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Clinical characterization of the first Belgian *SCN5A* founder mutation cohort

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Aims We identified the first Belgian *SCN5A* founder mutation, c.4813 + 3_4813 + 6dupGGGT. To describe the clinical spectrum and disease severity associated with this mutation, clinical data of 101 *SCN5A* founder mutation carriers and 46 non-mutation carrying family members from 25 Belgian families were collected.

Methods and results The *SCN5A* founder mutation was confirmed by haplotype analysis. The clinical history and electrocardiographic parameters of the mutation carriers and their family members were gathered and compared. A cardiac electrical abnormality was observed in the majority (82%) of the mutation carriers. Cardiac conduction defects, defined as PR or QRS prolongation on electrocardiogram (ECG), were most frequent, occurring in 65% of the mutation carriers. Brugada syndrome (BrS) was the second most prevalent phenotype identified in 52%, followed by atrial dysrhythmia in 11%. Overall, 33% of tested mutation carriers had a normal sodium channel blocker test. Negative tests were more common in family members distantly related to the proband. Overall, 23% of the mutation carriers were symptomatic, with 8% displaying major adverse events. As many as 13% of the patients tested with a sodium blocker developed ventricular arrhythmia. One family member who did not carry the founder mutation was diagnosed with BrS.

Conclusion The high prevalence of symptoms and sensitivity to sodium channel blockers in our founder population highlights the adverse effect of the founder mutation on cardiac conduction. The large phenotypical heterogeneity, variable penetrance, and even non-segregation suggest that other genetic (and environmental) factors modify the disease expression, severity, and outcome in these families.

Keywords Brugada syndrome • *SCN5A* • Founder mutation • Sudden death • Cardiac conduction defects • Atrial dysrhythmia

Introduction

Sudden cardiac death (SCD) in the young (<40 years) is predominantly caused by inherited cardiac disorders that predispose to the development of life-threatening arrhythmias, such as ventricular tachycardia (VT) or ventricular fibrillation (VF). These disorders comprise cardiomyopathies and primary electrical disorders (PED) with disturbances in the electric currents underlying the cardiac action potential. Primary electrical disorders are characterized by phenotypic and genetic heterogeneity,

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What's new?

- We report the first Belgian *SCN5A* founder mutation in the literature.
- Our cohort manifests high phenotypical heterogeneity with cardiac conduction disorders, Brugada syndrome, and atrial dysrhythmia as the three main clinical entities.
- We observed a relationship between the outcomes of the sodium channel blocker test and the degree of relatedness to the probands, with higher incidence of positive tests observed in probands and their first-degree relatives.
- Seven (13%) of the mutation carriers tested with sodium blocker challenge developed ventricular tachycardia and/or ventricular fibrillation. Two of these patients received appropriate ICD shocks later on.
- Two of the youngest, most severely affected patients carried additional genetic variants, which likely contributed to the aggravated phenotype.
- In one family, there was an additional patient diagnosed with Brugada syndrome who did not carry the founder mutation, confirming the complex genetic architecture of Brugada syndrome.

diagnostic overlap, variable expressivity, and reduced penetrance, often observed within the same family.[1,2]

The *SCN5A* gene encodes the alpha subunit of the human voltage-gated Nav1.5 cardiac sodium channel.[3] This gene is implicated in several forms of PED, such as Brugada syndrome (BrS) and long QT syndrome (LQTS). Loss-of-function variants in *SCN5A* are the most frequent genetic abnormality in BrS, accounting for 15–20% of BrS patients. These *SCN5A* pathogenic variants lead to a decrease in the upstroke velocity of the action potential through reduction of the inward sodium current across the cardiomyocyte membrane. This is presumed to be the pathogenic mechanism underlying BrS as well as familial progressive cardiac conduction disorders (CCD), sick sinus syndrome (SSS), and atrial standstill and has been implicated in some forms of familial atrial fibrillation.[3–6] Strikingly, a unique *SCN5A* pathogenic variant can cause a phenotypical spectrum of sodium channel diseases in one family. This has been demonstrated in several families where a single *SCN5A* variant resulted in BrS, atrial fibrillation, SSS, CCD, or even LQTS in a founder population.[1,2,7,8]

Previously, we developed and applied the PED next-generation sequencing (NGS) gene panel for molecular diagnostic screening of patients suffering from inherited primary arrhythmias.[9] This PED MASTR Plus panel encompasses 51 genes associated with inheritable primary arrythmias. Initially, we identified a recurrent c.4813 + 3_4813 + 6dupGGGT pathogenic variant in *SCN5A*, in 8 of the 180 index BrS patients. This pathogenic variant has previously been characterized in two Western European families and one sporadic patient.[10,11] It interferes with the splice donor site region downstream of exon 27, which results in the activation of a cryptic splice site within exon 27 and leads to a deletion of 96 basepairs of exon 27 at mRNA and therefore an in-frame deletion of 32 amino acids at protein level. Parts of Segment 2 and 3 of transmembrane domain IV of the Nav1.5 channel are deleted, resulting in a loss-of-function of the Nav1.5 channel.

By performing genetic testing on additional BrS patients in our clinics, we were able to identify new mutation carriers from different families. In total, we collected 25 different families with this specific pathogenic variant. Haplotype analysis demonstrated that these 25 families share a common ancestor, constituting a large founder population of a previously proven pathogenic *SCN5A* variant. Such populations are specifically important as they provide the unique opportunity to study genetic and environmental modifiers. As these patients carry the identical *SCN5A* mutation, other factors are likely to modify the disease expression, severity, and outcome.

Further cascade screening identified 101 carriers of this founder mutation in total. Clinical data of these founder mutation carriers and 46 non-mutation carrying family members were collected to gain more insight in the various clinical presentations and disease severity in *SCN5A* founder mutation carriers. The diverse clinical spectrum of this founder population clearly demonstrates the impact and complexity of *SCN5A*-related disease, with features of phenotypical heterogeneity, variable expressivity and penetrance, compound mutations, and additional variants in different genes implicated in SCD.

Methods

Haplotype analysis

Genomic DNA was extracted from blood from 25 mutation carriers (at least one mutation carrier per family) for haplotyping. We used Map viewer (NCBI, Build GRCh37) to select 11 highly polymorphic short tandem repeat markers (D3S1561, D3S3623, D3S1298, D3S1260, D3S3521, D3S3527, D3S3522, D3S2319, D3S3687, D3S3559, and D3S3647), flanking both sides of the *SCN5A* c.4813 + 3_4813 + 6dupGGGT pathogenic variant and primers were designed using Primer3. Forward primers were labelled with 6-fluorescein amidite. PCR products were analysed on an ABI3130XL (Applied Biosystems) in the presence of an internal sizing standard (ROX). Amplicon sizes were determined using ABI GeneMapper software v3.7 (Applied Biosystems).

The age of the most recent common ancestor (MRCA) was estimated using the function relating the length of a shared haplotype to the number of generations since the MRCA, as previously described.[12] The calculations are based on the concept that the segment of identical haplotype around the original pathogenic variant gets gradually shortened by random recombination events on both sides, leaving in present-day descendants a shared haplotype whose length is a function of the number of generations elapsed since the original mutation. This length should be expressed in centiMorgan (cM), since the distance between two markers expressed in centiMorgan equals the probability (in %) of observing a recombination event between them.

Clinical evaluation

Through collaboration between three Belgian centres (Cardiogenetics Clinic, Antwerp University Hospital, the Department of Cardiovascular Diseases, University Hospitas of Leuven and the Heart Center Hasselt), we gathered the medical records of 101 founder mutation carriers of which 25 were probands. Generally, either the PED MASTR Plus Assay or the inheritable cardiac arrhythmia NGS gene panel from the University Hospital Leuven (comprising 71 genes associated with PED and cardiomyopathies)[13] was used for genetic testing in the probands, while family members were tested by Sanger sequencing.

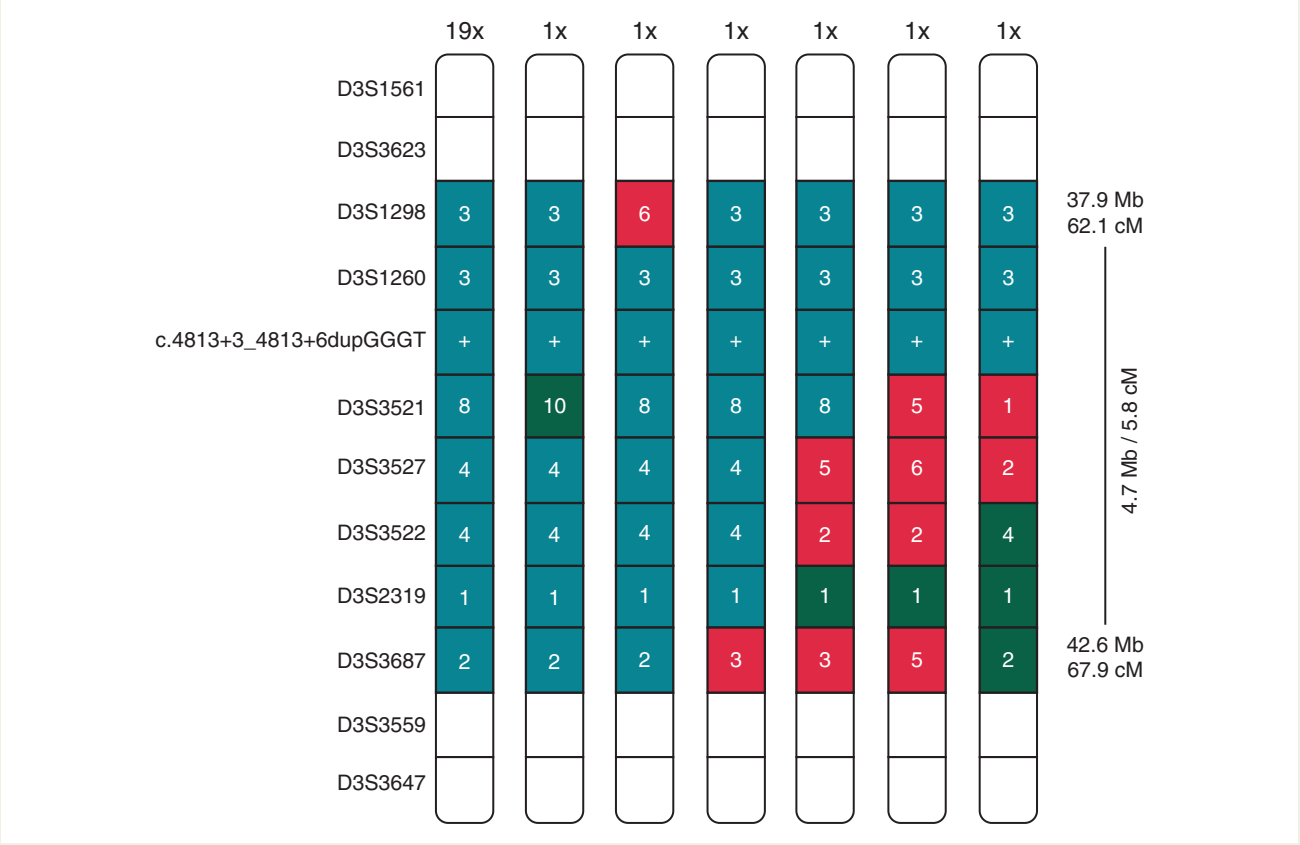

Figure 1 Haplotype analysis of 25 families. Shared haplotypes spanning seven markers derived from 25 probands of the 25 different families. From left to right: common haplotype found in 19 probands, haplotype containing a mutational event in marker D3S3521 found in one proband, 5 haplotypes of the 5 probands with recombination events.

Electrocardiographic data (resting 12-leads ECG) were available for 96 of the mutation carriers as well as 46 non-mutation carrying family members. The following ECG parameters were determined: PR interval, QRS duration and Bazett corrected QTc interval. Of the 101 mutation carriers, 55 underwent sodium blocker challenge using intravenous ajmaline ($n = 51$), procainamide ($n = 2$), or flecainide ($n = 2$), according to the consensus document.[14] Signal-averaged ECG (SAECG) data were available for 32 mutation carriers. Late potentials were considered positive when two of the standard SAECG criteria were met: filtered QRS duration >114 ms, terminal (40 ms) QRS root means square voltage <20 mcV, and high frequency low amplitude signal duration >37 ms.[15]

Patients were diagnosed with BrS if they demonstrated a Type 1 ECG-pattern spontaneously, during fever, or induced by a sodium channel blocker.[14] Patients with resting or Holter ECG prolongation of PR (>200 ms) or QRS (>120 ms) intervals were classified as CCD. Atrial dysrhythmia was defined as a current or past history of atrial fibrillation/flutter, atrial conduction abnormalities, and/or atrial bradyarrhythmia (sinus pause, atrial standstill, or symptomatic sinus bradycardia). Major adverse events (MAEs) were defined as sustained VT, sudden death/aborted cardiac arrest, an appropriate implantable cardioverter-defibrillator (ICD) shock, or atrial standstill.

Statistical analysis

Comparisons between groups were performed by the Mann–Whitney *U*-test with an alpha level of 0.05.

Results

SCN5A founder mutation

To investigate whether the *SCN5A* c.4813 + 3_4813 + 6dupGGGT pathogenic variant was inherited from a common founder, we haplotyped 11 markers flanking the pathogenic variant in 25 probands derived from the 25 families. We identified a shared haplotype spanning seven markers with a minimal region of 4.7 Mb or 5.8 cM (*Figure 1*).

Recombination had occurred in 5 of the 25 probands within the minimal region, while in 1 proband most likely a mutational event in marker D3S3521 had occurred. Based on the length of the region shared by 20 of the 25 families (5.8 cM) and the function of Marroni *et al.*, we estimated that the MRCA of these 20 families lived 20 generations ago [$x = (5.46 \times 27/5.8) - 5.46 = 19.957$]. Assuming that one generation spans 20 years, this is ~400 years ago. All identified families originated from one specific region in Belgium, 'de Kempen' (Campine) and most of them still live there (*Figure 2*).

Demographics and diagnostic spectrum

Of the 101 mutation carriers, 51 were female (*Table 1*). The mean age was 44 years ranging from 4 to 94 years. The initiation of cardiological follow-up of the mutation carriers ranged from 1996 to 2019. The average follow-up time for the patients was 3 years, ranging from

Figure 2 Map of residencies of the founder mutation families. Map of Belgium with Campine region in grey. Dots: residence of founder mutation families. The colour corresponds to the main diagnosis in the families.

Table 1 Demographic, clinical, and electrocardiographic data of mutation carriers 22 (28%)

	All mutation carriers ($n = 101$)	Asymptomatic ($n = 78$)	Syncope ($n = 15$)	MAE ($n = 8$)
Female	51 (51%)	42 (53%)	6 (40%)	3 (38%)
Age, mean (95% CI) (years)	44 (40–48)	44 (39–44)	42 (28–55)	46 (22–69)
No BrS/CCD	18 (18%)	16 (21%)	1 (7%)	1 (13%)
Isolated BrS	17 (17%)	13 (17%)	2 (13%)	2 (25%)
Isolated CCD	30 (30%)	26 (33%)	3 (20%)	1 (13%)
BrS and CCD	36 (36%)	23 (30%)	9 (60%)	4 (50%)
Spontaneous BrS ECG	22/101 (22%) (all carriers)[a] 22/53 (42%) (BrS patients)[a]	14 (18%)	5 (33%)	3 (38%)
BrS ECG induced by sodium blockers only	30/101 (30%) (all carriers) 30/53 (57%) (BrS patients)	3 (20%)	3 (38%)	
VT during sodium channel block	7 (7%)	2 (3%)	3 (20%)	2 (25%)
Mean PR (95% CI) (ms)	206 (196.6–215)	201.6 (191.3–212)	213.3 (193.8–232.8)	240.8 (180.3–301.4)
Mean QRS (95% CI) (ms)	115.7 (110.2–121.2)	114.3 (107.9–120.8)	117.9 (107.9–127.8)	127.3 (91.8–162.8)
Mean QTc (95% CI) (m, s)	417.5 (410.5–424.5)	414.3 (406.9–421.7)	418.3 (401–435.5)	455.3 (398.9–511.7)

BrS, Brugada syndrome; CCD, cardiac conduction defect; CI, confidence interval; ECG, electrocardiogram; MAE, major adverse events; VT, ventricular tachycardia.
[a]One patient had a fever-induced Type 1 BrS ECG.

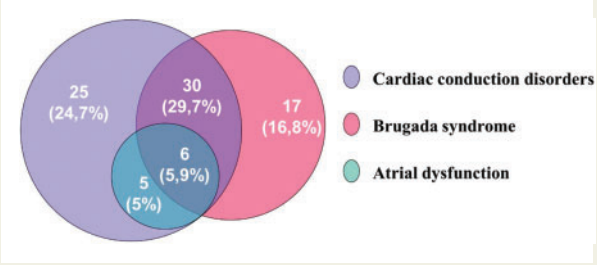

Figure 3 Diagnostic spectrum of the SCN5A c.4813 + 3_4813 + 6dupGGGT mutation carriers. Venn diagram displaying the diagnostic spectrum of the 101 SCN5A c.4813 + 3_4813 + 6dupGGGT mutation carriers, reflecting the absolute numbers (percentages between brackets) of mutation carriers with the diagnosis of cardiac conduction disorder, Brugada syndrome, and/or atrial dysfunction. One mutation carrier did not recieve clinical testing as he presented with SCD and seventeen mutation carriers displayed no cardiac abnormalities.

a single clinical visit to 18 years of follow-up. We identified a cardiac abnormality in the vast majority ($n = 83$; 82%) of the mutation carriers. The mutation carriers displayed a diagnostic spectrum of CCD ($n = 66$, 65%), BrS ($n = 53$, 52%), and atrial dysrhythmia ($n = 11$; 11%); 41 patients were diagnosed with more than one of these conditions (*Figure 3*). One of the mutation carriers could not receive clinical testing as he presented with SCD. Seventeen mutation carriers remained phenotype negative. Of the remaining patients, 13 did not receive ajmaline testing, because they were too young (<10 years old), because the test would not contribute to the therapeutic policy, or because they refused the test. The mean age of the phenotype negative founder mutation carriers was 39 years (range 5–96 years). The average follow-up time was 1 year.

Overall, mutation carriers without a cardiac diagnosis were younger than the patients with a diagnosis of CCD and/or BrS, with an average age of 39 years (range 5–96 years, 95% confidence interval 26–53) and 46 years (range 6–82 years, 95% confidence interval 41–50), respectively. The patients with CCD were also on average older (average age 47 years, range 6–82 years, 95% confidence interval 42–52 years) compared with those without CCD (average age 39, range 5–96 years 95% confidence interval 32–47 years). However, these differences were not significant (*P*-values of 0.278 and 0.053 and *U*-values of 814 and 1368 for cardiac diagnosis and CCD, respectively, with the Mann–Whitney *U*-test). This trend was not observed for BrS patients compared with those without BrS: average of 45 years (range 9–76 years, 95% confidence interval 40–50) and average of 44 years (range 5–96 years, 95% confidence interval 37–52), respectively.

The overall penetrance for BrS in the founder population was 53/101, with a penetrance of 55% for women and 50% for men (*Table 1*). When only the mutation carriers who either underwent sodium channel blocker testing or displayed a spontaneous BrS ECG pattern are taken into account, the observed penetrance amounts to 75% (53/71, *Table 1* and Supplementary material online), however this percentage is likely an overestimation as many untested individuals did not show a spontaneous ECG at follow-up and remained asymptomatic until old age.

Table 2 Demographic and electrocardiographic data of controls

	Controls ($n = 46$)
Female	23 (50%)
Age, mean (95% CI) (years)	42 (36–49)
Mean PR interval (95% CI) (ms)	155 (143.3–166.6)
Mean QRS interval (95% CI) (ms)	92.7 (88.2–97.2)
Mean QTc interval (95% CI) (ms)	418.8 (411.1–426.5)

CI, confidence interval.

Of the 55 patients undergoing sodium channel blocker testing, 7 (13%) individuals from 4 different families developed sustained VT and/or VF during provocation with ajmaline and were implanted with an ICD. Overall, the arrhythmia was unresponsive to cardioversion and only resolved after the effect of ajmaline wore out. The clinical and electrocardiographic characteristics of these seven mutation carriers did not differ significantly from those who did not develop arrhythmia (see Supplementary material online, *Table S1*). Two of them received appropriate ICD shocks later on.

In 18 of the 55 individuals (33%), the sodium blocker testing turned out to be non-diagnostic. The probands and 1st degree relatives of the probands had a higher incidence of positive sodium blocker challenge (88%) than the more distantly related family members (59%) ($P = 0.02$). Most of the mutation carriers with a non-diagnostic sodium blocker test (14/18, 78%) were diagnosed with CCD.

Of the 11 patients with atrial dysrhythmia, 5 were diagnosed with isolated atrial flutter/fibrillation, 3 with atrial bradyarrhythmias (1 with sinus pauses, 1 with atrial standstill, and 1 with sinus bradycardia and conduction abnormalities), and 3 with a combination of atrial flutter/fibrillation, 1st degree atrioventricular block and atrial bradyarrhythmias (sinus bradycardia, sinus pauses and sinus arrest).

Twenty-nine patients were implanted with an ICD, 5 of these patients had an isolated BrS phenotype and 19 had both BrS and CCD. Four only had a diagnosis of CCD. One of these patients had a negative ajmaline test, but displayed induced VT on electrophysiological testing and two presented with sudden syncopes. One patient was not diagnosed with BrS or CCD but had a history of multiple abrupt syncopes. Of the 22 patients with a spontaneous BrS ECG pattern, 9 (41%) received an ICD. Three BrS patients received appropriate ICD shocks whereas two other BrS patients received inappropriate therapy.

Symptoms and events

Twenty-three (23%) of the mutation carriers were symptomatic (syncope or MAE) (nine of these were probands) (*Table 1*). Eight of the mutation carriers (3 female, 5 male, age ranged from 2 to 76 years) experienced MAE. One patient, who carried the founder mutation and an additional likely pathogenic *de novo* variant in *SCN5A* (c.4711T>C or p. Phe1571Leu, transcript ID ENST00000333535) in trans, displayed both BrS and atrial dysfunction. He experienced continuous atrial standstill with two cerebral thrombo-embolic events, most likely secondary to bradyarrythmias, before the age of 3.[16] One patient presented with SCD at the age of 9. This patient carried both

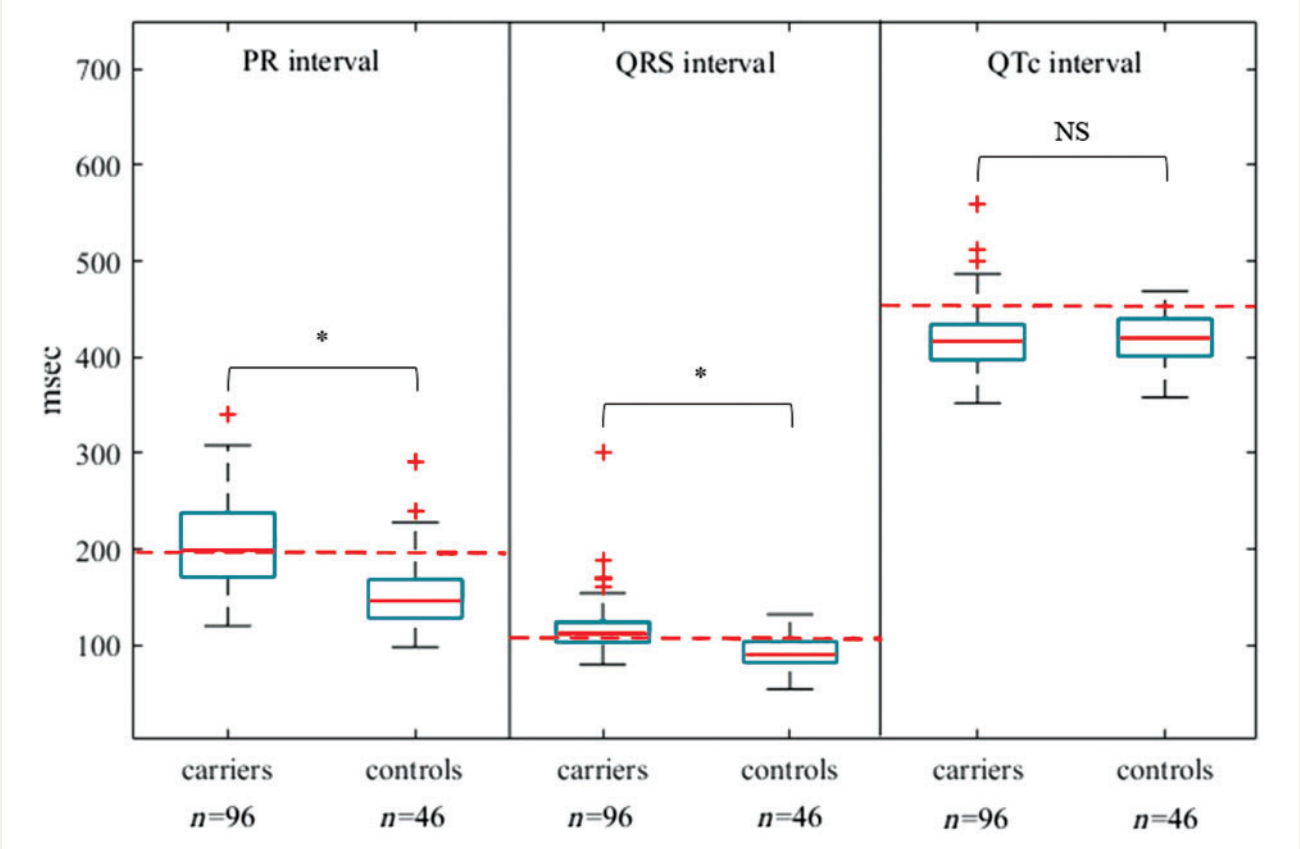

Figure 4 ECG parameters mutation carriers and controls. Box plots of the electrocardiographic data of the mutation carriers (n = 96) and the controls (negative family members, n = 46); Dashed red line: normal value in general population. '*' marks statistically significant ECG differences between the carrier and the control groups (P-value <0.001). ECG, electrocardiogram.

the maternally inherited founder *SCN5A* mutation and a paternal variant of uncertain significance in *TRPM4* (c.1744G>A or p. Gly582Ser, transcript ID ENST00000252826), a gene implicated in progressive familial heart block and BrS. The *TRPM4* variant was inherited from the father, who had a negative ajmaline test and only displayed atrioventricular conduction abnormalities. Therefore, it is likely that this variant aggravated the phenotype in view of an oligogenic disease architecture. Both the *SCN5A* c.4711T>C and the *TRPM4* c.1744G>A variants were detected by the PED MASTR Plus Assay.[9] The other MAEs encompassed one aborted sudden death (presenting symptom), two sustained VT and three appropriate ICD shocks. An additional 15 patients (6 female and 9 male) had a history of sudden syncopes without documented arrhythmia.

Within the group of the non-mutation carrying family members, one individual was diagnosed with BrS during the diagnostic work-up for sudden syncopes, based on a Type 1 ECG on ajmaline testing. No other pathogenic genetic variants were identified with the NGS-gene panel. The daughter of this patient presented with sudden syncope at the age of 4 years and showed non-diagnostic ECG abnormalities upon sodium blocker challenge (J-point elevation and pronounced QRS prolongation). Due to her young age, BrS could not be ruled out. Since both of them experienced sudden syncopes, they were treated with an ICD. They did not show either CCD or atrial dysrhythmia.

Electrocardiographic characteristics

As shown in *Table 2* and *Figure 4*, the PR interval in the mutation carrier group was significantly prolonged compared with the non-mutation carrying relatives [mean 206 ms (range 120–340 ms) vs. mean 155 ms (range 98–290 ms), $P < 0.001$]. The QRS duration was also significantly prolonged in the mutation carriers compared with the non-mutation carriers [mean 15.7 ms (range 80–300 ms) vs. mean 92.7 ms (range 54–132 ms), $P < 0.001$]. The QTc interval was similar in both groups.

Within the mutation carrier group, the symptomatic (syncope and/or MAE) mutation carriers had a prolonged average PR interval (222 vs. 202 ms), QRS (121 vs. 114 ms), and QTc (429 vs. 414 ms), compared with the asymptomatic mutation carriers, however, these differences were not statistically significant (*Figure 5*).

Signal-averaged electrocardiogram

Signal-averaged ECG was performed in 32 patients, 22 tested positive (69%). Of the 17 tested BrS patients, 11 were positive, as well as 17 of the 23 patients with CCD. Three mutation carriers with a normal phenotype were tested, two of these patients tested positive. Two of the patients with a positive SAECG experienced syncopes, none of them developed MAE.

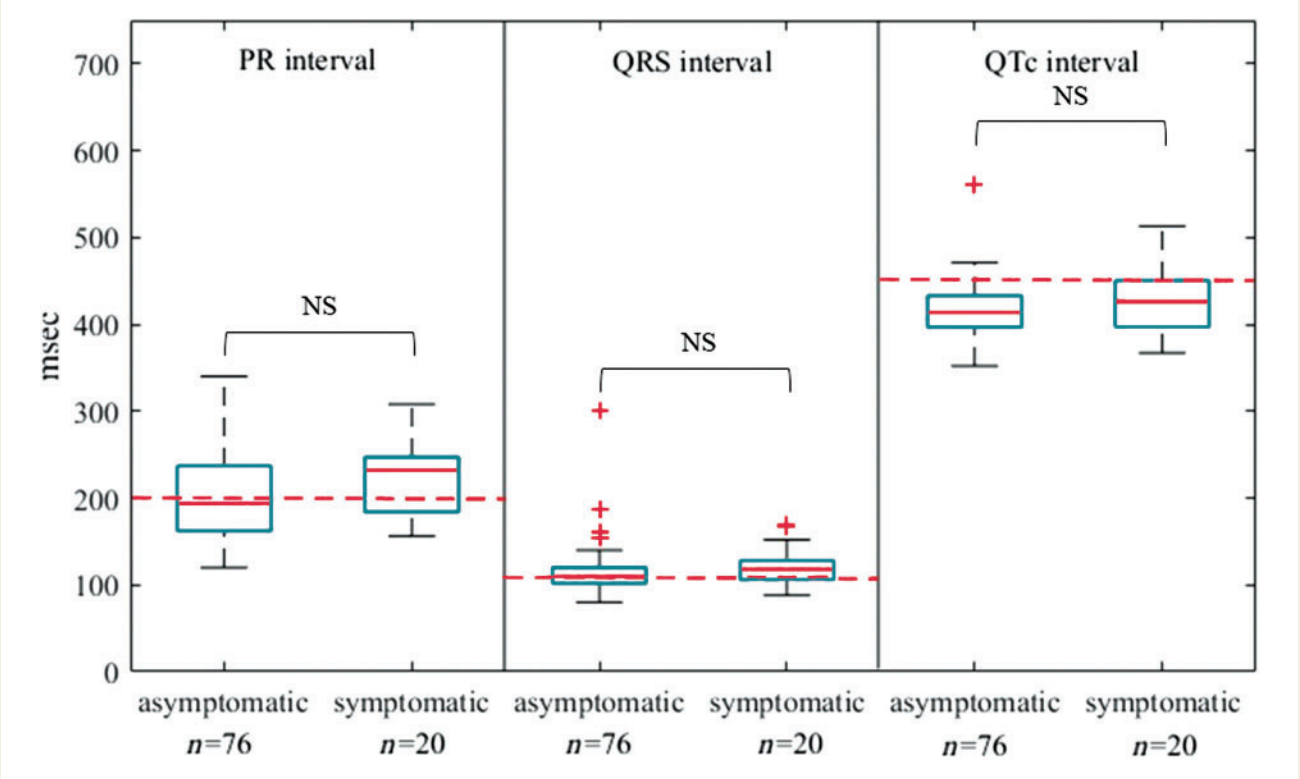

Figure 5 ECG parameters asymptomatic and symptomatic mutation carriers. Box plots of the electrocardiographic data of the symptomatic (n = 20) and asymptomatic (n = 76) mutation carriers; dashed red line: maximum normal value in general population. ECG, electrocardiogram.

Discussion

The *SCN5A* c.4813 + 3_4813 + 6dupGGGT intronic pathogenic variant causing a phenotypical spectrum of CCD, BrS, and atrial dysrhythmia is the first founder mutation related to inherited cardiac disorders described in Belgium. Our patient population is also the second largest *SCN5A* founder population described in the literature and the only *SCN5A* founder mutation not associated with LQTS3. Two different *SCN5A* founder mutations (c.4850_4852delTCT or p. Phe1617del[1] and c.5537insTGA or p. 1795insAsp[1,2,8]) have been described in the Netherlands, displaying a spectrum of phenotypes, ranging from CCD, BrS to LQTS3 (*Table 3*). Founder mutations have also been described for other cardiogenetic disorders, e.g. for hypertrophic cardiomyopathy in the Netherlands (p.Trp792fsX17, p. Pro955fsX95, and p. Arg943X in the *MYBPC3* gene), LQTS in Finland (Gly589Asp in the *KCNQ1* gene and Arg176Trp in the *KCNH2* gene), and LQTS in South Africa (p.Ala341Val in the *KCNQ1* gene).[17–19]

Our study shares some similarities with previously described patient populations with pathogenic variants in *SCN5A* (*Table 3*). In all studies, patients presented with large phenotypical heterogeneity. Especially conduction abnormalities, manifesting as prolongations in PR and QRS durations, are highly prevalent. In fact, the mean PR interval in this founder population exceeds the maximum value for the general population. We observed a trend towards an even more pronounced prolongation of the conduction parameters in symptomatic mutation carrying patients; however, this finding was not significant, possibly due to the small sample size of this subgroup.

The penetrance for BrS appears to be variable in the different populations. In our founder population, the 52% penetrance for BrS, detected by either a spontaneous Type 1 BrS ECG pattern or after provocation by sodium channel blockers, is lower than previously reported for *SCN5A* loss-of-function mutations (*Table 3*).[1,20] This lower penetrance might be influenced by a lack of sodium channel blocker testing in a part of our mutation carrier group, as evidenced by the increase in penetrance for BrS to 75% when only the mutation carriers who either underwent sodium channel blocker testing or displayed a spontaneous BrS ECG pattern are taken into account (*Table 1*). The percentage of patients who underwent sodium channel blocker testing in our mutation carrier group is lower than reported in other populations with *SCN5A* mutations (70% in our population, compared with 77%[20] and 90%[21] in prior publications). In the group of patients tested with sodium channel blockers, 67% developed a Type 1 BrS ECG. This is in line with previous studies showing that 49–80% of *SCN5A* mutation carriers develop Type 1 BrS ECG after sodium blocking challenge (*Table 3*).[20,21]

Interestingly, the sensitivity of the sodium channel blocker test was significantly higher for the probands and their first-degree relatives than the rest of the founder mutation carriers (respectively, 85% and 59%). As the probands are often more severely affected, it seems probable that they carry additional aggravating genetic modifiers, which can be shared by their first-degree relatives. As such they are also more likely to develop BrS and to demonstrate sensitivity to sodium blockade. This phenomenon was not examined in the previous studies.[1,2,20,21] This factor may also have contributed to the variability

Table 3 Comparison of clinical characteristics in studies of families with *SCN5A* mutations

Article	Ter Bekke *et al.*[1]	Postema *et al.*[2]	Hong *et al.*[21]	Probst *et al.*[20]	Our founder population
Mutation (number of mutation carriers included in study)	SCN5A c.4850_4852delTCT, p. Phe1617del (founder, 45)	c.5537insTGA, p.1795insAsp (founder, 149)	IV27S + 7insGGG[13] p.Arg367His[18] p.Arg769Cys[22] p.Thr1620Met[8]	c.612-2A>G[6] p.Gly1408Arg[14],[a] p.Arg225Trp[11],[a] p.Asn1722Asp[9] c.3963 + 2T>C[10] p.Ser1382Ile[9] p.Leu839Pro[5] p.Gly752Arg[6],[a] p.Ala665GlyfsX16[9] p.Arg535X7[5] p.1570-Phe1571insIle[11] p.Glu161Lys[11],[a]	c.4813 + 3_4813 + 6dupGGGT (founder, 101)
All BrS	11%	BrS phenotype present	79%	61%	52%
All CCD	33%	CCD phenotype present	Not mentioned	23% 1st degree AV block, 20% RBBB	65%
BrS +/CCD −	4%	Not mentioned	Not mentioned	Not mentioned	17%
CCD +/BrS −	27%	Not mentioned	Not mentioned	Not mentioned	30%
BrS+/CCD +	7%	Not mentioned	Not mentioned	Not mentioned	36%
LQTS	62%	Yes	Not mentioned	Not mentioned	0%
Normal	18%	Not mentioned	Not mentioned	Not mentioned	17%
Positive Na+ challenge	Not mentioned	Not mentioned	80% (28/35)	49%	67%
Symptoms	56%	Not mentioned	Not mentioned	Not mentioned	23%
MAE	20% (VT/Vfib/SCD)	Yes (SCD not in BrS patients)	Not mentioned	Not mentioned	8%
Affected non carrier family members[b]	0% BrS 38% CCD 8% LQTS	Not mentioned	6% (2/36) BrS All diagnosed on ajmaline testing	12% BrS (8/66) 3/8 spontaneous BrS ECG, 5/8 diagnosed on ajmaline testing	6% BrS (1/18) All diagnosed on ajmaline testing
VT/Vfib on Na+ challenge	Not mentioned	Not mentioned	Not mentioned	Not mentioned	13%
Number of mutation carriers	45	149	61	115	101

AV, atrioventricular; BrS, Brugada syndrome; CCD, cardiac conduction defects; ECG, electrocardiogram; LQTS, long QT syndrome; MAE, major adverse events; RBBB, right bundle branch block; SCD, sudden cardiac death; VT, ventricular tachycardia; Vfib, ventricular fibrillation.
[a]Variants that have been functionally tested. The other variants in this study are either frameshits or mutation carriers have, on average, longer PR intervals, and QRS durations than noncarriers.
[b]Calculated as the percentage of non-carriers diagnosed with BrS (either by a spontaneous BrS ECG or by a positive sodium blocker challenge) divided by the sum of non-carriers who underwent sodium blocker challenge or demonstrated a spontaneous BrS ECG.

in the previously reported sensitivity of ajmaline testing for the diagnosis of BrS, as well as the lower penetrance of BrS in our study.

Another observation in studies on large families with *SCN5A* pathogenic variants is non-segregation. Brugada syndrome was diagnosed in 6–12% of non-carrier family members who either displayed a spontaneous BrS ECG or were tested by sodium blocker challenge (*Table 3*).[20,21] This finding aligns well with the recent insights on the genetic architecture of BrS. While in the past it was assumed that BrS segregates as a Mendelian disease, it has since become clear that the inheritance of BrS is not purely monogenic. Although the *SCN5A* gene clearly plays a major role in the pathogenesis of BrS, modifier genes are also being increasingly recognized as important contributors to the phenotype.[22] It is therefore possible that a family member of a BrS patient would be more at risk to manifest BrS than the general population, even though they might not carry the presumed causal familial mutation. This elevated risk could be due to the contribution of harmful modifiers in other genes.

Our founder mutation population displays some unique characteristics. As many as 7 (13%) of the 55 patients tested with a sodium channel blocker developed ventricular arrhythmia during the course of the test, which is higher than the 2.4% found in the literature (tested in a population with previously unknown genotypes).[23] Two of the seven patients received appropriate ICD shocks later on. The prognostic value of ventricular arrhythmia following sodium channel

blockade is not clear. Previously, it has been speculated that the induction of ventricular arrhythmia by ajmaline testing does not affect the prognosis.[23] In a recent study, Ueoka *et al.* found that ventricular arrhythmia following sodium channel blockade with pilsicainide can be of prognostic value for BrS patients. However, the majority of the patients in this study displayed a spontaneous BrS Type 1 ECG pattern prior to testing, which was only the case for one of the seven patients in our founder population.[24]

Little is known about the relationship of the genotype to the development of ventricular arrhythmia during sodium channel blockade. There is no suggestion of this phenomenon in previous studies of families with *SCN5A* pathogenic variants (*Table 3*). Because of the development of these potentially life-threatening arrhythmias in our own population we recommend caution in performing sodium channel blocker challenge in carriers of this founder mutation. Our findings suggest that the development of arrhythmia upon ajmaline testing could be a marker of unfavourable disease expressivity and therefore may be a predictor of risk in our population. More studies with longer follow-up will be needed to explore whether this is applicable to other subtypes of *SCN5A* mutations.

Considering the rather limited follow-up time in our study (on average 3 years, 259 patient years), there was a high incidence of symptoms (23% of the mutation carriers), with 8% of the mutation carriers displaying MAEs, although additional genetic variants likely contributed to the severe phenotype in the two youngest individuals. These findings, together with the occurrence of ventricular arrhythmia following sodium blocker testing, highlight the malignant nature of the founder mutation. According to the experiments described by Rossenbacker *et al.*,[11] the founder mutation can give rise to four different splice effects: the main transcript with an in-frame deletion of 96 base pairs, two different out-of-frame insertions of intronic sequences, and (rarely) the wild-type splicing product. It is possible that the ratio between these splicing products in individual patients is partly responsible for the variability in the phenotype and disease severity observed in our patient population.

Conclusions

The high prevalence of symptoms and sensitivity to sodium channel blockers in our founder population highlights the adverse effect of the founder mutation on cardiac conduction. The large phenotypical heterogeneity, variable penetrance, and even non-segregation suggest that other genetic (and environmental) factors modify the disease expression, severity, and outcome in these families. Our ultimate aim is to make use of this unique founder population to identify genetic modifiers of BrS to develop combined clinical and genetic risk stratification models as well as to identify novel therapeutic targets applicable in the clinical setting. Further study of this founder population will hopefully help achieve those goals.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

Data availability

The data underlying this article are available in the supplementary material.

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Pneumothorax post-pacemaker implantation: the novelty of Heimlich valve

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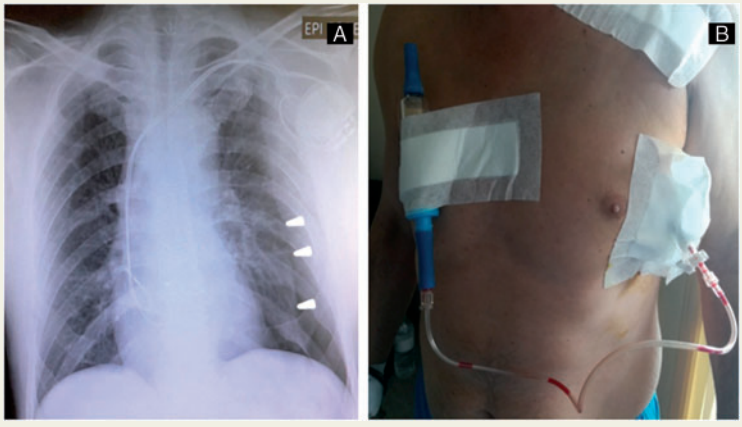

A 58-year-old male was referred for management of symptomatic bradycardia. The implantation of a permanent pacemaker was complicated by a haemodynamically significant left-sided pneumothorax (*Panel A*). An 8 Fr–16 cm radiopaque catheter-drain (Pneumothorax Kit, Arrow, Teleflex Medical, Ireland) was inserted into the left pleural cavity (over-the-needle technique) through the fourth intercostal space at the anterior-axillary line. The procedure was performed under ultrasound guidance, and resulted in patient's prompt clinical/haemodynamic improvement. The external end of the drain was connected to a Heimlich one-way valve (HV), through which air was expelled from the pleural space during expiration but was prevented from re-entering during the next inspiration. This drainage-system permitted the early ambulation of the patient (*Panel B*). A few hours later, air leakage (indicated by movement of the rubber tube of HV during exhalation/coughing) was not further detectable, and the left lung was totally re-expanded. The next morning, the system was removed and the patient was discharged.

Our report may suggest that iatrogenic pneumothorax post-pacemaker implantation can be easily and safely managed by the HV drain-technique. Cardiologists dealing with implantable devices should be familiar with this alternative method, as it can minimize patients' discomfort, inclination, length-of-stay, and cost-of-care.

The full-length version of this report can be viewed at: https://www.escardio.org/Education/E-Learning/Clinical-cases/Electrophysiology.