutations per phenotype are presented in Supplemental Table 14. *SCN5A* mutations were de novo variants in 69/442 patients (15.6%), whilst they were inherited in 347/442 patients (78.5%) and this was unclear in 26 patients. Of the 69 patients with a definite *de novo SCN5A* mutation, 21 had an overlap phenotype, 16 an isolated PCCD phenotype, 16 a negative ECG phenotype, 15 an isolated LQT3 phenotype and 1 an isolated SSS phenotype. *De novo SCN5A* mutations accounted for 40.0% of the 16% of patients with inaugural syncope and 66.1%% of the 14% of patients with inaugural aborted cardiac arrest.

SUPPLEMENTAL TABLES

Table 1: Clinical characteristics according to baseline ECG phenotypes (n=442)

	Negative ECG phenotype (n=196)	Isolated LQT3 (n=47)	Isolated BrS-1 (n=8)	Isolated PCCD (n=113)	Isolated SSS (n=6)	Isolated DCM (n=3)	Overlap phenotype (n=69)	p value
Male, n (%)	102 (52.0)	23 (48.9)	6 (75.0)	66 (58.4%)	4 (66.7%)	0 (0.0)	45 (65.2)	0.13
Median age at diagnosis, yrs (IQR)	8.8 (8.7)	10.1 (9.4)	8.9 (9.2)	6.8 (11.6)	13.4 (9.1)	7.0 (3.1)	5.8 (10.0)	0.32
Proband, n (%)	65 (33.2)	29 (61.7)	4 (50.0)	46 (40.7)	3 (50.0)	1 (33.3)	30 (43.5)	**0.02**
Mode of presentation								**<0.001** *
Cardiac arrest at diagnosis, n (%)	13 (6.6)	11 (23.4)	1 (12.5)	20 (17.7)	1 (16.7)	0 (0.0)	16 (23.2)	
Syncope at diagnosis, n (%)	26 (13.3)	14 (29.8)	2 (25.0)	13 (11.5)	1 (16.7)	0 (0.0)	14 (20.3)	
Asymptomatic at diagnosis, n (%)	157 (80.1)	22 (46.8)	5 (62.5)	80 (70.8)	4 (66.7)	3 (100.0)	39 (56.5)	
FH of SCD or ICD	108 (55.1)	22 (46.8)	4 (50.0)	66 (58.4)	4 (66.7)	2 (66.7)	37 (53.6)	0.88
FH of PCCD or PM	29 (14.8)	6 (12.8)	4 (50.0)	29 (25.7)	0 (0.0)	0 (0.0)	18 (26.1)	**0.03** *
Median FU length, yrs (IQR)	5.9 (5.1)	5.9 (9.2)	8.1 (8.4)	5.7 (5.9)	2.9 (6.3)	6.3 (1.8)	5.7 (7.4)	0.69
PM, n (%)	11 (5.6)	3 (6.4)	1 (12.5)	13 (11.6)	0 (0.0)	0 (0.0)	10 (14.7)	0.21
ICD, n (%)	26 (13.3)	11 (23.4)	3 (37.5)	18 (15.9)	2 (33.3)	0 (0.0)	17 (25.0)	0.08
SVT, n (%)	2 (1.0)	2 (4.3)	0 (0.0)	4 (3.5)	0 (0.0)	0 (0.0)	7 (10.1)	**0.04**
First MCE, n (%)	40 (20.4)	25 (53.2)	3 (37.5)	38 (33.6)	2 (33.3)	0 (0.0)	31 (44.9)	**<0.001** *¥
Death or transplantation, n (%)	1 (0.5)	6 (12.8)	0 (0.0)	3 (2.6)	0 (0.0)	0 (0.0)	4 (5.8)	**0.01** *¥

LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; PCCD: progressive cardiac conduction defect; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; FH: family history; FU: follow-up; PM: pacemaker; ICD: implantable cardioverter defibrillator; SVT: supraventricular tachycardia; MCE: major cardiac event; Transplantation: orthotopic heart transplantation because of intractable ventricular arrhythmias; SD= sudden death.

* Analysis with exclusion of BrS-1, DCM and SSS

¥ Cox proportional hazards regression analysis

Table 2: Characteristics of cardiac events in the 28 patients who received appropriate ICD shocks

ECG phenotype	Age at first MCE (years)	Type of first MCE	Age at ICD implant (years)	FU length (years)	MCEs after ICD implant (N)	TdP (N)	VT (N)	VF (N)	Electrical storm (N)	Heart transplant (N)	Alive at last follow-up

Patient	Diagnosis		Event										Status
Patient 1	Isolated PCCD	0.2	Syncope	14.3	20.4	9	0	0	0	1	0		Alive
Patient 2	Overlap	0.2	ACA	7.2	10.4	8	1	1	6	0	0		Alive
Patient 3	Isolated BrS1	11.5	Syncope	11.5	5.4	1	0	0	0	0	0		Alive
Patient 4	Overlap	1.7	ACA	4.5	12.6	2	0	0	0	0	0		Alive
Patient 5	Negative	9.5	ACA	13.5	23.7	4	0	1	1	2	0		Alive
Patient 6	Isolated PCCD	12.1	ACA	16.1	17.7	1	1	0	2	0	0		Alive
Patient 7	Overlap	0.0	ACA	0.1	2.0	5	2	0	0	0	1		Dead
Patient 8	Isolated PCCD	14.8	Syncope	15.4	19.3	1	0	1	1	0	0		Alive
Patient 9	Isolated PCCD	6.0	VF	14.0	13.6	0	0	0	0	0	0		Alive
Patient 10	Isolated LQT3	1.0	Syncope	1.1	9.0	5	0	0	3	2	0		Alive
Patient 11	Isolated PCCD	9.1	Syncope	14.5	19.7	4	0	0	4	0	0		Alive
Patient 12	Negative	0.2	ACA	3.7	21.2	8	0	0	8	0	0		Alive
Patient 13	Negative	0.0	ACA	1.2	5.3	2	1	0	0	1	0		Alive
Patient 14	Isolated PCCD	0.0	ACA	1.2	5.3	10	0	7	7	2	1		Dead
Patient 15	Negative	0.0	ACA	1.1	5.3	2	0	1	1	1	0		Alive
Patient 16	Negative	13.6	ACA	14.7	14.8	3	1	0	1	1	0		Alive
Patient 17	Negative	14.4	Syncope	14.7	8.5	0	0	0	1	1	0		Alive
Patient 18	Overlap	8.9	Syncope	8.9	5.7	15	5	2	8	0	0		Alive
Patient 19	Negative	10.8	Syncope	12.5	3.1	1	0	1	0	0	0		Alive
Patient 20	Overlap	14.7	Syncope	16.3	3.7	3	0	1	1	1	0		Alive

Patient	Phenotype		Event								Status
Patient 21	Isolated LQT3	2.5	ACA	2.6	1.9	2	1	0	0	0	Alive
Patient 22	Overlap	14.6	Syncope	15.1	1.6	0	0	0	0	0	Alive
Patient 23	Overlap	11.7	Syncope	14.8	6.7	0	0	0	0	0	Alive
Patient 24	Overlap	11.6	Syncope	11.7	4.2	0	0	0	0	0	Alive
Patient 25	Negative	11.2	ACA	11.2	5.6	1	0	0	1	0	Alive
Patient 26	Overlap	0.0	VT	3.4	5.1	0	0	0	0	0	Alive
Patient 27	Isolated SSS	0.2	VF	0.8	1.9	0	0	0	0	0	Alive
Patient 28	Negative	6.0	Syncope	6.0	11.2	0	0	0	0	0	Alive

LQT3: long QT syndrome type 3; BrS1: Brugada syndrome type 1; PCCD: progressive cardiac conduction defect; SSS: sick sinus syndrome; Negative: negative ECG phenotype; Overlap: overlap phenotype; ICD: implantable cardioverter defibrillator; MCE: major cardiac event; ACA: aborted cardiac arrest; VT: ventricular tachycardia; TdP: polymorphic VT with torsades de pointes; VF: ventricular fibrillation; FU: follow-up

Table 3: Phenotypes and family history (n=442)

Phenotype and family history	Baseline, n (%)	At last follow-up, n (%)
Phenotype		
Negative phenotype	196 (44.3)	143 (32.4)
LQT3	78 (17.6)	110 (24.9)
Isolated LQT3	47 (10.6)	50 (11.3)
Overlap phenotype including LQT3	31 (7.0)	60 (13.6)
BrS-1	38 (8.6)	65 (14.7)
Isolated spontaneous BrS-1	8 (1.8)	14 (3.2)
Overlap phenotype including BrS-1	30 (6.8)	51 (11.5)
PCCD	172 (38.9)	220 (49.8)
Isolated PCCD	113 (25.6)	119 (26.9)
Overlap phenotype including PCCD	59 (13.3)	101 (22.8)
SSS	23 (5.2)	24 (5.4)
Isolated SSS	6 (1.4)	4 (0.9)
Overlap phenotype including SSS	17 (3.8)	20 (4.5)
DCM	3 (0.7)	7 (1.6)
Isolated DCM	3 (0.7)	2 (0.5)
Overlap phenotype including DCM	0 (0.0)	5 (1.1)
Overlap phenotype	69 (15.6)	110 (24.9)
LQT3 and BrS-1	4 (0.9)	6 (1.4)
LQT3 and PCCD	20 (4.5)	36 (8.1)
LQT3 and SSS	4 (0.9)	2 (0.4)
LQT3 and DCM	1 (0.2)	0 (0.0)
BrS-1 and PCCD	24 (5.4)	37 (8.4)
PCCD and SSS	10 (2.3)	8 (1.8)
PCCD and DCM	3 (0.7)	4 (0.9)
LQT3 and BrS-1 and PCCD	0 (0.0)	6 (1.4)
LQT3 and BrS-1 and SSS	1 (0.2)	1 (0.2)
LQT3 and PCCD and SSS	1 (0.2)	8 (1.8)
LQT3 and PCCD and DCM	0 (0.0)	1 (0.2)
BrS-1 and PCCD and SSS	1 (0.2)	1 (0.2)
Family history of		
Syncope	156 (35.3)	
Atrial fibrillation	18 (4.1)	
SSS	63 (14.3)	
PCCD	62 (14.0)	
DCM	14 (3.2)	
MEPPT	1 (0.2)	
SCD	134 (30.3)	
Including SIDS	22 (5.0)	
Aborted cardiac arrest	72 (16.3)	
PM implantation	65 (14.7)	
ICD implantation	138 (31.2)	

LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; PCCD: progressive cardiac conduction defect; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; MEPPT: multifocal ectopic Purkinje-related premature contractions; SCD: sudden cardiac death; SIDS: sudden infant death syndrome; PM: pacemaker; ICD: implantable cardioverter defibrillator.

Table 4: Clinical characteristics of isolated LQT3 patients who experienced cardiac events (n=25)

	SCN5A mutation (c.)	Age at first MCE (years)	Type of first MCE	Age at first recurrence	Type of first recurrence	Drugs at the time of first recurrence	Other treatment	Length of FU (years)	Total number of MCEs	Alive at last FU
Patient 1	c.4519_4527del	5.3	Syncope	n/a	n/a	n/a	ICD	8.0	1	Alive
Patient 2	c.5329G>A	15.5	ACA	n/a	n/a	n/a	ICD	3.3	1	Alive
Patient 3	c.5236G>A	7.8	Syncope	n/a	n/a	n/a	n/a	22.9	1	Alive
Patient 4	c.4901T>C	6.0	Syncope	n/a	n/a	n/a	n/a	6.2	1	Alive
Patient 5	c.4458C>A	1.1	Syncope	7.4	VF	mexiletine	ICD, LCSD	9.0	6	Alive
Patient 6	c.5350G>A	10.9	Syncope	n/a	n/a	n/a	n/a	2.3	1	Alive
Patient 7	c.5287G>A	0.1	ACA	1.2	TdP	propranolol (2 mg/kg/d)	n/a	12.5	3	Dead
Patient 8	c.1231G>A	11.8	Syncope	21.8	TdP	nadolol (1 mg/kg/d)	n/a	25.7	2	Alive
Patient 9	c.1231G>A	7.1	Syncope	n/a	n/a	n/a	ICD	6.1	1	Alive
Patient 10	c.5296A>C	2.5	ACA	3.3	VF	mexiletine + propranolol (2 mg/kg/d)	ICD	1.9	3	Alive
Patient 11	c.5287G>A	0.7	SCD	n/a	n/a	n/a	ICD	0.0	1	Dead
Patient 12	c.1231G>A	5.3	ACA	n/a	n/a	n/a	ICD	3.6	1	Alive
Patient 13	c.2065C>T	10.1	ACA	n/a	n/a	n/a	n/a	14.9	1	Alive
Patient 14	c.3556G>A	15.2	Syncope	n/a	n/a	n/a	n/a	3.8	1	Alive
Patient 15	c.1273G>A	13.6	Syncope	n/a	n/a	n/a	n/a	5.0	1	Alive
Patient 16	c.5350G>A	14.3	Syncope	n/a	n/a	n/a	PM	5.9	1	Alive
Patient 17	c.5287G>A	0.1	ACA	n/a	n/a	n/a	ICD	0.8	1	Alive
Patient 18	c.4442G>A	0.6	ACA	n/a	n/a	n/a	n/a	12.1	1	Alive

Patient	Variant					Treatment				Outcome
Patient 19	c2821_2822delTCinsAA	0.1	ACA	0.2	VF	mexiletine + propranolol (2 mg/kg/d)	n/a	4.7	6	Dead
Patient 20	c5300A>G	14.0	Syncope	26.8	SCD	no treatment*	n/a	12.8	2	Dead
Patient 21	c1231G>A	10.9	Syncope	11.9	TdP	propranolol (2 mg/kg/d)	n/a	10.7	3	Alive
Patient 22	c4519_4527del	16.2	Syncope	n/a	n/a	n/a	LCSD	26.5	1	Alive
Patient 23	c4519_4527del	16.6	SCD	n/a	n/a	n/a	PM	13.0	1	Dead
Patient 24	c5972G>A	16.2	Syncope	16.3	TdP	propranolol (2 mg/kg/d)	ICD	2.0	4	Alive
Patient 25	c3989C>A	0.0	ACA	0.1	Syncope	mexiletine + propranolol (unknown dose)	n/a	0.2	3	Dead

All but one patient (receiving propranolol 1 mg/kg/d) had no treatment at the time of first MCE

*betablocker voluntarily interrupted by the patient who died off treatment.

LQT3: long QT syndrome type 3; PM: pacemaker; ICD: implantable cardioverter defibrillator; MCE: major cardiac event; SCD: sudden cardiac death, SIDS: sudden infant death syndrome; LCSD: left cardiac sympathetic denervation; FU: follow-up.

Table 5: SCN5A mutations (442 patients, 445 mutations, 185 unique mutations)

SCN5A mutation (c.)	exon	Mutant (p.)	Functional effect	n
Truncation mutations [n=81 mutations, 44 distinct mutations]				
c127C>T	2	pArg43*	Loss of function	1
c268del	2	pGln90Trpfs*14	Loss of function	1
c393-1C>T	4		Loss of function	1
c468G>A	4	pTrp156*	Loss of function	1
c611+1G>A	5		Loss of function	2
c703+1G>A	6		Loss of function	1
c870del	7	pAsn291Thrfs*52	Loss of function	2
c934+1G>A	7		Loss of function	2
c1036G>T	9	pGlu346*	Loss of function	1
c1603C>T	12	pArg535*	Loss of function	4
c1890G>A	12	pThr631Valfs*101	Loss of function	3
c1936del	13	pGln646Argfs*5	Loss of function	7
c2274delG	15	pIle759Phefs*6	Loss of function	3
c2320del	15	pTyr774Thrfs*28	Loss of function	2
c2335C>T	15	pGln779*	Loss of function	2
c2520del	16	pAsn841Thrfs*2	Loss of function	1
c2550del_2551dupGT	16	pPhe851Cysfs*19	Loss of function	1
c2582_2583del	16	pPhe861Trpfs*90	Loss of function	6
c2998C>T	17	pGln1000*	Loss of function	1
c3045_3046del	17	pGu1015Aspfs*14	Loss of function	1
c3175C>T	17	pGln1059*	Loss of function	1
c3207_3211dup	17	pGlu1071Glyfs*76	Loss of function	1
c3313G>T	18	pGlu1105*	Loss of function	1
c3318dup	18	pGlu1107Argfs*24	Loss of function	2
c3319G>T	18	pGlu1107*	Loss of function	1
c3352C>T	18	pGln1118*	Loss of function	2
c3491dup	19	pGlu1165Argfs*6	Loss of function	2
c3572G>A	20	pTrp1191*	Loss of function	2
c3666+1del	20	pLeu1222Leufs*7	Loss of function	2
c3840+1G>A	21		Loss of function	5
c3900_3903dup	22	Leu1302Valfs18	Loss of function	1
c4105G>T	23	pGly1369*	Loss of function	1
c4118del	23	pLeu1373*	Loss of function	4
c4245+1G>T	23		Loss of function	1
c4299+1dup	24	pGly1031fs*27	Loss of function	1
c4423del	24	pGln1475Asnfs*6	Loss of function	1
c4437+5G>A	25		Loss of function	1
c4845C>G	28	pTyr1615*	Loss of function	1
c4867C>T	28	pArg1623*	Loss of function	1
c5083C>T	28	pGln1695*	Loss of function	1
c5321_5324dup	28	pPhe1775Leufs*15	Loss of function	2
c5433T>G	28	pTyr1811*	Loss of function	1
c5830C>T	28	pArg1944*	Loss of function	2
c6017del	28	pPro2006Leufs*32	Loss of function	1

Table 5 (suite)

SCN5A mutations	Exon	Aminoacid changes	Effect	n
Missense pathogenic mutations [n=285 mutations, 95 distinct mutations]				
c278T>C	3	pPhe93Ser	Loss of function	3
c362G>A	3	pArg121Gln	Loss of function	1
c481G>A	4	pGlu161Lys	Loss of function	2
c635T>C	6	pLeu212Pro	Gain of function	2
c665G>A	6	pArg222Gln	Gain of function	1
c673C>T	6	pArg225Trp	Gain and loss	4
c718G>A	7	pVal240Met		1
c827T>C	7	pLeu276Pro		2
c844C>T	7	pArg282Cys	Loss of function	1
c1007C>T	9	pPro336Leu	Loss of function	1
c1018C>T	9	pArg340Trp	Gain of function	1
c1066G>A	9	pAsp356Asn	Loss of function	3
c1099C>T	9	pArg367Cys	Loss of function	1
c1100G>A	9	pArg367His	Loss of function	1
c1106T>A	9	pMet369Lys	Loss of function	3
c1109C>T	9	pThr370Met	Gain of function	4
c1120T>G	9	pTrp374Gly	Loss of function	2
c1126C>T	9	pArg376Cys	Loss of function	3
c1218C>A	10	pAsn406Lys	Gain of function	2
c1231G>A	10	pVal411Met	Gain of function	10
c1540G>T	12	pGly514Cys	Loss of function	2
c2047T>C	14	pCys683Arg	Gain of function	6
c2150C>T	14	pPro717Leu		2
c2204C>T	14	pAla735Val	Loss of function	1
c2441G>A	16	pArg814Gln		3
c2516T>C	16	pLeu839Pro	Loss of function	1
c2632C>T	16	pArg878Cys	Loss of function	1
c2674T>A	16	pPhe892Ile	Loss of function	1
c2677C>T	16	pArg893Cys	Loss of function	1
c2690G>A	16	pGly897Glu	Loss of function	1
c2701G>A	16	pGlu901Lys	Loss of function	7
c2780A>G	16	pAsn927Ser	Loss of function	1
c2821T>A and c2822C>A	17	pSer941Asn	Gain of function	1
c2822C>T	17	pSer941Phe	Gain of function	1
c2893C>T	17	pArg965Cys	Loss of function	2
c3157G>A	17	pGlu1053Lys	Loss of function	1
c3556G>A	20	pAla1186Thr	Gain of function	2
c3662C>T	20	pAla1221Val		2
c3673G>A	21	pGlu1225Lys	Loss of function	3
c3694C>T	21	pArg1232Trp	Loss of function	1
c3718G>C	21	pGlu1240Gln		1
c3784G>A	21	pGly1262Ser	Loss of function	1
c3823G>A	21	pAsp1275Asn	Loss of function	4
c3911C>T	22	pThr1304Met	Gain of function	2
c3956G>T	22	pGly1319Val	Loss of function	8
c3974A>G	23	pAsn1325Ser	Gain of function	4
c3988G>A	23	pAla1330Thr	Gain of function	1
c3989C>A	23	pAla1330Asp	Gain of function	1
c3995C>A	23	pPro1332Gln		1

Table 5 (suite)

SCN5A mutations	Exon	Aminoacid changes	Effect	n
Missense pathogenic mutations (suite)				
c3995C>T	23	pPro1332Leu	Gain of function	1
c4000A>G	23	pIle1334Val	Gain of function	3
c4035G>T	23	pTrp1345Cys	Loss of function	2
c4037T>C	23	pLeu1346Pro	Loss of function	1
c4140C>G	23	pAsn1380Lys		2
c4216G>C	23	pGly1406Arg	Loss of function	2
c4222G>A	23	pGly1408Arg	Loss of function	2
c4282G>T	24	pAla1428Ser	Loss of function	1
c4346A>G	25	pTyr1449Cys	Loss of function	2
c4441G>A	26	pGly1481Arg		1
c4442G>A	26	pGly1481Glu		1
c4442G>T	26	pGly1481Val		1
c4458C>A	26	pPhe1486Leu	Gain of function	1
c4459A>C	26	pMet1487Leu	Gain of function	1
c4493T>C	26	pMet1498Thr		2
c4501C>G	26	pLeu1501Val		4
c4562T>A	27	pIle1521Lys	Loss of function	2
c4748G>A	27	pArg1583His	Loss of function	1
c4783G>A	27	pAsp1595Asn	Loss of function	1
c4868G>A	28	pArg1623Gln	Gain of function	6
c4876C>T	28	pArg1626Cys		1
c4892G>A	28	pGly1631Asp	Gain of function	1
c4895G>T	28	pArg1632Leu		1
c4931G>A	28	pArg1644His	Gain of function	2
c4978A>G	28	pIle1660Val	Loss of function	2
c5015C>A	28	pSer1672Tyr	Loss of function	1
c5129C>T	28	pSer1710Leu	Loss of function	4
c5164A>G	28	pAsn1722Asp	Loss of function	2
c5227G>A	28	pGly1743Arg	Loss of function	3
c5228G>A	28	pGly1743Glu	Loss of function	8
c5287G>A	28	pVal1763Met	Gain of function	6
c5287G>T	28	pVal1763Leu		1
c5296A>C	28	pMet1766Leu	Gain and loss	1
c5300A>G	28	pTyr1767Cys	Gain of function	2
c5302A>G	28	pIle1768Val	Gain of function	9
c5320A>C	28	pAsn1774His		1
c5320A>G	28	pAsn1774Asp		1
c5329G>A	28	pVal1777Met	Gain of function	8
c5329G>T	28	pVal1777Leu		1
c5350G>A	28	pGlu1784Lys	Gain and loss	69
c5357T>G	28	pLeu1786Arg		1
c5368G>A	28	pAsp1790Asn		1
c5369A>G	28	pAsp1790Gly	Gain of function	7
c5383T>A	28	pTyr1795Asn		1
c5384A>G	28	pTyr1795Cys	Gain of function	2
c5546A>G	28	pHis1849Arg	Gain of function	1

Table 5 (suite)

SCN5A mutations	Exon	Aminoacid changes	Effect	n
In-frame mutations [n=32 mutations, 11 distinct mutations]				
c2184_2186del	14	pLeu729del	Loss of function	1
c4015_4017del	23	pLeu1339del		1
c4140_4142del	23	pAsn1380del	Loss of function	3
c4456_4458del	26	pPhe1486del	Gain and loss	1
c4519-4527del	26	pGln1507_Pro1509del		9
c4708_4710dup	27	pIle1570dup	Loss of function	1
c4850_4852del	28	pPhe1617del	Gain and loss	4
c5242_5244del	28	pGly1748del		1
c5272_5274del	28	pIle1758del		3
c5385_5387dup	28	pTyr1795_Glu1796insAsp	Gain and loss	7
c5972G>A	28	pArg1991Gln		1

SCN5A mutations	Exon	Aminoacid changes	Effect	n
Unknwon functional effect [n=47 mutations, 35 distinct mutations]				
c10T>G	2	pPhe4Val		1
c670C>T	6	pLeu224Phe		1
c680T>C	6	pLeu227Pro		1
c725C>A	7	pAla242Asp		1
c787G>A	7	pVal263Ile		1
c994G>A	8	pAla332Thr		1
c1022G>A	9	pCys341Tyr		2
c1063T>A	9	pPhe355Ile		2
c1201T>C	10	pSer401Pro		1
c1237G>A	10	pAla413Thr		2
c1273G>A	10	pAla425Thr		1
c1889C>T	12	pThr630Met		2
c2065C>T	14	pArg689Cys		1
c2207T>C	14	pLeu736Pro		1
c2335C>A	15	pGln779Lys		2
c3067C>T	17	pArg1023Cys		2
c3220A>G	17	pSer1074Gly		1
c3236C>A	18	pSer1079Tyr		2
c3236C>T	18	pSer1079Phe		2
c3598C>T	20	pHis1200Tyr		2
c3626C>G	20	pThr1209Arg		1
c3629T>C	20	pPhe1210Ser		1
c3665T>G	20	pLeu1222Arg		1
c4380C>A	25	pPhe1460Leu		1
c4424A>T	25	pGln1475Leu		1
c4473G>T	26	pGln1491His		1
c4510A>G	26	pLys1504Glu		1
c4571T>C	27	pIle1524Thr		2
c4901T>C	28	pLeu1634Pro		1
c5236G>A	28	pAla1746Thr		2
c5239G>A	28	pVal1747Met		1
c5246T>A	28	pIle1749Asn		1
c5378T>A	28	pMet1793Lys		1
c5431T>A	28	pTyr1811Asn		1
c5689C>T	28	pArg1897Trp		2

Table 6: Comparison between VUS and other mutations (n=442)

	Gain of function, loss of function or both gain and loss of function (n=350)	Variants of unknown significance (n=92)	p value
Diagnosis			
Male, n (%)	161(46)	35(38)	0.19
Proband, n (%)	128(37)	50(54)	**0.003**
Age at diagnosis, yrs (IQR)	8.0(9.2)	8.6(10.1)	0.64
Diagnosis ≤1year, n (%)	58(17)	17(18)	0.64
Mode of presentation, n (%)			0.55
Cardiac arrest at diagnosis, n (%)	50(14)	12(13)	
Syncope at diagnosis, n (%)	52(15)	18(20)	
Asymptomatic at diagnosis, n (%)	24871)	62(67)	
Phenotype			
Isolated LQT3 at baseline, n (%)	33(9)	14(15)	0.13
Isolated BrS-1 at baseline, n (%)	7(2)	1(1)	0.48
Isolated PCCD at baseline, n (%)	91(26)	22(24)	0.40
Isolated DCM at baseline, n (%)	3(1)	0(0)	0.50
Isolated SSS at baseline, n (%)	5(1)	1(1)	0.64
Overlap phenotype at baseline, n (%)	53(15)	16(17)	0.35
Negative phenotype at baseline, n (%)	158(45)	38(41)	0.29
ECG parameters			
Median age at ECG, yrs (IQR)	8.0(9.4)	8.(10.3)	0.37
Median heart rate, bpm (IQR)	78.9(34.8)	78.9(34.8)	0.72
Median PR interval, ms (IQR)	160(48)	160(60)	0.83
Median QRS complex, ms (IQR)	80(30)	80(32)	0.92
Median QT interval, ms (IQR)	360(100)	385(120)	0.07

QTc: corrected QT interval; AV block: atrioventricular block; RBBB: right bundle branch block; LBBB: left bundle branch block; SVT: supraventricular tachycardia; BrS1: Brugada syndrome type 1; LQT3: long QT syndrome type 3.

Table 7: Clinical characteristics according to *SCN5A* mutation location (domains) (N=442)

	N-terminus (n=7)	DI-DIV (n=325)	C-terminus (n=110)	p value	Analysis	HR (95%IC)
Diagnosis						
Male, n (%)	5 (71.4)	183 (56.3)	58 (52.7)	0.77		
Proband, n (%)	3 (42.9)	141 (43.4)	34 (30.9)	0.06		
FH of CCD-PM, n (%)	0 (0.0)	58 (17.8)	28 (25.4)	0.11		
FH of SCD-ICD, n (%)	3 (42.9)	170 (52.3)	70 (63.6)	0.13		
Median age at diagnosis, yrs (IQR)	7.4 (10.6)	7.1 (9.9)	10.1 (6.7)	0.01		
Diagnosis ≤1year, n (%)	2 (28.6)	62 (19.1)	11 (10.0)	0.08		
Mode of presentation, n (%)				0.001		
Cardiac arrest at diagnosis, n (%)	2 (28.6)	56 (17.2)	4 (3.6)			
Syncope at diagnosis, n (%)	1 (14.3)	54 (16.6)	15 (13.6)			
Asymptomatic at diagnosis, n (%)	4 (57.1)	215 (66.1)	91 (82.7)			
Phenotype						
Isolated LQT3 at baseline, n (%)	0 (0.0)	31(9.5)	16 (14.5)	0.29		
Isolated BrS-1 at baseline, n (%)	0 (0.0)	7 (2.2)	1 (0.9)	0.72		
Isolated PCCD at baseline, n (%)	1 (14.3)	87 (26.8)	25 (22.7)	0.64		
Isolated DCM at baseline, n (%)	0 (0.0)	3 (0.9)	0 (0.0)	0.59		
Isolated SSS at baseline, n (%)	0 (0.0)	5 (1.5)	1 (0.9)	0.56		
Overlap phenotype at baseline, n (%)	2 (28.6)	51 (15.7)	16 (14.5)	0.55		
Negative ECG phenotype at baseline, n (%)	4 (57.1)	141 (43.4)	51 (46.4)	0.67		
Outcomes						

Median FU length, yrs (median, IQR)	4.7 (5.9)	5.7 (5.8)	7.2 (6.3)	0.06		
MCE, n (%)	3 (42.9)	115 (35.4)	21 (19.1)	**0.0002*** (cox) 0.1	DI-DIV vs C-term N-term vs C-term	2.9 (1.7-4.9) 4.5 (1.1-18.6)
ICD implantation, n (%)	3 (42.9)	52 (16.0)	22 (20.0)			
At least one appropriate shocks, n (%)	0 (0.0)	24 (46.2)	4 (18.2)	**0.03**		
Death or transplantation, n (%)	0 (0.0)	14 (4.3)	0 (0.0)	n/a		n/a

FH: family history; PCCD: progressive cardiac conduction disorder; PM: pacemaker; SCD: sudden cardiac death; ICD: implantable cardioverter defibrillator; LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; FU: follow-up; MCE: major cardiac event; Transplantation: orthotopic heart transplantation because of intractable ventricular arrhythmias; n/a = not applicable. * Cox proportional hazards regression analysis

Table 8: Phenotype and outcomes according to *SCN5A* mutation location (segments) (n=241)

	S1-S4 (n=80)	S5-S6 (n=161)	p-value
Diagnosis			
Male, n (%)	38 (47.5)	91 (56.5)	0.12
Proband, n (%)	34 (42.5)	69 (42.9)	0.53
FH of CCD-PM, n (%)	11 (13.7)	25 (15.5)	0.85
FH of ICD-SCD, n (%)	35 (43.7)	88 (54.7)	0.13
Median age at diagnosis, yrs (IQR)	8.4 (9.2)	6.9 (10.1)	0.08
Diagnosis ≤1year, n (%)	12 (15.0)	34 (21.1)	0.3
Symptomatic, n (%)	33 (41.2)	56 (34.8)	0.39
Mode of presentation, n (%)			0.55
Cardiac arrest at diagnosis, n (%)	12 (15.0)	27 (16.8)	
Syncope at diagnosis, n (%)	17 (21.2)	25 (15.5)	
Asymptomatic at diagnosis, n (%)	51 (63.7)	109 (67.7)	
Phenotype			
Isolated LQT3 at baseline, n (%)	2 (2.5)	16 (9.9)	0.04
Isolated BrS-1 at baseline, n (%)	3 (3.8)	3 (1.9)	0.4
Isolated PCCD at baseline, n (%)	20 (25.0)	43 (26.7)	0.88
Isolated DCM at baseline, n (%)	0 (0.0)	0 (0.0)	-
Isolated SSS at baseline, n (%)	1 (1.3)	1 (0.6)	0.56
Overlap phenotype at baseline, n (%)	13 (16.3)	28 (17.4)	0.49
Negative phenotype at baseline, n (%)	41 (51.3)	70 (43.5)	0.27
Outcomes			
Median FU length, yrs (IQR)	5.7 (5.1)	5.8 (6.3)	0.76
MCE, n (%)	29 (36.3)	57 (35.4)	0.52*
ICD implantation, n (%)	13 (16.2)	32 (19.9)	0.6
At least one appropriate shocks, n (%)	10 (76.9)	12 (37.5)	**0.02**
Death or transplantation, n (%)	1 (1.3)	6 (3.7)	0.36*

LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; PCCD: progressive cardiac conduction disorder; CA: cardiac arrest (includes aborted cardiac arrest and sudden cardiac death); MCE: major cardiac event; ICD: implantable cardioverter defibrillator.
*Cox proportional hazards regression analysis

Table 9: Clinical characteristics according to *SCN5A* mutation function (N=442)

Phenotype	Gain of function (n=87)	Loss of function (n=178)	Gain and loss of function (n=85)	Unknown functional effect (n=92)	p value	HR (95% IC)
Diagnosis						
Male, n (%)	43(49.4)	79(44.4)	39(45.9)	35(38.0)	0.48	
Proband, n (%)	43(49.4)	58(32.6)	27(31.8)	50(54.3)	0.001	
FH of PCCD or PM, n (%)	7(8.0)	44(24.7)	20(23.5)	15(16.3)	0.005	
FH of SCD or ICD, n (%)	44(50.6)	109(61.2)	52(61.2)	38(41.3)	0.008	
Median age at diagnosis, yrs (IQR)	7.0(11.8)	7.0(8.9)	9.8(6.7)	8.6(10.2)	0.19	
Diagnosis ≤1year, n (%)	23(26.4)	25(14.0)	10(11.8)	17(18.5)	0.05	
Mode of presentation, n (%)					<0.001	
Cardiac arrest at diagnosis, n (%)	26(29.9)	22(12.4)	2(2.4)	12(13.0)		
Syncope at diagnosis, n (%)	14(16.1)	27(15.2)	11(12.9)	18(19.6)		
Asymptomatic at diagnosis, n (%)	47(54.0)	129(72.5)	72(84.7)	62(67.4)		
Phenotypes						
Isolated LQT3 at baseline, n (%)	23(26.4)	1(0.6)	10(11.8)	16(17.4)	<0.001	
Isolated BrS-1 at baseline, n (%)	1(1.1)	8(4.5)	4(4.7)	1(1.1)	0.28	
Isolated PCCD at baseline, n (%)	10(11.5)	68(38.2)	19(22.4)	22(23.9)	<0.001	
Isolated DCM at baseline, n (%)	1(1.1)	1(0.6)	0(0.0)	0(0.0)	0.67	
Isolated SSS at baseline, n (%)	3(3.4)	0(0.0)	0(0.0)	1(1.1)	0.03	
Overlap syndrome at baseline, n (%)	7(8.0)	34(19.1)	12(14.1)	16(17.4)	0.11	
Negative ECG phenotype at baseline, n (%)	39(44.8)	48(27.0)	30(35.3)	26(28.3)	0.02	
Outcomes						
Median FU length, yrs (IQR)	5.8(5.9)	4.7(5.8)	7.0(5.6)	6.3(7.3)	0.02	
MCE, n (%)	41(47.1)	52(29.2)	14(16.5)	32(34.8)	<0.001 (cox)	Gain vs loss 2.3(1.4-3.9) Gain and loss vs loss 0.4(0.2-0.8) Unknown vs loss 1.2(0.7-2.1)
ICD implantation, n (%)	30(34.5)	23(13.1)	12(14.1)	12(13.0)	<0.001	
At least one appropriate shock, n (%)	14(46.7)	9(39.1)	3(25.0)	2(16.7)	0.25	
Death or transplantation, n (%)	6(6.9)	3(1.7)	1(1.2)	4(4.3)	0.18 (cox)	

FH: family history; PCCD: progressive cardiac conduction defect; PM: pacemaker; SCD: sudden cardiac death; ICD: implantable cardioverter defibrillator; Group 1: cardiac arrest as first symptom; Group 2: syncope as first symptom; Group 3: asymptomatic at diagnosis; LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; FU: follow-up; MCE: major cardiac event; Transplantation: orthotopic heart transplantation because of intractable ventricular arrhythmias.

Table 10: Clinical characteristics according to *SCN5A* mutation type (N=442)

Phenotype	Non missense pathogenic mutation (n=113)	Missense pathogenic mutation (n=283)	Unknown (n=46)	p-value
Diagnosis				
Male, n (%)	61 (54.0)	158 (55.8)	27 (58.7)	0.85
Proband, n (%)	36 (31.9)	119 (42.0)	23 (50.0)	0.06
FH of PCCD or PM, n (%)	36 (31.9)	42 (14.8)	8 (17.4)	**0.001**
FH of SCD or ICD, n (%)	70 (61.9)	155 (54.8)	18 (39.1)	**0.03**
Median age at diagnosis, yrs (IQR)	5.8 (9.7)	8.6 (9.4)	10.3 (8.7)	**0.02**
Diagnosis ≤1year, n (%)	24 (21.2)	49 (17.3)	2 (4.3)	**0.02**
Mode of presentation, n (%)				0.47
Cardiac arrest at diagnosis, n (%)	19 (16.8)	37 (13.1)	6 (13.0)	
Syncope at diagnosis, n (%)	17 (15.0)	42 (14.8)	11 (23.9)	
Asymptomatic at diagnosis, n (%)	77 (68.1)	204 (72.1)	29 (63.0)	
Phenotypes				
Isolated LQT3 at baseline, n (%)	7 (6.2)	34 (12.0)	6 (13.0)	0.17
Isolated BrS-1 at baseline, n (%)	2 (1.8)	5 (1.8)	1 (2.2)	0.87
Isolated PCCD at baseline, n (%)	42 (37.2)	61 (21.6)	10 (21.7)	**0.006**
Isolated DCM at baseline, n (%)	2 (1.8)	1 (0.4)	0 (0.0)	0.30
Isolated SSS at baseline, n (%)	1 (0.9)	5 (1.8)	0 (0.0)	0.83
Overlap syndrome at baseline, n (%)	23 (20.4)	39 (13.8)	7 (15.2)	0.27
Negative ECG phenotype at baseline, n (%)	36 (31.9)	138 (48.8)	22 (47.8)	**0.007**
Outcomes				
Median FU length, yrs (IQR)	6.3 (6.0)	5.9 (5.6)	4.7 (5.6)	0.14
MCE, n (%)	39 (34.5)	83 (29.3)	17 (37.0)	0.51*
ICD implantation, n (%)	18 (16.1)	54 (19.1)	5 (10.9)	0.38
At least one appropriate shock, n (%)	6 (33.3)	21 (38.9)	1 (20.0)	0.78
Death or transplantation, n (%)	3 (2.7)	10 (3.5)	1 (2.2)	0.84*

FH: family history; PCCD: progressive cardiac conduction defect; PM: pacemaker; SCD: sudden cardiac death; ICD: implantable cardioverter defibrillator; Group 1: cardiac arrest as first symptom; Group 2: syncope as first symptom; Group 3: asymptomatic at diagnosis; LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; FU: follow-up; MCE: major cardiac event; Transplantation: orthotopic heart transplantation because of intractable ventricular arrhythmias.

* Cox proportional hazards regression analysis

Table 11: Clinical characteristics according to specific mutations

SCN5A mutation	absence	presence	P value	Analysis	HR (95%CI)
pGlu1784Lys					
Proband, n(%)	159 (89.3)	19 (10.7)	0.02		
Median age at diagnosis, yrs (IQR)	7.4 (9.9)	10.5 (5.9)	0.002		
Mode of presentation					
cardiac arrest, n (%)	62 (100.0)	0 (0.0)	<0.001		
syncope, n (%)	61 (87.1)	9 (12.9)			
asymptomatic, n (%)	250 (80.6)	60 (19.4)			
MCE, n(%)	129 (92.8)	10 (7.2)	0.0002*	absence vs presence	3.7 (1.8-7.6)
pGly1319Val					
Proband, n(%)	171 (96.1)	7 (3.9)	0.008		
pVal1763Met					
Proband, n(%)	173 (97.2)	5 (2.8)	0.04		
Median age at diagnosis, yrs (IQR)	8.1 (9.2)	0.5 (1.6)	<0.001		
Mode of presentation			<0.001		
cardiac arrest, n (%)	56 (90.3)	6 (9.7)			
syncope, n (%)	70 (100.0)	0 (0.0)			
asymptomatic, n (%)	310 (100.0)	0 (0.0)			
MCE, n(%)	133 (95.7)	6 (4.3)	<0.0001*	Presence vs absence	15.4 (5.4-43.4)
pVal411Met					
Proband, n(%)	168 (94.4)	10 (5.6)	<0.001		
Mode of presentation			<0.001		
cardiac arrest, n (%)	58 (93.5)	4 (6.5)			
syncope, n (%)	65 (92.9)	5 (7.1)			
asymptomatic, n (%)	309 (99.7)	1 (0.3)			
MCE, n(%)	130 (93.5)	9 (6.5)	<0.0001*	Presence vs absence	5.1 (2.3-11.4)
pTyr1795_Glu1796insAsp					
Median age at diagnosis, yrs (IQR)	8.2 (9.5)	0.7 (7.1)	0.02		

Age at diagnosis is expressed in years; SD: standard deviation; CE: cardiac event; Med.: median; MCE: major cardiac event.

*Cox proportional hazards regression analysis

Table 12: Multivariable analysis on first CE (n=424)

	HR	95% CI	p value
Genotype			0.03
Single *SCN5A* mutation	1		
Double *SCN5A* mutation	2.1	0.3-13.9	0.45
Compound genotype	3.7	1.2-12.0	0.03
***SCN5A* mutation functional effect**			0.001
Loss-of-function	1		
Gain-of-function	1.8	0.9-3.31	0.07
Both gain- and loss-of-function	0.5	0.2-0.9	0.04
Variants of unknwon significance	0.8	0.4-1.4	0.4
Interaction Age ≤1 year at diagnosis and Proband status			0.0002
Age ≤1 year at diagnosis in proband patients	35.4	16.2-77.6	<0.0001
Age ≤1 year at diagnosis in relative patients	3.2	1.1-9.1	0.03

Multivariable analysis was stratified on baseline phenotype.

Table 13: Considered cut-off values for definition of cardiac conduction abnormalities according to age

		Infants and young children <4 yrs	Children and teenagers ≥4 yrs and <16 yrs	Adults ≥ 16 yrs
1st-degree AV block	PR interval, ms	≥ 160	≥ 180	≥ 200
Left axis deviation	QRS axis, °	-30° and beyond	-30° and beyond	-30° and beyond
Right axis deviation	QRS axis, °	+180° and beyond	+140° and beyond	+90° and beyond
Incomplete RBBB	QRS complex, ms	80 ≤ QRS < 90 *	90 ≤ QRS < 100 *	110 ≤ QRS < 120 *
Complete RBBB	QRS complex, ms	QRS ≥ 90 **	QRS ≥ 100 **	QRS ≥ 120 **
Incomplete LBBB	QRS complex, ms	80 ≤ QRS < 90 #	90 ≤ QRS < 100 #	110 ≤ QRS < 120 #
Complete LBBB	QRS complex, ms	QRS ≥ 90 ##	QRS ≥ 100 ##	QRS ≥ 120 ##
Non-specific IVCA	QRS complex, ms	QRS ≥ 80 †	QRS ≥ 90 †	QRS ≥ 110 †
Left anterior FB	QRS complex, ms	< 120 ‡	< 120 ‡	< 120 ‡
Left posterior FB	QRS complex, ms	< 120 ‡‡	< 120 ‡‡	< 120 ‡‡

BBB: bundle branch block; IVCA: intraventricular conduction abnormality; FB: fascicular block.

* and rsr', rsR' or rSR' in leads V1 or V2.
** and rsr', rsR' or rSR' in leads V1 or V2.
# and absent q wave in leads I, V5 and V6; and R peak time > 60 ms in leads V5 and V6 but normal in leads V1, V2 and V3.
and broad notched or slurred R wave in leads I, aVL, V5 and V6, eventually associated with a RS pattern in V5 and V6; and absent q wave in leads I, V5 and V6; and R peak time > 60 ms in leads V5 and V6 but normal in leads V1, V2 and V3.
† and no criteria for RBBB or LBBB.
‡ and frontal plane axis between -45° and -90°; and qR pattern in lead aVL; and R peak time ≥ 45 ms in aVL.
‡‡ and Frontal plane axis between 100° and 180°; and rS pattern in leads I and aVL; and qR pattern in leads III and aVF

Adapted from [Priori *et al*, 2015; Surawicz *et al*, 2009; Schwartz *et al*, 2002; Rijnbeek *et al*, 2001]

Table 13bis: Baseline ECG characteristics according to main ECG phenotypes and age groups (n=442)

ECG characteristics	Negative ECG phenotype	Isolated LQT3	Isolated BrS-1	Isolated PCCD	Overlap phenotype
Infants and young children <4 yrs	n=53	n=10	n=2	n=39	n=27
Heart rate, bpm	108.4 ± 28.0	123.2 ± 19.3	116.5 ± 16.3	102.3 ± 27.2	107.2 ± 28.1
ECG intervals, ms					
PR	127.9 ± 18.9	112.8 ± 27.4	135.0 ± 21.2	167.7 ± 22.5	173.7 ± 32.3
Conducted QRS	73.1 ± 13.6	65.8 ± 15.8	70.0 ± 14.1	85.5 ± 24.7	98.0 ± 34.6
Corrected QT	426.2 ± 35.7	545.6 ± 37.9	392.6 ± 3.4	426.6 ± 27.8	486.9 ± 84.71
Children and teenagers ≥4 yrs and <16 yrs	n=143	n=37	n=6	n=74	n=42
Heart rate, bpm	78.0 ± 21.6	73.6 ± 22.7	71.7 ± 12.6	72.8 ± 16.2	82.8 ± 51.2
ECG intervals, ms					
PR	142.6 ± 21.5	142.3 ± 20.7	150.0 ± 24.5	196.5 ± 40.6	176.5 ± 30.4
Conducted QRS	79.5 ± 15.0	78.2 ± 13.7	73.3 ± 10.3	111.8 ± 20.8	103.0 ± 30.5
Corrected QT	426.3 ± 29.7	535.4 ± 45.6	403.5 ± 42.6	436.5 ± 24.5	462.6 ± 65.5

LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; PCCD: progressive cardiac conduction defect; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy; N/A: not applicable.

Table 14: Most common *SCN5A* mutations per phenotype (n=442)

	Negative ECG phenotype (n=196)	Isolated LQT3 (n=47)	Isolated BrS-1 (n=8)	Isolated PCCD (n=113)	Isolated SSS (n=6)	Isolated DCM (n=3)	Overlap phenotype (n=69)	p value
pGlu1784Lys	29 (15)	13 (28)	0 (0)	17 (15)	0 (0)	0 (0)	10 (14)	0.33
pIle1768Val	6 (3)	1 (2)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	0.78
pGly1743Glu	6 (3)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)	0.69
pVal411Met	2 (1)	5 (11)	0 (0)	2 (2)	0 (0)	0 (0)	1 (1)	0.04
pGln1507_Pro1509del	4 (2)	5 (11)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	**0.01**
pGly1319Val	6 (3)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0.61
pTyr1795_Glu1796insAsp	4 (2)	0 (0)	1 (13)	1 (1)	0 (0)	0 (0)	1 (1)	0.34
pAsp1790Gly	5 (3)	0 (0)	0 (0)	1 (1)	1 (17)	0 (0)	0 (0)	0.18
pAsp356Asn	1 (1)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	0 (0)	0.65
pGln646Argfs5	0 (0)	0 (0)	0 (0)	5 (4)	0 (0)	0 (0)	2 (3)	**0.05**
pGlu901Lys	3 (2)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	3 (4)	0.47
pVal1763Met	2 (1)	3 (6)	0 (0)	0 (0)	1 (17)	0 (0)	0 (0)	**0.01**

LQT3: long QT syndrome type 3; BrS-1: Brugada syndrome type 1; PCCD: progressive cardiac conduction defect; SSS: sick sinus syndrome; DCM: dilated cardiomyopathy.

SUPPLEMENTAL FIGURES

Figure 1: Mode of presentation at diagnosis of cardiac sodium channelopathy

The diagnosis of cardiac sodium channelopathy was most often made because of a family history and an abnormal electrocardiogram obtained as a screening tool (red area, 67.9%). In green are the patients diagnosed after presentation for syncope (15.8%). Sudden cardiac death or resuscitated cardiac arrest was the cause of diagnosis in 14.0% of patients.

Figure 2: Location of *SCN5A* variants to the protein topology

Cardiac sodium channel is constituted by four domains (DI to DIV), each of them consisting of six transmembrane segments (S1 to S6), which are interconnected by extracellulat and cytoplasmic loops. Of the 241 cases whose *SCN5A* mutations were localized to one of the 4 transmembrane-spanning regions, 80 (33.2%) localized to either DI S1-S4, DII S1-S4, DIII S1-S4, or DIV S1-S4 and 161 (66.8%) localized to the S5, P-loop, and S6 regions containing the pore and selectivity filter of the sodium channel (DI S5-S6, DII S5-S6, DIII S5-S6, or DIV S5-S6)

Adapted from van Hoeijen DA et al. Expert Opin Pharmacother. 2014;15:1875-87.

Figure 3: Freedom from major cardiac event according to *SCN5A* mutation functional effect

Occurrence of MCE also differed according to *SCN5A* mutation functional effect (p<0.0001)

Figure 4: Freedom from major cardiac event according to *SCN5A* mutation type

Mutation type did not associate with outcomes (p=0.52).

group_avec_palpitation
- Cardiac arrest
- Syncope
- Familial screening
- Palpitations

14,0%

15,8%

67,9%

2,3%

Figure 1

Figure 2

Nb at risk (free from CE in %+/-SD)

loss	178	142(85,2+/-2,7)	109(79,2+/-3,1)	53(67,2+/-4,3)
gain	87	62(75,8+/-4,6)	49(68,0+/-5,1)	28(51,2+/-5,9)
gain and loss	85	78(96,5+/-2,0)	70(94,0+/-2,6)	40(81,2+/-4,8)
unkwown	92	73(83,7+/-3,9)	58(75,3+/-4,6)	35(65,4+/-5,5)

Figure 3
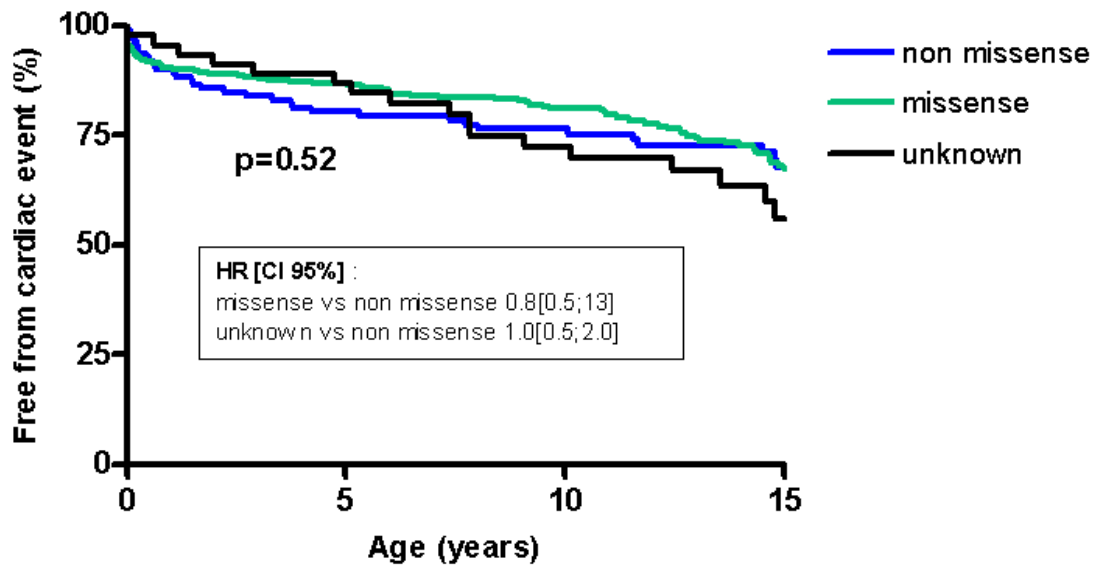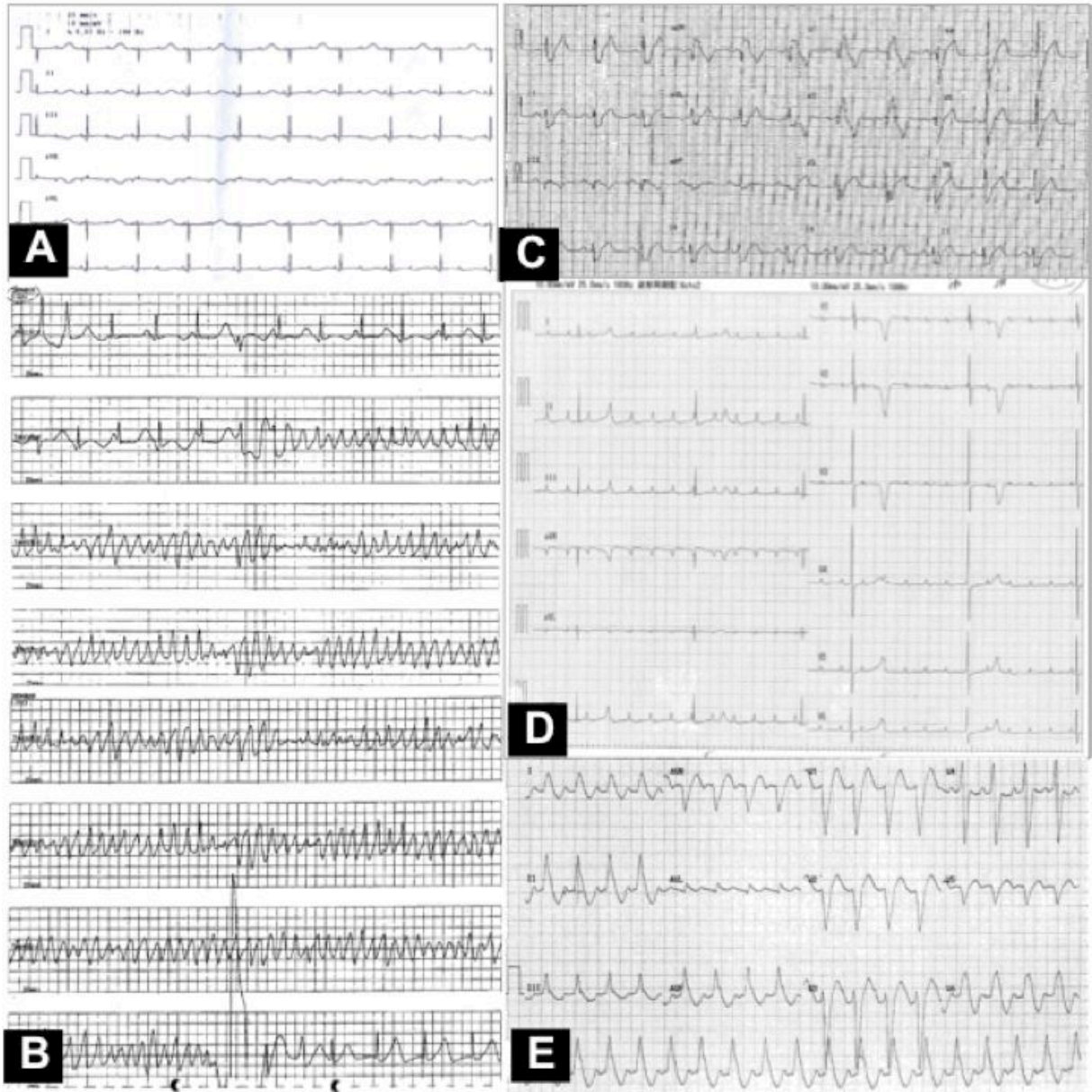
Figure 4

Figure 5

LIMITATIONS

The 25 years of data collected for this study represents a limitation, since clinical practice has evolved and considerable progress has been made in medical management of probands, screening of relatives and early cardiac pacing and/or ICD implantation. In addition, since patients were included from 25 tertiary, high-volume hospitals, young and/or symptomatic children were more likely to be included in the database, constituting a bias in inclusion. Data on genotype-positive adult relatives were not available.

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Baruteau AE et al: *SCN5A* Mutations in 442 Neonates and Children: Genotype-Phenotype Correlation and Identification of Higher-Risk Subgroups

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2 Correlation and Identification of Higher-Risk Subgroups

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5 **ABSTRACT -** 248 words

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8

9 **Aims:** To clarify the clinical characteristics and outcomes of children with *SCN5A*-mediated

10 disease and to improve their risk stratification.

11

12 **Methods and Results:** A multicenter, international, retrospective cohort study was conducted

13 in 25 tertiary hospitals in 13 countries between 1990-2015. All patients ≤16 years of age

14 diagnosed with a genetically confirmed *SCN5A* mutation were included in the analysis. There

15 was no restriction made based on their clinical diagnosis.

16 A total of 442 children [55.7% boys, 40.3% probands, median age: 8.0 (IQR: 9.5) years] from

17 350 families were included; 67.9% were asymptomatic at diagnosis. Four main phenotypes

18 were identified: isolated progressive cardiac conduction disorders (25.6%), overlap phenotype

19 (15.6%), isolated long QT syndrome type 3 (10.6%), and isolated Brugada syndrome type 1

20 (1.8%); 44.3% had a negative ECG phenotype. During a median follow-up of 5.9 (IQR: 5.9)

21 years, 272 cardiac events occurred in 139 (31.5%) patients. Patients whose mutation localized

22 in the C-terminus had a lower risk. Compound genotype, both gain- and loss-of-function

23 *SCN5A* mutation, age ≤1 year at diagnosis in probands and age ≤1 year at diagnosis in non-

24 probands were independent predictors of cardiac event.

25

26 **Conclusion:** In this large pediatric cohort of *SCN5A* mutation-positive subjects, cardiac

27 conduction disorders were the most prevalent phenotype; cardiac events occurred in about one-

28 third of genotype-positive children and several independent risk factors were identified,

29 including age ≤1 year at diagnosis, compound mutation and mutation with both gain- and loss-

30 of-function.

31

34