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Systematic analysis of SCN5A variants associated with inherited cardiac diseases

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Keywords:

SCN5A; domains; overlap syndrome; phenotypes; pleiotropy.

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Abstract

Background:

SCN5A variants are associated with a spectrum of cardiac electrical disorders with clear phenotypes. However, they may also be associated with complex phenotypic traits like overlap syndromes, or pleiotropy, which have not been systematically described. Additionally, the involvement of SCN5A in dilated cardiomyopathies (DCM) remains controversial.

Objective:

We aimed to (1) evaluate the different phenotypes associated with pathogenic (P)/likely pathogenic (LP) SCN5A variants and (2) determine the prevalence of pleiotropy in a large multicentric cohort of P/LP SCN5A variant carriers.

Methods:

The DNA of 13,510 consecutive probands (9960 with cardiomyopathies) was sequenced using a custom panel of genes. Individuals carrying a heterozygous single P/LP SCN5A variant were selected and phenotyped.

Results:

The study included 170 P/LP variants found in 495 patients. Among them, 119 (70%) were exclusively associated with a single well-established phenotype: 91 with Brugada syndrome, 15 with type 3 long QT syndrome, six with progressive cardiac conduction disease, four with multifocal ectopic Purkinje-related premature contraction, and three with sick sinus syndrome. Thirty-two variants (19%) were associated with overlap syndromes and/or pleiotropy. The 19 remaining variants (11%) were associated with atypical or unclear phenotypes. Among those, eight were carried by eight patients presenting with DCM with a debatable causative genotype/phenotype link.

Conclusion:

Most P/LP SCN5A variants were found in patients with primary electrical disorders, mainly Brugada syndrome. Nearly 20% were associated with overlap syndromes or pleiotropy, underscoring the need for comprehensive phenotypic evaluation. The concept of SCN5A variants causing DCM is extremely rare (8/9960), if not questionable.

Introduction

The SCN5A gene encodes the alpha subunit of the main cardiac sodium channel Nav1.5. This channel is predominant for the inward sodium current and plays a critical role in the regulation of cardiac electrophysiologic function.

Pathogenic (P)/likely pathogenic (LP) variants of SCN5A have been shown to be associated with a spectrum of cardiac electrical disorders, including type 3 long QT syndrome (LQT3), Brugada syndrome (BrS), progressive cardiac conduction disorders (PCCDs), atrial standstill and sick sinus syndrome (SSS), familial atrial fibrillation (AF), multifocal ectopic Purkinje-related premature contractions (MEPPC), and sudden death syndromes. However, the involvement of SCN5A in cardiomyopathies, including dilated cardiomyopathy (DCM), is still debated.¹

BrS-related SCN5A variants are mostly loss-of-function variants.^{2,3} All studies on these variants have shown that BrS associated P/LP variants affect various regions throughout the structure of the channel and that the pore is a hot spot.^{2–6} In addition, transmembrane regions⁶ (in particular, the voltage-sensing domain [VSD]² and extracellular loops⁵) and the interdomain linkers (IDLs) DI–DII, DIII–DIV,³ and DIII³ have been proposed as hot spots in several studies. Transmembrane regions are enriched in rare variants.⁶ LQT3 is caused by gain-of-function variants in SCN5A. The IDLs have been identified as hot spots for LQT3-related SCN5A variants in various studies,⁷ in particular DIII–DIV.^{3,5} However, such variants have also been identified in the pore,^{5,8} segment 4 (S4),³ C-terminal region,³ and S4–S5⁵ MEPPC variants are almost exclusively located in or near S4 of VSDs.⁹

It is well known that SCN5A variants can lead to overlap syndromes, that is, associated phenotypes in the same individual, such as cardiac conduction disorders and BrS. However, only a few studies have focused on pleiotropy associated with SCN5A variants, that is, the association of distinct phenotypes among different index cases (from different families) or individuals of the same family for the same variant, and its frequency.^{10–13}

Here, we studied a large French multicentric cohort of consecutive patients referred for inherited cardiac diseases who carry a P/LP variant of the SCN5A gene. The objectives were to examine the phenotypes associated with P/LP SCN5A variants, in particular according to their localization, and to establish the frequency of interfamilial and intrafamilial pleiotropy.

Methods

Study design and population

We considered consecutive patients with cardiomyopathies or inherited cardiac arrhythmias referred from 2015 to 2023 for genetic testing at 3 tertiary university hospitals, all members of the Cardiogen French national network for inherited cardiac diseases: Bichat University Hospital (Paris, France), Pitié-Salpêtrière University Hospital (Paris, France), and Amiens-Picardie University Hospital (Amiens, France). Patients originated from 15 hospitals throughout the French territory. We included all probands (defined as the first patient in a

family diagnosed with an SCN5A-mediated cardiac disease) and their relatives carrying a P/LP variant of the SCN5A gene.

The exclusion criteria were patients carrying a second P/LP variant in another gene (ie, double heterozygous) and their relatives to avoid heterogeneity in the phenotypes, patients carrying a second P/LP variant in the SCN5A gene, absence of clinical data, and refusal of the patient to participate. All patients or, for children, authorized family members provided written informed consent for genetic testing and the use of genetic data for research purposes. The study protocol complied with the ethical principles of the 1975 Declaration of Helsinki and its subsequent amendments. Following the regulations on routine clinical practice studies in France, the study protocol obtained approval from the clinical research unit (at Amiens-Picardie University Hospital). Furthermore, the study database was anonymized and registered with the French National Data Protection Commission (Commission nationale de l'informatique et des libertés [Paris, France]; reference No. PI2022_843_0059).

Genetic analysis and variant interpretation

Genetic analysis and variant interpretation are detailed in the Supplemental Methods.

Evaluation of phenotype at time of diagnosis

We analyzed the patients' clinical data, including their personal and family medical histories. Data from all cardiac examinations performed at the time of diagnosis were collected by reviewing the medical records: 12-lead electrocardiogram, sodium channel blocker challenge, transthoracic echocardiography, cardiac magnetic resonance imaging, and electrophysiologic studies. We collected data on age, sex, date and mode of diagnosis, electrocardiographic data, right and left ventricular dimensions and functional indices, and presence of late gadolinium enhancement. Phenotypic evaluation was not standardized but left to the discretion of the treating cardiologist.

Definitions used to diagnose conduction disorders, BrS, LQT3, PCCD, MEPPC syndrome, DCM, hypertrophic cardiomyopathy, sudden cardiac death, sudden infant death syndrome, and idiopathic ventricular fibrillation are detailed in the Supplemental Methods.

Pleiotropy and overlap syndromes

We defined interfamilial pleiotropy as a single variant causing distinct phenotypes in different probands. Intrafamilial pleiotropy was defined as variants associated with different phenotypes in at least 1 relative compared with the proband. For this analysis, we considered the following phenotypes: BrS, LQT3, PCCD, MEPPC, SSS, AF, and overlap syndrome. Overlap syndromes were defined as the coexistence of at least 2 distinct phenotypes among BrS, LQT3, PCCD, and SSS within the same individual.

Results

Study population

In total, 3550 probands with a primary electrical disorder and 9960 with cardiomyopathy underwent genetic screening including that for SCN5A. Among them, 362 SCN5A variants were detected and initially classified as P/LP or of unknown significance (Supplemental Figure 1). After review of the variants, 156 were considered to be benign or of unknown significance and 206 P or LP. Twenty-four were associated with another P/LP variant and were therefore excluded, and 12 were excluded because of the absence of clinical data. In total, 170 P/LP variants (found in 495 patients) were included in the study (Supplemental Table 1).

SCN5A variants leading to well-established single phenotypes

In total, 119 variants (70%) were exclusively associated with a single well-established phenotype (Figure 1) in 239 patients: 91 with BrS (166 patients), 15 with LQT3 (31 patients), 6 with PCCD (16 patients), 4 with MEPPC syndrome (19 patients), and 3 with SSS (7 patients). On a review of the phenotypes in both probands and relatives, no evidence of overlap syndrome or pleiotropy was observed for these variants.

Ninety-one variants were associated with BrS and distributed among 109 probands and in 30 of their relatives. Of these 139 patients, 72 had a spontaneous type 1 electrocardiogram and therefore a class I diagnosis of BrS. Of the remaining 67 patients, without type 1 and with a positive ajmaline test response, 2 also had a class I diagnosis (personal history of cardiac arrest) and 28 had a class IIa diagnosis (personal history of presumed arrhythmic syncope or family history of definite BrS). The remaining 37 patients had a class IIb diagnosis of BrS. We also counted 27 (47%) relative carriers with no BrS phenotype: 8 from 7 families (7 different variants) with a negative result on ajmaline challenge and 19 patients (13 families, 12 different variants) without a provocation test. Of the BrS-associated variants, most were missense ($n = 61$ [67%]; Table 1), with half ($n=30$ [49%]) located in the extracellular loops, 18 (29.5%) in the pore domain, and 9 (15%) in the voltage sensor (Figure 2). Twenty-six (28%) of the BrS associated SCN5A variants were truncating variants.

Fifteen variants were associated with LQT3 (20 probands, 2 relatives): 14 (93%) missense variants and 1 in-frame deletion. Of these 15 variants, 9 (60%) were located in a transmembrane domain (of which 5 variants were located in S4–S5 [33%] and 4 [27%] in the pore), and 4 (27%) were located in the DIII–DIV IDL. None were found in the voltage sensor. In addition to the 2 relatives with LQT3, 9 relatives from 5 families had concealed LQT3.

Six variants were associated with PCCD only (6 probands, 3 relatives), including 3 truncating variants and 3 missense variants. Two of the missense variants were located in the pore domain and the others in the voltage sensor. In addition to the 3 relatives with a PCCD, 5 relatives from 3 families had a normal electrocardiography recording and 2 relatives from 2 families showed conduction disturbances that were insufficient to make a diagnosis of PCCD.

Four variants, all missense, were associated with MEPPC syndrome, distributed between 11 probands and 7 relatives. Three were located in the voltage sensor and 1 in the C terminus. In

addition to the 7 relatives with MEPPC syndrome, 1 relative presented with a healthy phenotype.

Three variants (carried by 3 probands), 2 missense and 1 truncating variant, were associated with SSS only. The 3 relatives, from 2 families, carrying 2 different SSS-related variants, were healthy carriers.

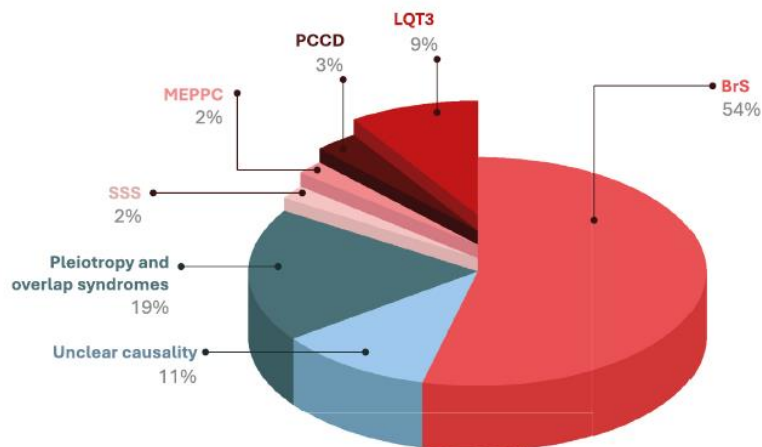


Figure 1 Distribution of the phenotypes in relation to the 170 variants. Red: Brugada syndrome (BrS); dark red: type 3 long QT syndrome (LQT3); burgundy: progressive cardiac conductive disorder (PCCD); light red: multiple ectopic Purkinje-related premature contractions (MEPPC); pink: sick sinus syndrome (SSS); gray: pleiotropy and overlap syndrome; light blue: unclear causality.

SCN5A variants leading to pleiotropy and overlap syndrome in primary electrical disorder phenotypes

In total, 32 SCN5A variants (19%) were associated with overlap syndromes or pleiotropy. Nine variants, including 7 (78%) truncating variants carried by 9 probands and 1 relative, led to overlap syndromes (ie, a mixed phenotype within a single individual). Twenty-three variants were associated with intrafamilial pleiotropy (n = 10) or interfamilial pleiotropy (n = 16), with 1 variant leading to different distinct phenotypes (BrS, LQT3, PCCD, MEPPC, SSS, AF, overlap syndrome) among the probands or their relatives (Supplemental Table 2). We identified 2 clusters of pleiotropic missense variants: S5–S6 (n = 8 variants) and the C terminus (n = 4 variants).

Of patients with overlap syndromes, the most frequent phenotypic associations were BrS1PCCD (n = 11 patients) and BrS1SSS (n = 7 patients), followed by BrS1LQT3 (n = 5 patients).

The number of index cases and relatives who underwent sodium blocker challenge, transthoracic echocardiography, and cardiac magnetic resonance imaging is presented according to the final diagnosis retained in Supplemental Tables 3 and 4, respectively. Only 77 relatives underwent a sodium blocker challenge, suggesting that the rate of overlap syndrome and pleiotropy may be higher than that observed herein.

SCN5A variants associated with other phenotypes

The 19 remaining SCN5A P/LP variants were associated with unclear or atypical phenotypes. Eight SCN5A variants were carried by at least 1 patient with idiopathic ventricular fibrillation/ sudden death. Sodium channel blocker challenge was performed on 3 patients and showed no BrS or premature ventricular contraction. Four of these variants were also found in another patient with BrS.

Eight variants were carried by at least 1 patient presenting with DCM (Supplemental Table 5). The p.(Ala385Thr) and p.(Gln1695His) variants were found in patients with secondary DCM, resulting from massive ventricular hyperexcitability and extensive myocarditis, respectively. These cases are detailed in the Supplemental Results. The third variant, p.(Gln1491Arg), was found in a patient with AF-induced DCM. Indeed, left ventricular ejection fraction recovered after successful AF ablation. The 5 other variants were carried by at least 1 patient (4 probands, 1 relative) with DCM in association with PCCD. These 5 variants were also found in 3 probands with isolated BrS, 2 relatives with isolated PCCD, and 1 proband presenting with overlap syndrome with BrS and PCCD.

One variant, p.(Gly1262Ser), was found in a proband presenting with hypertrophic cardiomyopathy, fortuitously diagnosed at 55 years of age. This case is detailed in the Supplemental Results.

Two truncating SCN5A variants were found in newborns with atypical phenotypes, detailed in the Supplemental Results.

Analysis of phenotypes according to variant functional effect and location

Of the 170 variants, 57 have been functionally studied in the literature. The 10 gain-of-function variants were identified in LQT3 patients, whereas the 47 loss-of-function variants were found in patients with BrS, PCCD, SSS, and an overlap between these phenotypes.

SCN5A variants located in the pore led to all primary electrical disease phenotypes (BrS, LQT3, PCCD, and SSS) except MEPPC syndrome. Only 2 variants located in the pore were in the selectivity filter, found in patients with BrS, SSS, and overlap syndrome phenotypes. Variants located in the VSD led to all phenotypes except LQT3. Most of the variants found in the inactivation gate region (IDL DIII–DIV; n = 10) were responsible for LQT3 (n 5 4). Only 1 variant, identified in a sudden cardiac death victim, was localized in the inactivation particle.

Truncating variants were found in patients presenting with BrS, PCCD, SSS, and overlap phenotypes and also led to pleiotropy.

Discussion

In this large multicenter registry of P/LP SCN5A variants, 70% of the variants led solely to primary electrical disorders (BrS, LQT3, PCCD, MEPPC, and SSS), BrS being the most frequent (n 5 91/170 [53.5%]). Conversely, 30% of P/LP SCN5A variants were associated with more complex phenotypes. Thirty-two (19%) variants led to overlap syndromes or pleiotropy. For 19 (12%) variants, the associated phenotype was atypical, and therefore the causality of the variant was unclear. Of such variants, 8 were found in patients with DCM, frequently in association with PCCD.

Table 1 Type of variant and location of missense and in-frame deletion variants leading to the principal phenotypes

	Variants leading to BrS (n = 91)	Variants leading to LQT3 (n = 15)	Variants leading to MEPPC (n = 4)
Mutation type			
Missense variants	61 (67.0)	14 (93.3)	4 (100)
Truncating variants	26 (28.5)	0 (0)	0 (0)
Frameshift	12	0	0
Nonsense	9	0	0
Splice (out of frame)	4	0	0
Synonymous	1	0	0
In-frame deletion	0	1 (6.6)	0
Splice	4 (4.5)	0	0
Mutation location, structural	(n = 61)	(n = 15)	(n = 4)
Transmembrane domain	51 (83.6)	9 (60.0)	3 (75.0)
DI	5 (8.2)	0 (0)	1 (25.0)
DIS1	0 (0)	0 (0)	0 (0)
DIS2	1 (1.6)	0 (0)	0 (0)
DIS3	0 (0)	0 (0)	0 (0)
DIS4	2 (3.3)	0 (0)	1 (25.0)
DIS5	0 (0)	0 (0)	0 (0)
DIS6	2 (3.3)	0 (0)	0 (0)
DII	3 (4.9)	2 (13.3)	2 (50.0)
DIIS1	0 (0)	0 (0)	0 (0)
DIIS2	0 (0)	0 (0)	0 (0)
DIIS3	0 (0)	0 (0)	0 (0)
DIIS4	2 (3.3)	0 (0)	2 (50.0)
DIIS5	0 (0)	1 (6.7)	0 (0)
DIIS6	1 (1.6)	1 (6.7)	0 (0)

DIII	6 (9.8)	0 (0)	0 (0)
DIIIS1	0 (0)	0 (0)	0 (0)
DIIIS2	2 (3.3)	0 (0)	0 (0)
DIIIS3	0 (0)	0 (0)	0 (0)
DIIIS4	1 (1.6)	0 (0)	0 (0)
DIIIS5	2 (3.3)	0 (0)	0 (0)
DIIIS6	1 (1.6)	0 (0)	0 (0)
DIV	3 (4.9)	2 (13.3)	0 (0)
DIVS1	0 (0)	0 (0)	0 (0)
DIVS2	0 (0)	0 (0)	0 (0)
DIVS3	0 (0)	0 (0)	0 (0)
DIVS4	1 (1.6)	0 (0)	0 (0)
DIVS5	2 (3.3)	0 (0)	0 (0)
DIVS6	0 (0)	2 (13.3)	0 (0)
Extracellular loops	30 (49.2)	0 (0)	0 (0)
S1S2	4 (6.6)	0 (0)	0 (0)
S3S4	0 (0)	0 (0)	0 (0)
S5S6	26 (42.6)	0 (0)	0 (0)
Intersegment linker	4 (6.6)	5 (33.3)	0 (0)
S2S3	0 (0)	0 (0)	0 (0)
S4S5	4 (6.6)	5 (33.3)	0 (0)
Cytoplasmic	10 (16.4)	6 (40.0)	1 (25.0)
N terminus	2 (3.3)	0 (0)	0 (0)
Interdomain linker	3 (4.9)	5 (33.3)	0 (0)
DIDII	0 (0)	1 (6.7)	0 (0)
DIIDIII	1 (1.6)	0 (0)	0 (0)
DIIIDIV	2 (3.3)	4 (26.7)	0 (0)
C terminus	5 (8.2)	1 (6.7)	1 (25.0)
Mutation location, functional			
Voltage-sensing domain (S1–S4)	9 (14.8)	0 (0)	3 (75)
Pore domain (S5-p loop-S6)	18 (29.5)	4 (26.7)	0 (0)
Selectivity filter	0 (0)	0 (0)	0 (0)

Values are reported as n (%).

BrS = Brugada syndrome; D = domain; LQT3 = type 3 long QT syndrome;

MEPPC = multiple ectopic Purkinje-related premature contractions;

S = segment.

Well-established phenotypes

Most of the SCN5A P/LP variants (53.5%) were carried only by patients with a pure phenotype of BrS. In our study, as in previously published studies, these variants were most commonly missense variants (67%). Kapplinger and associates² found 71% of variants in the transmembrane domains. Similarly, Walsh and coworkers⁶ also found enrichment in these regions. Our study yielded similar results as 84% of the identified missense variants were situated in the transmembrane regions. More specifically, the extracellular loops were the preferential localization of the BrS variants in our study (49.2%), in accordance with the findings of Li and colleagues.⁵ From a functional point of view, the pore region was a hot spot for variants in our study (29.5%) as well as in published studies.^{2–5} In addition to the pore, Kapplinger and associates² also proposed the VSD as a hot spot (31%), whereas we found only 15% of the BrS P/LP variants in the VSD. Contrary to previous findings that

identified the DI–DII and DIII–DIV IDLs as hot spots,³ our study did not yield similar results as only 5% of BrS variants were observed in the IDLs.

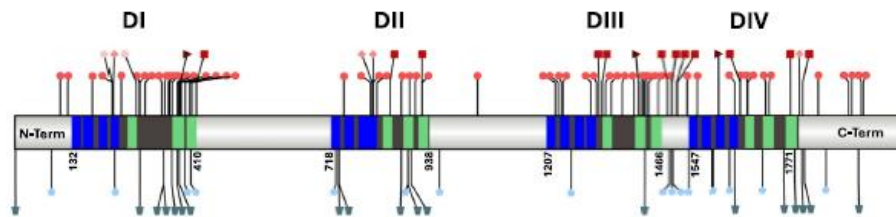


Figure 2 Representation of the missense variants along Nav1.5. Red circle: Brugada syndrome; dark red rectangle: type 3 long QT syndrome; burgundy triangle: progressive cardiac conductive disorder; light red rhombus: multiple ectopic Purkinje-related premature contractions; pink octagon: sick sinus syndrome; gray trapezoid: pleiotropy and overlap syndrome; light blue pentagon: unclear causality. The parts of the protein highlighted in blue represent the voltage-sensing domains, and the parts highlighted in green represent the pore domains. Extracellular loops were a hot spot for Brugada syndrome, whereas variants associated with type 3 long QT syndrome were mostly found in S4–S5 and the DIII–DIV interdomain linker. Multiple ectopic Purkinje related premature contraction syndrome was associated with specific variants, mostly located in the S4 domain. This figure was created with IBS 2.0.

We reported 15 variants exclusively associated with LQT3. IDLs have been identified as a hot spot for LQT3-related SCN5A variants in several studies,^{7,8} in particular the DIII–DIV IDL.^{3,5} Moreover, such variants have also been identified in the pore,^{5,8} S4,³ and C-terminal region.³ Li and colleagues⁵ found variants clustered within S4–S5 and S5 and S6 of DIII and DIV. In our study, we confirmed the preferential localization of variants leading to LQT3 in S4–S5 (n=55 [33%]) and the DIII–DIV IDL (n=54 [27%]). We also confirmed that LQT3 can be caused by variants in the pore (n=54 [27%]). We did not detect any variants in the VSD.

We found 6 variants exclusively associated with PCCD phenotypes. There are no published cohorts of P/LP SCN5A variant carriers with PCCD phenotypes. Clinical cases have shown such patients to be mainly carriers of truncating mutations^{14,15} or compound heterozygous/homozygous variants,¹⁶ although missense variants have also been reported.¹¹ Of the 6 variants reported here, 3 were truncating variants and 3 missense variants.

We found 4 variants associated with MEPPC syndrome. The phenotype was highly penetrant among relatives (7 of 8 affected relatives), as previously reported in the literature.¹ MEPPC syndrome is caused by variants that produce no change in inward sodium current density but an increased window current. The variants associated with MEPPC that have been reported are mostly located in or near S4 of DI and DII.⁹ In our cohort, we found patients with MEPPC carrying the already described p.(Arg222Gln) and p.(Arg814Trp) variants located in S4. We also report, for the first time, the p.(Leu812Pro) variant, also located in S4, in 1 proband with MEPPC. Two of his relatives carried the variant and exhibited the phenotype. We also found the previously reported p.(Thr1779Met) variant, located in the C terminus, present in 2 sisters with a MEPPC phenotype. Three variants outside of S4 have previously been recognized to be responsible for MEPPC phenotypes.⁹

Three variants (2 missense and 1 truncating) led to SSS. The first description of SCN5A-related SSS was published by Benson and coworkers¹⁷ in 2003, with 3 index cases with

compound heterozygosity. Another case with compound variants was also reported¹⁸ as well as heterozygous variants associated with familial SSS.¹⁹

Overlap syndromes and pleiotropy

We documented 9 variants that resulted in overlap syndromes. The concept of overlap syndrome was introduced in 1999 with the description of the p.(1795insAsp) variant by Bezzina and coworkers.¹⁰ This variant was shown to be associated with the phenotypes of LQT3, BrS, and PCCD, sometimes in association, in the same family. Similar to our cohort, the most frequently described phenotypes in clinical cases involve the association of BrS and PCCD,¹¹ followed by BrS and LQT3.^{12,20} Of our 9 variants, 7 (77.8%) were truncating variants, suggesting that haploinsufficiency is a possible disease mechanism (when BrS and PCCD are involved), although this has been little reported.²¹

Furthermore, SCN5A is known to be a pleiotropic gene^{10–13} as gain-of-function variants lead to LQT3 and loss-of-function variants lead to BrS or PCCD. In 1999, Bezzina and coworkers¹⁰ described an 8-generation kindred in which the p.(1795insAsp) carriers exhibited LQT3, BrS, or sudden cardiac death. The team of Probst¹¹ also highlighted the phenomenon of intrafamilial pleiotropy, showing that p.(Gly1406Arg), located in S5–S6 of DIII, can lead to BrS or PCCD in the same family. Then, the same team²² highlighted the intimate bond between BrS and PCCD in 16 families of P/ LP SCN5A variant carriers. Grant and coworkers¹² reported the p.(Lys1500del) variant (in the DIII–DIV IDL) in a large family in which they observed intrafamilial pleiotropy (BrS, LQT3, and PCCD) and overlap syndromes. Makita and coworkers²³ studied 41 carriers of the p.(Glu1784Lys) variant: 93% had LQT3, 22% BrS, and 39% SSS. We also found this variant to be responsible for pleiotropy as well as for overlap phenotypes.

We identified 23 variants (13.5%) responsible for pleiotropy. Although this phenomenon is distinct from overlap syndrome, 10 of the 23 variants (43.5%) were found at least once in an individual with overlap syndrome. We identified 2 clusters of pleiotropic missense variants: S5–S6 and the C terminus.

More than the localization of the variant, the heterogeneous biophysical properties of certain P/LP SCN5A variants, such as the 1795insD²⁴ variant, which can cause gain or loss of function, provide a better explanation for overlap syndromes.²⁵ Compound heterozygosity, alternative splicing,²⁶ and copy number variations²⁷ are the other genetic mechanisms that could explain overlap syndrome. There are also clinical modifiers of phenotypic expression that could explain overlap syndromes. For example, Beaufort-Krol and colleagues²⁸ showed that the 1795insD mutation is associated with the age-dependent penetrance of electrocardiographic alterations. The same observation was made for an intronic variant.¹⁵ Gender is also involved.¹¹ Similar arguments are often used to explain pleiotropy. The most common is the ability of certain variants to cause a defect in inactivation, resulting in a persistent sodium current, or to enhance slow inactivation, with reduced channel availability.¹⁰ Moreover, some studies have highlighted the role of single-nucleotide polymorphisms that perhaps modify clinical expression of a variant, as shown for KCNH2,²⁹ or those that rescue a variant.³⁰

SCN5A variants associated with other phenotypes

Nineteen variants were found at least once among individuals with a phenotype that we were not able to directly link to the P/LP variant found in SCN5A. The absence of segregation data prevents the determination of variant causality.

One interesting finding of our study concerned DCMs. Indeed, outside the context of DCM secondary to MEPPC syndrome, we found 8 variants that were carried by at least 1 individual with DCM. One patient had secondary DCM due to massive ventricular hyperexcitability and 1 linked to rapid AF. Indeed, they recovered a normal ejection fraction after successful ablation. Another had DCM that we linked to myocarditis rather than to a direct effect of the variant, although we cannot rule this out, given the extent of fibrosis never described at this level in the literature for DCM-associated SCN5A variants. The 5 other variants were found in patients with PCCD. In other words, we did not find any cases of primary DCM. Peters and coworkers¹ published an extensive review of DCM and P/LP SCN5A variants. They found 18 unique SCN5A variants in 29 kindreds. Arrhythmia and conduction disorders were the presenting complaints in all probands. Our data along with the findings of Peters and coworkers indicate that SCN5A does not lead to isolated DCM but that DCM may occur in association with arrhythmic manifestations, such as MEPPC, PCCD, or ventricular/atrial arrhythmias.

Phenotypic evaluation of SCN5A P/LP variant carriers

There are currently no recommendations concerning the evaluation to perform after documentation of a P/LP SCN5A variant in an individual. The proportion of variants (19%) responsible for overlap syndrome or pleiotropy argues for the complete phenotypic evaluation of patients carrying a P/LP SCN5A variant.

These patients should have repeated electrocardiography and Holter electrocardiography to search for BrS, LQT3, and the appearance or evolution of conductive disorders. They should also undergo regular cardiac echography, especially those with a PCCD phenotype, as cases of DCM associated with PCCD and MEPPC phenotypes have been reported.

Patients with LQT3, who may be receiving treatment with class I antiarrhythmic drugs, should undergo a sodium channel blocker challenge. The systematic use of this test and its practical usefulness are more debatable for other phenotypes, in particular for relatives because of its lack of specificity³¹ and the low risk of serious complications in SCN5A carriers.³²

Finally, we suggest that all patients with a P/LP SCN5A variant should respect the list of drugs contraindicated for BrS, with the exception of patients with LQT3 and a negative ajmaline test result.

Limitations

This study had several limitations. A small proportion of patients considered to have BrS had only a class IIa or IIb diagnosis according to the latest recommendations. However, the diagnosis is reinforced by the presence of the SCN5A P/ LP variant.

Phenotypic evaluation was not standardized, which may have led to an underestimation of the pleiotropy. Not all patients had a sodium channel blocker test, which may have led to an underestimation of the number of variants leading to overlap syndromes or pleiotropy. Similarly, a number of the BrS patients and some healthy relatives showed minor conductive disorders, which may be progressive over time. Thus, PCCD may develop in some patients during follow-up. This also may have potentially caused underestimation of the number of variants leading to overlap syndromes or pleiotropy.

There was a small number of relatives, making it impossible to establish reliable penetrance rates.

Conclusion

In this large multicenter registry, most of the P/LP SCN5A variants were found in patients with primary electrical disorders, the most frequent being BrS. Extracellular loops were a hot spot for BrS, whereas variants associated with LQT3 were mostly found in S4–S5 and the DIII–DIV IDL.MEPPC syndrome was associated with specific variants, mostly located in the S4 domain. A significant proportion of variants were found in patients with overlap syndromes or pleiotropy, indicating that carriers of P/LP SCN5A variants require a complete evaluation. Finally, certain SCN5A variants led to phenotypes for which the causality of the variant was unclear, in particular for DCM.

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