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ARTICLE

SCN4A variants and Brugada syndrome: phenotypic and genotypic overlap between cardiac and skeletal muscle sodium channelopathies

Véronique Bissay*[,1,7], Sophie CH Van Malderen[2,3,7], Kathelijn Keymolen[4], Willy Lissens[4], Uschi Peeters[4], Dorien Daneels[4], Anna C Jansen[5], Gudrun Pappaert[2], Pedro Brugada[2], Jacques De Keyser[1,6] and Sonia Van Dooren[4]

SCN5A **mutations involving the α-subunit of the cardiac voltage-gated muscle sodium channel (NaV1.5) result in different cardiac channelopathies with an autosomal-dominant inheritance such as Brugada syndrome. On the other hand, mutations in *SCN4A* encoding the α-subunit of the skeletal voltage-gated sodium channel (NaV1.4) cause non-dystrophic myotonia and/or periodic paralysis. In this study, we investigated whether cardiac arrhythmias or channelopathies such as Brugada syndrome can be part of the clinical phenotype associated with *SCN4A* variants and whether patients with Brugada syndrome present with non-dystrophic myotonia or periodic paralysis and related gene mutations. We therefore screened seven families with different *SCN4A* variants and non-dystrophic myotonia phenotypes for Brugada syndrome and performed a neurological, neurophysiological and genetic work-up in 107 Brugada families. In the families with an *SCN4A*-associated non-dystrophic myotonia, three patients had a clinical diagnosis of Brugada syndrome, whereas we found a remarkably high prevalence of myotonic features involving different genes in the families with Brugada syndrome. One Brugada family carried an *SCN4A* variant that is predicted to probably affect function, one family suffered from a not genetically confirmed non-dystrophic myotonia, one family was diagnosed with myotonic dystrophy (*DMPK* gene) and one family had a Thomsen disease myotonia congenita (*CLCN1* variant that affects function). Our findings and data suggest a possible involvement of *SCN4A* variants in the pathophysiological mechanism underlying the development of a spontaneous or drug-induced type 1 electrocardiographic pattern and the occurrence of malignant arrhythmias in some patients with Brugada syndrome.**
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INTRODUCTION

Sodium channel disorders are rare autosomal-dominant inherited diseases. They are caused by mutations in one of the subunit isoforms of voltage-gated sodium channels present in specific tissues such as the skeletal muscle, the cardiac muscle and the nervous system.[1]

SCN5A mutations involving the α-subunit of the cardiac voltage-gated muscle sodium channel (NaV1.5) result in different cardiac channelopathies with an autosomal-dominant inheritance such as Brugada syndrome (BS). This syndrome is associated with syncope, life-threatening ventricular arrhythmias and sudden cardiac death. In the majority of patients with BS, no gene mutation is identified and only 30% of cases has an *SCN5A* mutation.[2] The exact prevalence of this orphan disease is unknown, although it has been estimated to affect 1 in 2000 people worldwide.[3]

SCN4A encodes the α-subunit of the voltage-gated sodium channel (NaV1.4) in skeletal muscles. Mutations in this gene are responsible for muscular sodium channelopathies, encompassing (non-dystrophic) sodium channel myotonia (SCM), hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PMC) and a small percentage of hypokalemic periodic paralysis (HypoPP), as well as congenital myasthenia syndrome. The prevalence of muscular sodium channelopathies in the general population has been estimated to be $<1/100\,000$.[4]

Except for nonspecific cardiac arrhythmias described in two *SCN4A*-associated case reports,[5,6] no overlapping phenotypes between muscular and cardiac sodium channelopathies have been reported. Based on a personal observation of a patient with a genetically confirmed SCM and BS (patient A1 in Table 1), we investigated the possible role of *SCN4A* mutations in the pathophysiology of BS. The possible association between these two rare diseases is supported by several publications, demonstrating the expression of skeletal muscle voltage-gated sodium channels in the cardiac muscle.[5,7,8] To determine whether cardiac arrhythmias or channelopathies such as BS can be part of an *SCN4A*-associated phenotype, we performed a cardiac work-up in the family of this patient as well as in six other families with an *SCN4A* variant. To increase the power of the tested hypothesis, a second cross-sectional

[1]Department of Neurology, Center for Neurosciences, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [2]Department of Cardiology, Heart Rhythm Management Center, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [3]Department of Electrophysiology, Thorax Center, Erasmus MC, Rotterdam, The Netherlands; [4]Center for Medical Genetics, Reproduction and Genetics; Genetics and Regenerative Medicine, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [5]Pediatric Neurology Unit, Department of Pediatrics, UZ Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium; [6]Department of Neurology, University Medical Center Groningen, Groningen, The Netherlands
*Correspondence: Dr V Bissay, Department of Neurology, Center for Neurosciences, UZ Brussel, Vrije Universiteit Brussel (VUB), Laarbeeklaan 101, Brussels 1090, Belgium. Tel: +32 2 4763408; Fax: +32 2 4776800; E-mail: veronique.bissay@uzbrussel.be
[7]These authors contributed equally to this work.
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Table 1 Clinical and genetic findings in families with muscular sodium channelopathies

						SCN4A genetic analysis					*SCN5A genetic analysis*	
Patient	*Age (years)*	*Sex*	*MCP*	*Muscular features*	*SET room temp/ cooling*[9,10]	*cDNA position*	*Protein position*	*HC*[9a]	*Cardiac features*	*Ajm*	*cDNA position*	*Protein position*
A1	64	M	SCM	CA MS and pain at rest, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)[13,14]	AFu	Syncopes, NCWU (EPS negative)	+	c.[1673A>G];[=] (DP) c.[1141−3C>A];[=] (DP)[16]	p.His558Arg[15] NA
A2	38	F	No	No	Not done	− (for c.[4307T>C];[=])	NA	NA	AS, NCWU	+	c.[1673A>G];[1673A>G] c.[1141−3C>A];[=]	p.His558Arg NA
A3	36	F	SCM	Mild CA MS at rest, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU	+	c.[1673A>G];[=] c.[1141−3C>A];[=]	p.His558Arg NA
A4	32	F	No	No	Not done	− (for c.[4307T>C];[=])	NA	NA	AS, NCWU	−	c.[1673A>G];[1673A>G] c.[1141−3C>A];[=]	p.His558Arg NA
B1	74	M	SCM	CA MS and pain at rest, WUP	Not done	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU	−	− (no VLAF or mutation)	NA
B2	50	M	SCM	MS at rest, pain, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU, type 2 ECG Brugada pattern	−	− (no VLAF or mutation)	NA
B3	48	M	SCM	Mild MS at rest, WUP	Not done	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	Palpitations, 15 s AF	−	− (no VLAF or mutation)	NA
B4	25	M	SCM	Mild CA MS at rest, WUP	Not done	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	Dizziness, NCWU	−	− (no VLAF or mutation)	NA
C1	70	F	SCM	CA MS and pain at rest, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU	−	− (no VLAF or mutation)	NA
C2	47	M	SCM	CA MS at rest, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	Palpitations, NCWU	−	− (no VLAF or mutation)	NA
C3	43	M	SCM	No symptoms but myotonic discharges on EMG	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU	−	c.[1673A>G];[=] (DP) c.[1141−3C>A];[=] (DP)	p.His558Arg NA
D1	41	M	SCM	CA MS and pain at rest, WUP	Type III/type III	c.[4379G>A];[=]	p.(Arg1460Gln)[18]	VUS2	Syncopes, NSVT(15 beats), ILR implanted	−	− (no VLAF or mutation)	NA
E1	35	F	PMC/ SCM overlap	CA MS of hands and face, MS of the lower limbs at rest, no paradoxical myotonia nor episodic weakness	Type III/type I	c.[4373T>A];[=]	p.(Val1458Asp)[b]	VUS3	AS, NCWU	−	Not done	NA
F1	45	F	PMC	MS during physical activity evolving in episodic weakness lower limbs, CA MS of hands and face	Type III/type I	c.[.4342C>T];[=]	p.(Arg1448Cys)[5,17c]	VUS3	AS, NCWU	−	Not done	NA
G1	37	M	SCM	CA MS at rest, WUP	Type III/type III	c.[4307T>C];[=]	p.(Leu1436Pro)	AFu	AS, NCWU	+	− (no VLAF or mutation)	NA

Abbreviations: AF, atrial fibrillation; aFu, affects function; Ajm, Ajmaline challenge test; AS, asymptomatic; BS, Brugada syndrome; CA, cold aggravated; DP, disease polymorphism; ECG, electrocardiogram; EMG, electromyography; EPS, electrophysiological study; F, female; HC, Hofman Classification; homoz, homozygote; ILR, implantable loop recorder; M, male; MCP, muscular channelopathy; MS, muscular stiffness; NA, not applicable; NCWU, normal cardiac work-up (besides performance of the Ajmaline Challenge Test); NSVT, non-sustained ventricular tachycardia; Path, pathogenic; PMC, paramyotonia congenita; SET, short exercise test; SCM, sodium channel myotonia; VLAF, variants likely to affect function; VUS, variant of unknown significance; WUP, warm-up phenomenon; Y, years.
[a]Scoring details of all described variants are available in the Supplementary Table (Hofman classification).
[b]New variant.
[c]dbSNP ID rs121908544.

study was conducted to assess the prevalence of muscular sodium channelopathies in BS.

MATERIALS AND METHODS

Both studies were performed in accordance with the Declaration of Helsinki and approved by the Ethical Committee of the UZ Brussel. Written informed consent was obtained from all patients. All data have been submitted to the Leiden Open Variation database v.3.0 (http://chromium.liacs.nl/LOVD2/cancer/home.php). All identified variants are classified according to Wallis *et al.* and scored according to Hofman *et al.*[9,10] With these scoring list (scoring lists 1 for missense mutations and 2 for non-sense and frame-shift variants), the variants were classified into five different classes: no functional effect, variant of unknown clinical significance 1, 2 and 3 (VUS1, probably no functional effect; VUS2, unknown; VUS3, probably affects function) or affects function (for scoring details see Table 3 in Supplementary Material).

First case/index family

The hypothesis for this study was based on observations compiled from a 64-year-old male (patient A1 in Table 1). Electromyography (EMG) findings showed diffuse myotonia without myopathic features, and a short exercise test[11,12] suggested an SCM. Subsequent *SCN4A* analysis confirmed the presence of the c.4307T>C variant p.(Leu1436Pro), a variant previously described by Matthews *et al.*[13] Detailed description of the neurological symptoms of this patient and his family has been published elsewhere.[14] One year earlier, the index was diagnosed with BS (Figure 1) after repeated syncopes and he underwent implantation of a cardioverter defibrillator (ICD). Genetic analysis showed no *SCN5A* variants that are likely to affect function. However, two polymorphisms (c.1673A>G (p.His558Arg) and c.1141−3C>A), described as possible disease-modifying variants, were found. These *SCN5A* polymorphisms are also present in 20% of the general population.[15,16]

Consecutively, his three daughters (patient A2, A3 and A4) were screened for BS and both polymorphisms, regardless of the presence of the muscular channelopathy.

First study: screening for BS in patients with *SCN4A* variants

Eleven neurologically affected members in six other unrelated families carrying an *SCN4A* variant (families B to G) underwent a cardiac work-up (Figure 2). Echocardiography demonstrated neither underlying cardiomyopathy nor structural heart disease in any patient. None of the included patients took any antiarrhythmic drugs at inclusion. The *SCN4A* phenotypes and genotypes of the different families are described in Table 1.[5,9,13–18] The diagnosis of BS was confirmed if patients had either a spontaneous or a drug-induced ST segment elevation with a type 1 morphology of >2 mm in >1 lead among the right precordial leads (V1–V3).[19] If no spontaneous type I morphology was seen on the electrocardiogram (ECG), a standardized Ajmaline Challenge Test was performed conforming to current guidelines to unmask any concealed forms of BS.[20] In case of established BS, *SCN5A* genetic analysis was performed, according to the recommendations from the Heart Rhythm Society/European Heart Rhythm Association.[21] *SCN5A* variant detection in genomic DNA was carried out via high-resolution melting-curve analysis (HRMCA), followed by direct bidirectional Sanger sequencing analysis of aberrant HRMCA melting patterns and of exons and flanking intron regions for which HRMCA was not available. The interpretation was realized through SeqPilot v.4.0.1. using reference transcript NM_198056.2 and NG_008934.1. Other genes associated with BS, accounting for only 5% of all BS, were not screened in the myotonia probands.

Second study: screening for muscular sodium channelopathy in patients with BS

One hundred and sixty-nine adult BS patients of 107 Brugada families were recruited between October 2010 and March 2012 from the outpatient clinic of the UZ-Brussel. BS was previously diagnosed in the same manner as in the first study. In addition, a type C BS gene panel analysis of 16 extra BS-associated genes was performed in *SCN5A*-negative probands who consented for further genome-wide genetic testing. BS gene panel variants were detected via Roche SeqCap v.3 target enrichment (Roche, Vilvoorde, Belgium) and 100 bp paired-end sequencing on an Illumina HiSeq1500 machine (Illumina, Eindhoven, The Netherlands). The in-house developed next-generation sequencing data analysis pipeline uses bwa v.0.7.10-r789,[22] picard-tools v.1.97,[23] samtools v.0.1.19,[24] GATK v.2.7[25] and Alamut-HT v.1.1.11 (Interactive Biosoftware, Rouen, France). Detected variants that may affect function were confirmed by Sanger sequencing and SeqPilot v.4.0.1. (JSI Medical Systems, Ettenheim, Germany) data analysis.

Forty-two percent (71/169) of the patients received an ICD based on international recommendations at the time of implant.[26] All patients underwent a clinical and electrophysiological assessment to detect a muscular sodium channelopathy (SCM, PMC, HyperPP and HypoPP) (Figure 2). They were interviewed by one of the investigators to assess the presence of episodic weakness and myotonia, the latter by asking about stiffness and pain, as well as alleviating and precipitating factors. All patients were tested for clinical myotonia, including evaluation of hand grip and lid lag, as well as eye closure and percussion myotonia over the thenar eminence. To detect the presence of myotonic discharges, seen in SCM and PMC, as well as sometimes in HyperPP, a standardized EMG was performed by an experienced neurophysiologist in all patients. This electrophysiological test was carried out at room temperature in a proximal and distal muscle of the upper and lower limb and after cooling of the hand muscle to 22 °C.[18] The prolonged exercise test[11] was only proposed in patients with a history of episodic muscle weakness, with the aim to confirm the phenotype of HyperPP or HypoPP.

Only in the presence of clinical and/or electrophysiological features suggesting a muscular sodium channelopathy, genetic testing of the *SCN4A* gene was performed (Figure 2). Analysis for detection of *SCN4A* mutations was carried out by PCR amplification and Sanger sequencing of all 24 exons and parts (30 bp) of the flanking introns of the *SCN4A* gene (NM_000334.4 and NG_011699.1). In the absence of *SCN4A* variants that may affect function in patients with myotonic features, the *CLCN1* (chloride channel, voltage sensitive 1, NM_000083.2 and NG_009815.1), which is the other gene involved in non-dystrophic myotonia (NDM) was explored (Figure 2).[27] *CLCN1* was sequenced by PCR amplification and Sanger sequencing of all 23 exons and parts of the flanking introns. As NDM are considered to be highly penetrant genetic disorders, *SCN4A* or *CLCN1* mutations are neither expected nor tested in the absence of clinical and/or electrophysiological features.[27]

One family was indicative for *DMPK* testing (Figure 2). Detection of the CTG repeat expansion in the 3′-UTR of the *DMPK* gene was performed with PCR techniques described by Brook *et al.*[28] and with triplet repeat primed PCR techniques described by Warner *et al.*[29] adapted for use on the ABI3130 genetic analyzer.

RESULTS

Screening for BS in patients with muscular sodium channelopathies

In the index family (family A in Table 1), the three cardiac asymptomatic daughters (A2–A4) all carried the *SCN5A* polymorphisms c.1673A>G (p.H558R) and c.1141−3C>A. Daughters A2 and A3 had a positive Ajmaline Challenge Test, revealing BS (Table 1 and Figure 1). During a subsequent electrophysiological study (EPS), no sustained ventricular arrhythmia could be induced in both patients. Therefore, ICD implantation was not indicated and follow-up once a year was proposed. One of both cardiac-affected daughters (A3) carried the same *SCN4A* variant and the matching neurological phenotype as the father. The youngest daughter (A4) had normal cardiac tests and did not carry this *SCN4A* variant. One out of 11 patients of 6 additional families, all carrying an *SCN4A* variant, had a positive Ajmaline Challenge Test (Figure 1). No cardiac symptoms or suspicious family history was reported for this patient (G1). The subsequent EPS and *SCN5A* analyses of this patient were normal and no ICD implantation was proposed at that time.

One cardiac asymptomatic patient (B2) had a baseline Brugada type 2 pattern, which remained unchanged after the challenge test with ajmaline. This ECG pattern is therefore considered to be a normal

variant rather than a specific predictor of life-threatening arrhythmia.[30]

Extensive cardiac work-up in four patients (B3, B4, C2 and D1) with palpitations, syncope or dizziness only demonstrated the occurrence of a non-sustained ventricular tachycardia in one patient with syncope (D1; Table 1). Ajmaline testing and *SCN5A* analysis demonstrated no underlying cardiac channelopathy. All other patients had normal cardiac findings.

Screening for muscular sodium channelopathies in patients with BS

Periodic paralysis was not diagnosed in any Brugada patient. Ten Brugada patients from four families had electrophysiological myotonic features (Table 2). In one family, a known *SCN5A* variant was identified and a second family carries a novel *SCN5A* variant. Both variants were predicted to affect function.[2,31,32] In the other two families, no BS-associated variants were identified in *SCN5A* (H1) and the BS gene panel of 16 additional genes (I1). Clinical, neurophysiological features and genetic diagnoses are described in detail in Table 2. There was an important intra- and interfamilial variation in severity and distribution of myotonic features (Table 2 and Figure 3). Four out of ten patients (H1, I1, I3 and K1) had typical symptoms of myotonia and one (K1) had objective myotonic signs during physical examination. None of them had consulted a physician for these complaints in the past, suggesting a relatively mild impact on their quality of life.

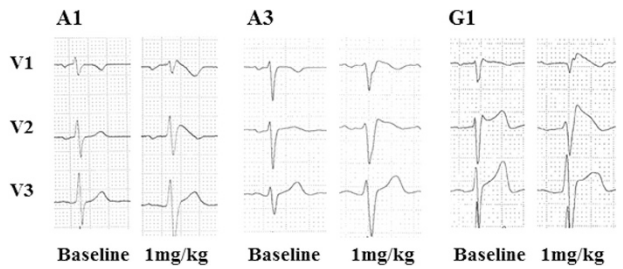

Figure 1 Right precordial ECG tracings (V1–V3) in three patients with an *SCN4A*-associated non-dystrophic myotonia and Brugada syndrome. The baseline ECG pattern in patient A1 and A3 is normal. Patient G1 demonstrates a type 2 baseline ECG (not diagnostic). After a maximal administered dose of ajmaline (1 mg/kg), baseline ECG patterns are converted into a diagnostic type 1 ECG pattern, which consist of a coved-type ST segment elevation.

In the proband of the first *SCN5A*-negative Brugada family (H1), a c.2341G>A p.(Val781Ile) in exon 4 of *SCN4A* was detected. This variant, currently classified as having no functional effect (Hofman classification; Table 2), was previously reported in association with hyperkalemic and normokalemic periodic paralysis.[33,34] However, a functional expression study supported the hypothesis that this variant should be classified as a rare benign polymorphism rather than a causative mutation.[35] No other family members were included in that study to evaluate segregation. The *CLCN1* gene analysis was not performed because no patient consent was obtained. Therefore, we cannot confirm the involvement of *SCN4A* p.(Val781Ile) in the muscular phenotype of this patient.

Two of the three family members (I1 and I3) of the second *SCN5A*-negative family (I) had myotonic discharges on EMG and a novel c.3901_3903delCAG (p.(Gln1301del)) variant in the intracellular domain 3–4 of *SCN4A*. The localization of this amino-acid deletion as well as the phenotype/genotype concordance in the family members advocates the probable function affecting the role of this genetic change.

In the third Brugada family (J), positive for the known *SCN5A* variant c.2632C>T p.(Arg878Cys),[32] no *SCN4A* mutation was found in the index patient (J1) who presented myotonic features on EMG. Consequently, the *CLCN1* gene was analyzed. All the family members with myotonic features on EMG (J1, J2, J7, J8 and J12) are heterozygous carriers of a c.774+1G>A variant in intron 6, which has not been described before. In this large family, there was a 100% genotype–phenotype concordance for myotonia as none of the myotonia-negative members who were tested (J3, J4 and J9–J11) carried the *CLCN1* variant, therefore confirming the causative role for this variant. Based on these findings, we can conclude that some family members, besides having a BS, suffer from

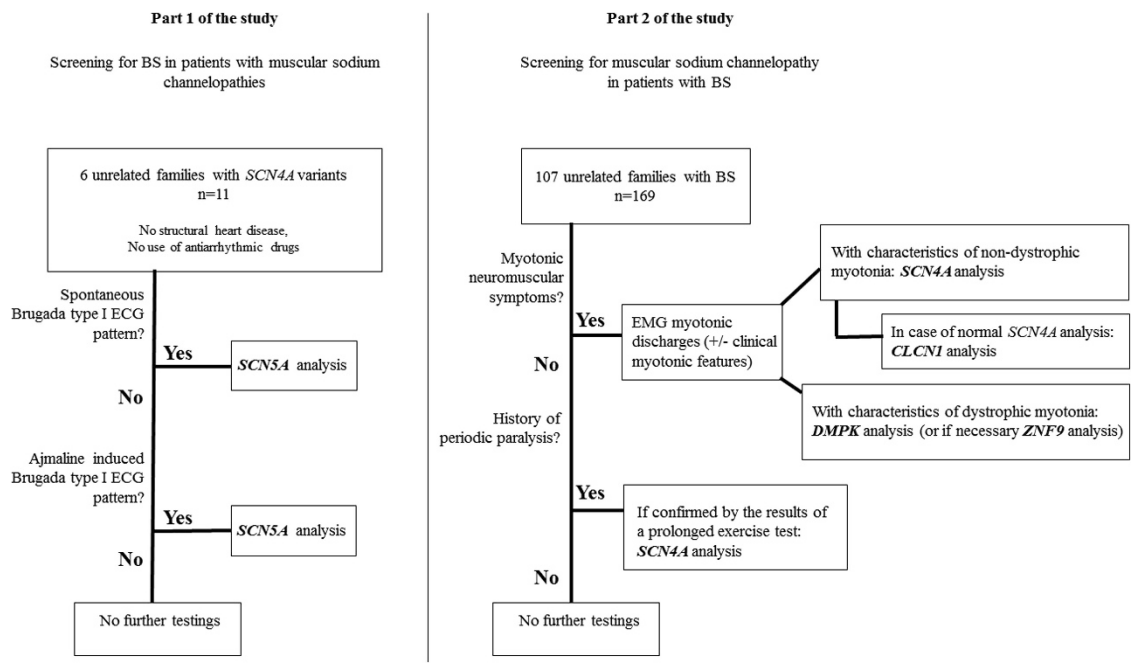

Figure 2 Study design.

Table 2 Clinical and genetic findings in families with Brugada syndrome

							SCN5A genetic analysis					*Myotonia genetics*		
Patient	*Age (years)*	*Sex*	*Sp type 1 ECG*	*Ajm*	*Cardiac features*	*ICD*	*c.DNA position*	*Protein position*	*HC*[9a]	*EMG MD*	*Muscular features*	*c.DNA position*	*Protein position*	*HC*[9a]
H1	62	M	–	+	Syncope	Yes	– (no VLAF or mutation)	NA	NA	+ After cooling	MS at rest after exercise, WUP	*SCN4A*: c.[2341G>A];[=]	*SCN4A* p.(Val781Ile)[b] 25–27	No funct effect
I1	57	M	+	NA	ACA	Yes	– (no VLAF or mutation)	NA	NA	+	MS at rest after exercise	*SCN4A*: c.[3901_3903delCAG];[=][c]	*SCN4A* p.(Gln1301del)	VUS3
I2	54	M	–	+	AS	No	Not done	NA	NA	–	None	– (for c.3901_3903delCAG)	NA	NA
I3	53	F	–	+	AS	No	Not done	NA	NA	+	Calf/hand MS at rest after exercise, WUP	*SCN4A*: c.[3901_3903delCAG];[=]	*SCN4A* p.(Gln1301del)	VUS3
J1	50	F	–	+	AS	No	c.[2632C>T];[=]	p.(Arg878Cys)[d]	VUS3	+	Sporadic MS of the thumb	*SCN4A*: no VLAF or mutation *CLCN1*: c.[744+1G>A];[=][c]	NA	AFu
J2	45	M	–	+	AS	No	– (for c.[2632C>T];[=])	NA	NA	+	Sporadic cramps calfs at night	*CLCN1*: c.[744+1G>A];[=]	NA	aFu
J3	46	F	–	+	AS	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	– (for *CLCN1* c.[744+1G>A];[=])	NA	NA
J4	27	F	–	+	NSVT at Ajm	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	– (for *CLCN1* c.[744+1G>A];[=])	NA	NA
J5	50	F	–	+	AS	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	Not done	NA	NA
J6	42	F	–	+	Syncope	Yes	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	Not done	NA	NA
J7	49	F	–	+	RBBB, AS	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	+	None	*CLCN1*: c.[744+1G>A];[=]	NA	aFu
J8	24	M	–	+	AS	No	– (for c.[2632C>T];[=])	NA	NA	+	Sporadic cramps at night	*CLCN1*: c.[744+1G>A];[=]	NA	aFu
J9	21	F	–	+	AS	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	– (for *CLCN1* c.[744+1G>A];[=])	NA	NA
J10	51	F	–	+	SSS, AF	Yes	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	– (for *CLCN1* c.[744+1G>A];[=])	NA	NA
J11	71	F	+	NA	Syncope, EPS +	Yes	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	– (for *CLCN1* c.[744+1G>A];[=])	NA	NA
J12	46	F	–	+	VF	Yes	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	+	None	*CLCN1*: c.[744+1G>A];[=]	NA	aFu
J13	24	F	–	+	AS	No	c.[2632C>T];[=]	p.(Arg878Cys)	VUS3	–	None	Not done	NA	NA
K1	54	M	+	NA	VF during fever	Yes	c.[5189C>A];[=]	p.(Pro1730-His)[c]	VUS3	+	CA MS, myopathic face, weakness/wasting hands/feet	Expanded repeats *DMPK*	Expanded repeats *DMPK* (1700 bp)	NA
K2	22	F	–	+	AS	No	– (for c.[5189C>A];[=])	NA	NA	+	Discrete ptosis, temporal muscle wasting	Expanded repeats *DMPK*	Expanded repeats *DMPK* (900 bp)	NA

Abbreviations: ACA, aborted cardiac arrest; aFu, affects function; Ajm, Ajmaline Challenge Test; AF, atrial fibrillation; AS, asymptomatic; BS, Brugada syndrome; CA, cold aggravated; DMPK, dystrophia myotonica protein kinase; ECG, electrocardiogram; EPS+, positive electrophysiological study with induction of a sustained ventricular arrhythmia; F, female; funct, functional; ICD, implantable cardioverter defibrillator; M, male; MD, myotonic discharges; MS, muscle stiffness; NSVT, non-sustained ventricular tachycardia; NA, not applicable; SSS, sick sinus syndrome; Sp, spontaneous; VLAF, variants likely to affect function; VF, ventricular fibrillation; WUP, warm-up phenomenon; Y, years.
[a]Scoring details of all described variants are available in the supplementary table (Hofman classification).
[b]dbSNP ID rs62070884.
[c]New variant.
[d]dbSNP ID rs199473168.

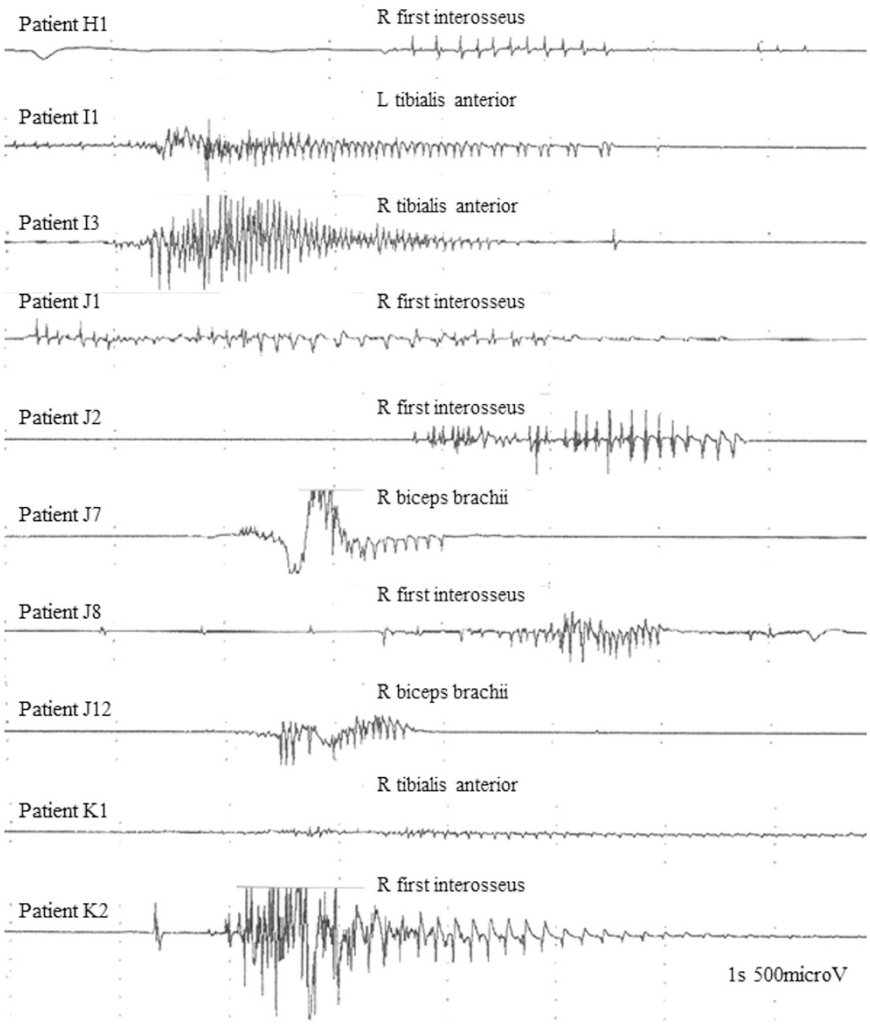

Figure 3 Myotonic discharges in electromyography of Brugada patients.

autosomal-dominant myotonia congenita (Thomsen disease). However, most of them were clinically asymptomatic or described minimal nonspecific muscular symptoms. Interestingly, not all the Brugada patients in this family have the known c.2632C>T p.(Arg878Cys) *SCN5A* variant. Some of them only have the *CLCN1* variant (Table 2), do not display malignant cardiac events (J2 and J8), but have a positive Ajmaline Challenge Test in addition to their myotonic features.

The fourth family (K1 and K2) had typical clinical features of a myotonic dystrophy (DM1), including ptosis and atrophy of the temporal and sternocleidomastoid muscle. Discrete distal muscular weakness and percussion myotonia were only observed in the oldest patient (K1). His major complaints were cramps in both hands and grip myotonia, exacerbated by cold. The analysis of the *DMPK* gene (dystrophia myotonica protein kinase) showed an expansion of the CTG trinucleotide repeat in the 3′ end of the gene in both father (1700 bp) and daughter (900 bp), the presence of which confirmed the diagnosis of DM1 in both patients. In the cardiac symptomatic proband (K1), additional *SCN5A* gene analysis revealed a novel variant c.5189C>A (p.(Pro1730His)), which probably affects the function. His cardiac asymptomatic daughter (K2) did not carry this variant despite a positive Ajmaline Challenge Test. This finding demonstrates that in this family this *SCN5A* variant is not disease causing, as it not segregating correctly.

DISCUSSION

We report on three families (families A, G and I) with an *SCN4A*-related NDM and one family (H) with a possible *SCN4A*-related NDM in association with BS. Two out of five patients with predicted gain-of-function *SCN4A* variants, and BS, had cardiac malignant events (A1 and I1). Furthermore, some family members of two other *SCN4A*-positive families (B and D) displayed cardiac arrhythmia symptoms. One had palpitations and a short documented episode of atrial fibrillation (B3). The second had syncopes and a documented non-sustained ventricular tachycardia (D1). No function affecting *SCN5A* variant was found in their families. Although two case studies[5,6] suggested a possible role of *SCN4A* in cardiac arrhythmogenesis, this association has not yet been documented.

Because *SCN4A* and *SCN5A* are not genetically linked to each other (chromosome 17q23 and 3q12, respectively),[36] we need to take a closer look at the role and the localization of Nav1.4 channels in the human cardiac muscle to find a possible explanation for these findings. Distribution of voltage-gated sodium channels differs between different mammalian species. Although ischemic rat hearts

do not express functional NaV1.4 channels,[37] they are present in mouse, pig and human cardiac tissue.[7] By staining the α-subunit in human atrial tissue, Kaufmann and *et al.*[8] demonstrated that the tetrodotoxin (TTX)-sensitive Nav1.4 and the non-TTX-sensitive Nav1.5 channels accounted for 2.6% and 87.7% of total sodium channel staining, respectively. The human ventricle also expresses the *SCN4A* α-subunit gene,[5] and in whole human heart samples, the transcript level of Nav1.4 was 1.1%.[7] Unlike other α channels, both Nav1.4 and Nav1.5 are present in a striated pattern on the cell myocyte surface, in register with the z-lines.[8] How Nav1.5 and Nav1.4 interact with each other is not clear, but their colocalization, which is also similar to the localization of Cav1.2 calcium channels in atrial tissue, could ensure rapid activation of the calcium channels and thus contraction. Whether *SCN4A* mutations, resulting in dysfunctional Nav1.4 channels, could influence this interchannel interaction and result in a reduced net depolarization current, as seen in BS[38,39] and/or aberrant calcium channel activation, needs further investigation including functional expression studies and wedge preparation models.

However, we should also emphasize that in contrast to the loss-of-function of NaV1.5 sodium channels in the *SCN5A*-associated BS, muscular sodium channelopathies with myotonic features typically result from a gain-of-function of Nav1.4 channels. Therefore, gain-of-function *SCN4A* mutations in cardiac tissue are less likely to result in a reduced net depolarization current, as seen in BS. In patients with PMC, prolonged QTc intervals were observed in association with a gain-of-function *SCN4A* mutation (p.(Arg1448Cys))[5]. Such repolarization abnormalities have also been seen in long QT syndrome type 3 (LQTS3) as a result of gain-of-function *SCN5A* mutations.[40] We were, however, unable to demonstrate prolonged QTc intervals in association with p.(Val781Ile), p.(Gln1301del), p.(Leu1436Pro), p.(Arg1448Cys), p.(Val1458Asp) and p.(Arg1460Gln). In LQTS3, prolongation of the QT interval is expected to result from decreased K^+ repolarization currents, increased Ca^{2+} entry or a sustained entry of Na^+ (late I_{Na}) into the cardiomyocyte.[40] Whether a parallelism can be drawn for *SCN4A* and if *SCN4A* mutations might result in overlapping clinical properties of different syndromes such as BS and LQTS3[41] requires further investigation.

Our data support the utility of a cardiac work-up in patients with *SCN4A* mutation or variants. Class Ic antiarrhythmic drugs such as flecainide and propafenone, which are used for the symptomatic treatment of myotonia, are contraindicated in Brugada syndrome.[42,43] Caution during a challenge test is therefore required. In contrast, Mexiletine, a class Ib antiarrhythmic, is considered to be safe in reducing myotonia[44] and is known to restore trafficking defects in BS.[45,46]

More intriguing is the presence of a rare pathology such as Thomsen disease ($<1/100\,000$) in one BS family (J). It is caused by an *CLCN1* variant encoding for the ClC-1 voltage-gated chloride channel.[4] Several chloride channels in the heart, including ClC-2 and ClC-3 voltage-gated chloride channels, have been previously reported as contributors to arrhythmogenesis.[47] Only recently, the expression of *CLCN1* mRNA transcripts has been reported in the human brain and heart.[48] Therefore, no clear explanation can be found for the concomitant occurrence of both diseases.

DM1 was diagnosed in one family (K). In recent publications,[49,50] a significantly high frequency of spontaneous and ajmaline-induced type 1 Brugada ECG pattern was detected in DM1 patients (prevalence: 0.5–3%), suggesting that our findings are not a coincidence. Patient K1 presented with a VF episode during a febrile episode, a typical phenomenon seen in BS. A possible explanation underlying the link between DM1 and BS could be that the CTG repeats in *DMPK* result in the accumulation of nuclear mRNA sequences, facilitating abnormal splicing of several genes such as *SCN5A*.[49] Others suggest that because both DM1 and BS are associated with focal fibrosis and fatty infiltration,[51,52] their coexistence could amplify the subsequent arising conduction delay in the right ventricle (RV). Such RV delay has been thought responsible for the arrhythmogenesis in BS.[39]

The 'pure' self-sufficient causative role of loss-of-function mutations of the *SCN5A* gene in the Brugada ECG pattern or syndrome has been challenged and BS is no longer thought to be a pure monogenic disorder. Phenotype-positive genotype-negative family members (genotype–phenotype discordance), *SCN5A* mutation carriers without a spontaneous or induced type 1 BS ECG pattern (incomplete penetrance) and family members who carry the same *SCN5A* mutation and express wide-ranging clinical manifestations (variable expression) support this statement.[53]

Only about 35% of BS patients have been determined to have a genetic cause and nearly 30% carry a mutation in *SCN5A*. Besides the known mutations in several genes (*SCN5A*, *GPD1L*, *SCN1B*, *SCN2B*, *SCN3B*, *MOG1*, *RANGRF*, *SLMAP*, *KCNE3*, *KCNJ8*, *HCN4*, *KCNE5*, *KCND3*, *CACNA1C*, *CACNB2*, *CACNA2D1*, *SCN10A* and *TRPM4*),[2,3] other factors also have an important role in the resulting phenotype such as additional variants[54] (compound heterozygous disease-associated polymorphisms in family A),[15,16,55] epigenetic mechanisms (DNA methylation, posttranslational modifications and RNA mechanisms)[56] and phenotype modulators (vagal tone, sex hormones and febrile status).[57] It is likely that these additional factors influence the precise phenotypic expression and are therefore responsible for phenotypic overlap[41] and variable expressivity or incomplete penetrance[58] as seen in these families. As described for *SCN10*A, which encodes the sodium channel isoform Nav1.8,[54] the presence of an *SCN4A* variant or a *CLCN1* variant or the expansion of a CTG trinucleotide repeat in *DMPK*, is probably not solely responsible for an arrhythmic event, but would rather act as an additional modifier. Further studies, including genome-wide association studies, are required to further assess the influence of these additional variants on conduction and BS.

In conclusion, we report a high number of patients with coexisting BS and SCM. Our findings suggest a possible impact of *SCN4A* variants on the pathophysiological mechanism underlying the development of a type 1 ECG pattern and of malignant arrhythmia symptoms in some patients with BS.

CONFLICT OF INTEREST

The authors declare no conflict of interest. Prof Dr Pedro Brugada received grants and speaker fees from Biotronik, Medtronic, St Jude Medical and Boston Scientific. These disclosures are not related to the study.

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