**TITLE PAGE**

**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) and in frame indels; and variants of uncertain significance (VUS). Missense Variants were classified as pathogenic/likely pathogenic or VUS using generally accepted criteria:23 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 χ2 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, AV block, RBBB and SV arythmia. 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 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 were272 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 (Online Table 2). Inappropriate ICD shocks occured in 9 patients (12%; T wave oversensing in 7 patients, atrial fibrillation in 1, lead fracture in 1). The four ‘major’ ECG phenotypes at baseline developed as follows:

Isolated PCCD 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 <50bpm 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-chanel blocker challenge, all three patients maintained an isolated CCD 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 Online Table 3); 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, ≥2 recurrences in 5 patients). Three patients died from SCD and one required ECMO support and was then transplanted for intractable arrhythmias.

Isolated LQT3 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.

MCE occurred in 25 patients [53%, 5/25 (11%) ≤1 year of age, 1/25 (4%) on betablocker at the time of the event] (Online Table 4). 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 betablocker therapy (Online Table 5); three patients experienced several recurrences under a coprescription of betablocker and mexiletine. Seven ICD shocks (6 appropriate, 1 inappropriate) were delivered in 3/11 (27%) implanted patients. Six patients (13%) died throughout follow-up, three of them had experienced a MCE in the first year of life.

Isolated BrS1 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 5 remaining patients were asymptomatic and left untreated.

**Negative ECG phenotype patients.** 196 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 family history 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 betablocker. 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) yrs]. All but one were treated by betablocker 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 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 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 (500mg morning, 250mg afternoon, 500mg evening), leading to ICD implantation.

**Genetic characteristics.** The 442 *SCN5A* genotype-positive children had 185 independent *SCN5A* variants (Online Table 5). Three (0.7%) patients harbored 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 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 (Online Table 6). 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 Online Figure 2.

**Genotype- phenotype correlations.**

Mutation topological location (Online Table 7, Figure 2).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. No significant difference was found when comparing variants localized in S1-S4 to those localized in S5-S6 in the relevant 241 patients (Online Table 8).

Mutation functional effect (Online Table 9). 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%). 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 (Online Table 10).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). 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), 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) (Online Table 10). 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], proband status [HR (95%CI): 7.8(5.1-12.1), p<0.0001] (Figure 3), supraventricular tachycardia [HR (95%CI): 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 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] (Online Figure 3). Mutation type did not associate with outcomes (p=0.52) (Online Figure 4).

Five *SCN5A* mutations correlated with specific clinical characteristics (Online Table 11). 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 (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 BrS24,25 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 subjects28 and life-threatening events were higher among LQT3 men,29 our results are concordant with previous smaller pediatric reports24,30,31 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 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 PCCD3,12 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 greater impairment of INa 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.36 We found that the N-terminus domain, the DI-DIV region and the C-terminus domain were not overrepresented amongst the five main ECG phenotypes. No difference appeared when considering the 6 segments of the transmembrane domains. However, in a recent case/control study, Kapplinger et al. were able to identify regions of Nav1.5 associated with a high probability of pathogenicity in both BrS and LQT3.22 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 overrepresentation of LQT3-associated variants.22 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 pediatric multicenter international study26 and a recent multicenter series of 391 adult and pediatric LQT3 patients.27 However, the burden of events was higher than reported by other LQT3 or BrS series in the past.31,37,38 The rate of SCD or ACA in our cohort was 14%, similar to other recent reports on LQT3 patients26,27 but significantly higher than that reported in BrS children.24,31,39 This may reflect an overrepresentation 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.37,40,41 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 counseling young affected individuals and their families.

It is recognised that double *SCN5A* mutation carriers have a more severe phenotype with longer QTc intervals, a younger age at diagnosis and more CEs despite therapy.38

Schwartz et al. 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.41 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.42 Our results extend this observation to all pediatric *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.43 This is in keeping with the observation of *de novo* mutations in the *SCN5A* gene associated with early onset of sudden infant death.9,10,44 Our observation may therefore be due to a clustering of de novo mutations45 and *SCN5A* mutation-positive patients with no family history constitute a subgroup at high-risk of ACA and arrhythmic events and should be treated accordingly.

**CONCLUSIONS**

In this large pediatric 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.

**TAKE-HOME FIGURE AND ONE-SENTENCE SUMMARY**

**Take-home figure**



**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.

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**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 mutation, n (%) | 74 (24.4) | 39 (28.1) | reference | 1 |  |
| 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.