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Criteria to predict carriers of a novel *SCN5A* mutation in a large Portuguese family affected by the Brugada syndrome

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Aims Brugada syndrome (BrS) is a life-threatening arrhythmia disorder associated with autosomal-dominant mutations in the *SCN5A* gene. We aimed to characterize the diagnostic challenges and clinical manifestations of a novel *SCN5A* mutation associated with BrS.

Methods and results From a novel *SCN5A* mutation (c.664C>T; p.Arg222X) identified in a proband with the characteristic electrocardiographic pattern and the history of sudden collapse, 122 family members were studied including 40 carriers of the mutation. The electrocardiographic diagnosis of BrS requires type 1 Brugada electrocardiogram (ECG) pattern in >1 right precordial lead (V1–V3), but recently an isolated lead with coved-type ECG was proposed to be enough for the diagnosis. In this family, these proposed criteria (PC) were more sensitive in detecting mutation carriers than the conventional criteria without repercussion on the specificity. Carriers had, on average, longer P-wave duration, PR, and QRS intervals and higher transmural dispersion of repolarization. The prevalence of late potentials was higher in carriers, and individual signal average ECG (SAECG) parameters (QRSf, LAS, and RMS40) also were related to *SCN5A* gene mutation. Three non-carriers were found to be affected by BrS, two with a spontaneous type 1 ECG with alternative placement of the precordial electrodes, and one only after the pharmacological provocative test, suggesting that other genes may play a role in the pathophysiology of this disease.

Conclusion The PC for BrS diagnosis should be implemented. Some parameters from the spontaneous ECG and the SAECG are more effective tools than the characteristic repolarization pattern to discriminate between carriers of *SCN5A* mutations.

Keywords Brugada syndrome • *SCN5A* mutation • ECG • SAECG • Diagnostic criteria • Sudden cardiac death

Introduction

Brugada syndrome (BrS) is characterized by a distinctive coved-type ST-segment elevation in the right precordial leads and a high risk of ventricular arrhythmias and sudden cardiac death (SCD) in individuals with a structurally normal heart.[1–4] The worldwide prevalence is difficult to determine because the electrocardiogram (ECG) pattern may be dynamic or concealed, but it is estimated at 1–5 per 10 000 habitants, with male dominance (8 : 1 ratio), uneven geographical distribution, and with lower prevalence in the West and higher in Asia.[5] The BrS is a hereditary condition (although acquired forms have been reported) with autosomal-dominant inheritance and incomplete penetrance (it is estimated that up to 25% of individuals genetically affected do

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not have BrS). Mutations in the *SCN5A*, the gene that encodes the alpha subunit of the cardiac sodium channel gene, have been found in 18–30% of patients.[6–8]

Arrhythmic events tend to manifest around third and fourth decades of life and BrS is thought to be responsible for 4–12% of all cases of SCD and up to 20% in those with structurally normal hearts. Assessing the disease-associated arrhythmic risk, especially in asymptomatic patients, is a controversial debate and currently is based on the spontaneous ECG, the history of syncope or aborted SCD, and the inducibility of ventricular arrhythmias during electrophysiological study.[9] This paper presents a large Portuguese family with a novel *SCN5A* mutation related to BrS and evaluates different diagnostic criteria regarding this entity and the ability to predict *SCN5A* mutation carriers.

Materials and methods

All investigations conformed to principles defined in the Helsinki Declaration. One hundred and twenty-two members of a three-generation Portuguese family were investigated, and informed written consent was obtained from each member. Genetic counselling was offered to every family member. The clinical assessment included a complete medical history, physical examination, 12-lead ECG recordings obtained at different times, and echocardiographic scanning. Electrocardiogram recordings were performed in the absence of antiarrhythmic drugs and at normal electrolyte levels. All ECGs were recorded in a supine position at normal body temperatures ($<37.5^{\circ}$C). Recording speed was 25 mm/s, and all ECGs were analysed by two experienced physicians. Only coved-type ECG pattern with >2 mm ST-segment elevation with an upward convexity to an inverted T-wave in at least two right precordial leads was defined as diagnostic (type 1 ECG). Types 2 and 3 patterns have a saddle back ST-T-wave configuration in which the elevated ST-segment descends towards the baseline and then rises again to an upright or biphasic T-wave. The ST-segment is elevated ≥ 1 mm in type 2 and <1 mm in type 3, and these two patterns only were considered suggestive. Heart rate, width of P-wave, PR interval, QRS duration, and QT interval corrected for heart rate using Bazett's formula (QTc) were measured in limb lead II. QTc intervals were averaged from five consecutive beats. Signal average ECG (SAECG) was obtained with time-domain analysis, and three measures were made: filtered QRS (fQRS) duration, low-amplitude signal (LAS) duration, and root-mean-square (RMS) of the voltage in the last 40 ms of the fQRS (RMS 40). The graphic representation of late potentials as well as the numerical values of signal-averaged ECG parameters was manually checked. Late potentials were defined when at least two of the three criteria were found: fQRS >114 ms, RMS40 <20, and LAS >38 ms.

Genetic testing

Genomic DNA was extracted from 5 mL of peripheral blood using an automated procedure (Roche magnapure). Mutation screening was performed on the complete *SCN5A* coding sequence of the index case, and primers were designed to individually amplify each *SCN5A* exon, including exon/intron boundaries. The remaining members of the family were analysed only for exon 6. Amplification products were purified and sequenced using the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin-Elmer, Foster City, CA, USA) and an Applied Biosystems 3130xl Genetic Analyzer. Sequencing was performed on both strands using the original primers, and every case was polymerase chain reaction/sequenced twice independently.

Statistical analysis

Results for continuous measurements are given as mean $\pm$ standard deviation. Differences between means were accessed through the Mann–Whitney test, and comparisons of different sensitivities and specificities were performed using McNemar test and binominal test taking as reference value one of the sensitivity/specificity. A value of $P < 0.05$ was considered statistically significant. The SPSS® version 17 software package was used.

Results

Mutation screening

The proband (III25, *Figure 1*) was a 35-year-old man, taking no medication on a regular basis, with a routine ECG with type 1 Brugada pattern in leads V1 and V2, the characteristic phenotype of BrS. Seven months earlier, he had had an episode of syncope. The diagnosis also was supported by the patient's family history of five known cases of SCD under the age of 45 years, four during their sleep among relatives on the maternal side of the family: a male cousin, two uncles, and two great uncles. His maternal grandmother had five siblings (all deceased, including the above two great uncles), and his mother had 10 siblings and 21 cousins, making a total of 124 possibly affected individuals.

Genetic analysis led to the discovery of a nonsense mutation in exon 6 of the SCN5A gene, leading to the formation of a premature stop codon at position 222 of the protein (c.664C>T; p.Arg222X).

Genetic testing revealed 42 family members carrying this specific mutation of the *SCN5A* gene: 24 women and 18 men. Two women carrying the mutation refused to perform further analysis. Therefore, 122 individuals (*Figure 1*) were enrolled in additional studies, including 40 carriers of the *SCN5A* mutation (22 women, 34 $\pm$ 18.1 years, and 18 men, 27.9 $\pm$ 17.1 years) and 82 non-carriers (40 women, 31 $\pm$ 16.1 years, and 42 men, 28.6 $\pm$ 15.4 years).

Electrocardiogram screening

A spontaneous ECG was recorded in 122 family members with the right precordial leads (V1 and V2) carefully placed in the fourth intercostal space (IS) parasternally (using anatomic references). *Table 1* presents the number of mutation carriers detected with the conventional criteria (CC) (type 1 ECG >1 right precordial lead) against the proposed criteria[10] (PC) (type 1 ECG ≥ 1 right precordial lead) (*Figure 2*). Screening with a single ECG using CC found five mutation carriers and zero non-carriers. Screening with a single ECG using PC found 13 mutation carriers and 1 non-carrier. The sensitivity of the PC was significantly higher, and no significant differences were observed for specificity. Applying the CC for the three repolarization patterns (CC3R) (type 1, 2, or 3 ECG >1 right precordial lead) found nine mutation carriers with one non-carrier and with the PC for the three repolarization patterns (PC3R) (type 1, 2, or 3 ECG ≥ 1 right precordial lead) found 16 mutation carriers with 9 non-carriers, meaning significantly higher sensitivity but lower specificity. During follow-up (25.1 $\pm$ 14.3 months per patient), a total of 391 ECGs (3.2 $\pm$ 2.3 per patient) were made, and the cumulative results are presented in

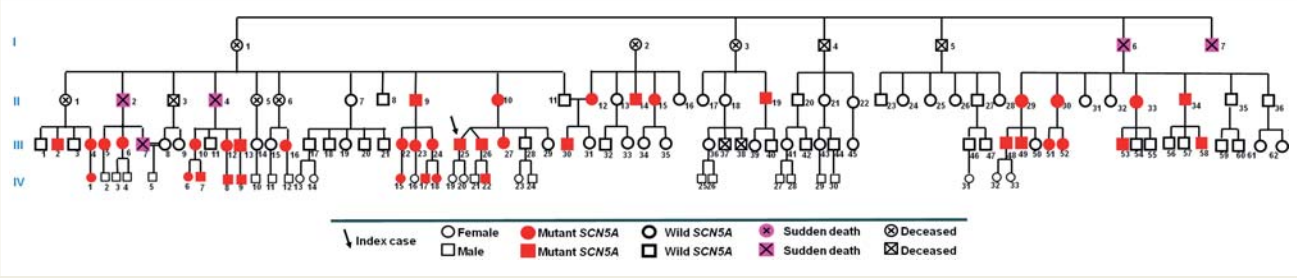

Figure 1 Genogram.

Table 1 Number of diagnosis in carriers and non-carriers with different criteria and with one electrocardiogram against several electrocardiograms during follow-up

		CC	PC	*P* value	CC3R	PC3R	*P* value
Single spontaneous ECG	Carrier	5	13	<0.05	9	16	<0.05
	Sensibility	12.5	32.5		22.5	40	
	Non-carrier	0	1	>0.05	1	9	<0.05
	Specificity	100	98.8		98.8	89	
Cumulative results during follow-up	Carrier	12	18	<0.05	20	23	>0.05
	Sensitivity	30	45		50	57.5	
	Non-carrier	0	1	>0.05	2	11	<0.05
	Specificity	100	98.8		97.6	86.6	

	One ECG	Several ECGs	*P* value
	Sensitivity		
CC	12.5%	30%	<0.05
PC	32.5%	45%	>0.05
CC3R	22.5%	50%	<0.05
PC3R	40%	57.5%	<0.05
	Specificity		
CC	100	100	–
PC	98.8	98.8	–
CC3R	98.8	97.6	>0.05
PC3R	89	86.6	>0.05

CC, conventional criteria (type 1 ECG > 1 right precordial lead); PC, proposed criteria (type 1 ECG ≥ 1 right precordial lead); CC3R, conventional criteria for the three repolarization patterns (type 1, 2, or 3 ECG > 1 right precordial lead).
PC3R proposed criteria for the three repolarization patterns (type 1, 2, or 3 ECG ≥ 1 right precordial lead).

Table 1. Conventional criteria found 12 and PC found 18 carriers (with one non-carrier in the second). The PC led to a significant increase in sensitivity without a significant decrease in specificity. The CC3R found 20 carriers with 2 non-carriers, and using the PC3R, the number of carriers was not significantly higher with a significant decrease in specificity.

With a single ECG, the PC had a positive likelihood ratio (PLR) of 27.1, negative likelihood ratio (NLR) of 0.68, positive predictive value (PPV) of 92.3%, and negative predictive value (NPP) of 75%. When several exams were available, the PC3R and the CC3R were not significantly different: PLR (37.5 vs. 20.8), NLR (0.56 vs. 0.51), PPV (94.7 vs. 90.1%), and NPV (80 vs. 78.6%). *Table 1* also presents the number of detected carriers with one ECG against several ECGs during follow-up.

Upward displacement of the right precordial leads to the second intercostal space

There is a concordant opinion that upward displacement of the right precordial leads increases the number of ECGs that are diagnostic for the BrS. The right ventricular outflow tract has been identified as the source of the electrocardiographic abnormalities and arrhythmic activity in patients with the BrS, and higher IS is anatomically related with it.[9,11] However, no data prove whether this manoeuvre increases the sensitivity to detect mutation carriers without affecting its specificity.[7] To ascertain this question, 70 family members (all older than 16 years), including 27 mutation carriers, underwent an ECG recording with conventional lead

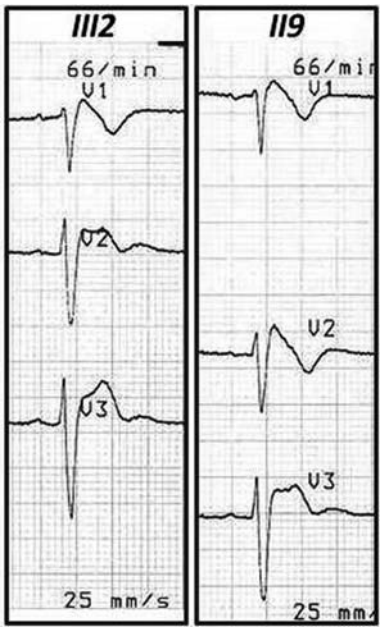

Figure 2 The electrocardiographic diagnosis of Brugada syndrome requires type 1 repolarization pattern in >1 right precordial lead (II9) but it was recently proposed that an isolated lead with coved-type electrocardiogram could be enough for the diagnosis (III2).

placement and then with upward displacement of precordial leads V1 and V2 to the second IS. Conventional lead position found three carriers and zero non-carriers with the CC (sensitivity 11.1% and specificity 100%). Positioning of the right precordial leads in the second IS leads to six more diagnosis (four carriers and two non-carriers), thus augmenting the sensitivity to 25.9% ($P = 0.1$ ns) with an inferior specificity (95.3%). Thus, we have an increase on sensitivity of 14.8% (95% confidence interval for the difference: −6 to 22%), whereas the decrease on the specificity lowers by only 4.7% ($P =$ ns).

Provocative test

Screening family members of patients with BrS with a non-diagnostic spontaneous ECG must include a pharmacological provocative test, and if a type 1 ECG is induced in more than one right precordial lead, the diagnostic is confirmed.[9] Twenty-three adults from this family (36.36 ± 12.34 years; nine men), including 14 mutation carriers (35.23 ± 12.10 years; four men) and nine non-carriers (38 ± 13.22 years; five men), all with a non-diagnostic spontaneous ECG, underwent provocative test using flecainide (2 mg/kg/10 min, intravenous). Nine mutation carriers developed a diagnostic pattern (type 1 ECG >1 right precordial lead), and one of the non-carriers had a positive result (asymptomatic male, 32-year-old: III17, *Figure 1*). This means that the provocative test used to detect mutation carriers has a sensitivity of 64.3% and specificity of 88.9%, with a PPV of 90% and NPV of 61.5%. All the 14 mutation carriers repeated the provocative test with flecainide after 1 year, and one (III58, *Figure 1*) presented a different result: the first test was negative, and the second was positive (repeatability 92.9%).

After the provocative test, both PR and QRS intervals are significantly longer in the carriers of loss-of-function *SCN5A* mutations leading to premature truncation of the protein.[12] In these 23 family members, before and after the provocative test, mutation carriers compared with the non-carriers had, on average, a significantly longer P-wave duration (before 111.5 ± 14 vs. 93.8 ± 14.1, $P = 0.01$ and after 122.3 ± 13 vs. 107.5 ± 17.5, $P = 0.03$), a significantly longer PR interval (before 194.6 ± 21.8 vs. 164.4 ± 26.1, $P = 0.008$ and after 224.6 ± 27 vs. 180 ± 35.5, $P = 0.004$), and a not significantly longer QRS duration (before 106.4 ± 41.4 vs. 91.1 ± 16.9, $P =$ ns and after 137.9 ± 44.1 vs. 122.5 ± 16.7, $P =$ ns). During pharmacological challenge, an ECG trend to higher PR interval (30 ± 24.2 vs. 20 ± 15, $P =$ ns) and QRS width (31.4 ± 9.5 vs. 30 ± 14.1, $P =$ ns) in carriers was noticed, but these slight interval changes were not significant to discriminate carriers vs. non-carriers.

Poor value of lead V3 in diagnosis Brugada syndrome

A spontaneous diagnosis was done in the conventional position (fourth IS) in 12 mutation carriers, and during follow-up, these patients did 74 ECGs. The type 1 repolarization pattern was far more frequent in leads V1 and V2: V1 with type 1 pattern in 54 (73%) ECGs and V2 with type 1 pattern in 49 (66.2%) ECGs. There were only three (4.1%) ECGs with coved pattern in lead V3, which means that this lead is less important for the diagnosis of mutation carriers.

Non-invasive markers

Several ECG parameters were measured and analysed: P-wave duration, PR interval, QRS duration, corrected QT, T peek-end distance, transmural dispersion of repolarization (TDR) between V1 and V3, and presence of aVR sign, fragmented QRS, and early repolarization. Signal average ECG was obtained from the adult population available (>16 years old). Mutation carriers had, on average, significantly longer P-wave duration and PR and QRS intervals and higher TDR compared with the non-carriers. The prevalence of late potentials was higher in mutation carriers, and individual SAECG parameters (QRSf, LAS, and RMS40) also were significantly related to *SCN5A* gene (*Table 2*).

Invasive markers

Twenty-four family members (19 carriers of the mutation) did EPS. Assessment of the ventricular arrhythmia inducibility was performed through ventricular programmed stimulation at two right ventricular sites (apex and outflow tract) at three basic drive cycle lengths (600, 500, and 400 ms) with up to three extra-stimuli. Ventricular arrhythmias were induced in three mutation carriers and in none of the non-carriers. All inducible patients had spontaneous Brugada ECG (*Table 3*). Mutation carriers compared with the non-carriers had, on average, a not significantly longer atria–His interval (98.94 ± 28.73 vs. 76.8 ± 3.9, $P =$ ns) and significantly longer His–ventrical interval (48.18 ± 7.15 vs. 38.8 ± 6.42, $P = 0.016$).

Table 2 Non-invasive risk markers measured and analysed among mutation carriers and non-carriers

	n	Carriers	Non-carriers	*P* value
P-wave	122	119.66 ± 15.47	91.96 ± 12.4	<0.001
PR interval		184.21 ± 34.92	148.1 ± 24.8	<0.001
QRS interval		98.97 ± 18.32	88.97 ± 15.08	0.003
QT corrected		404.07 ± 37.98	395.81 ± 34.27	ns
T peak-end distance		85.64 ± 25.83	85.56 ± 20.89	ns
TDR		46.41 ± 26.51	28,66 ± 18.63	<0.001
aVR sign		8	5	ns
Fragmented QRS		16	17	ns
Early repolarization		12	11	ns
Late potentials	46	52%	26.1%	<0.001
QRSf		104.74 ± 9.67	92.11 ± 12.91	<0.001
LAS		41.33 ± 9.64	32.43 ± 14.33	0.009
RMS40		18.78 ± 6.43	37.21 ± 24.64	<0.001

Symptoms

In the last 4 years among the 40 mutation carriers, 4 (10%) had symptoms that could be related to BrS: 3 with syncope (one presented at the emergency department with sustained VT), and 1 with an episode of nocturnal agonal respiration (witnessed by his wife and daughter): 2.5% event rate/year (*Figure 3*). Among non-carriers, 4 also had syncope, with an aetiology concluded to be vasovagal and with negative EPS. Events were between 16 and 61 years old.

Treatment

Four symptomatic patients with a diagnostic ECG received an implantable cardioverter defibrillator (ICD). Asymptomatic patients underwent EPS, and three were inducible for ventricular arrhythmias and received an ICD. One patient with type 1 ECG and asymptomatic and negative EPS received an ICD from a second opinion from a different centre (*Table 3*).

Discussion

The electrocardiographic diagnosis of BrS requires type 1 repolarization pattern in >1 right precordial lead (V1–V3), and type 2 or 3 repolarization patterns are only considered suggestive.[9] However, this expert consensus has never been evaluated nor validated: some authors believe that types 2 and 3 also are diagnostic, and other investigators recently proposed that an isolated lead with coved-type ECG could be enough for the diagnosis because these individuals have similar clinical profile and arrhythmic risk as those with two affected leads. In our study, the sensitivity of PC to detect carriers of the *SCN5A* mutation was significantly higher than CC with no significant differences in the specificity. The right ventricular outflow tract has been identified as the source of the electrocardiographic abnormalities and arrhythmic activity in this disease, and the presence of the pattern in one vs. two leads may be more related with anatomical interindividual variance than to a physiopathological condition.[7,10,11] Using the three repolarization patterns can be useful and appropriate in familiar screening, but the clinician must be aware that this approach can mean higher sensitivity with lower specificity, particularly the incomplete right bundle branch block pattern that is frequent in the young and can mimic type 3 Brugada repolarization pattern. Whatever criteria we use, the best single way to augment the sensitivity of spontaneous ECG seems to be doing several examinations related to the dynamic phenotypic presentation of this entity.

Specific *SCN5A* mutations correlate with the electrocardiographic phenotype. Carriers of loss-of-function or missense *SCN5A* mutations with more severe biophysical properties develop more severe phenotypes associated with conduction disorders, and this has been studied in terms of risk stratification.[12] As the sodium current dysfunction reflects on the fast response action potential characteristic of atrial and ventricular myocytes and in the fast-conducting Purkinje system, carriers have, on average, significantly longer P-wave duration and PR and QRS intervals and higher TDR compared with the non-carriers. The prevalence of late potentials is higher in mutation carriers, and individual SAECG parameter (QRSf, LAS, and RMS40) also is significantly related to *SCN5A* gene mutation. These electrocardiographic parameters can participate as a screening tool to help the clinician distinguish between possible carriers and non-carriers. The presence of a normal-high intra-atria and intra-ventricular conduction (normal-high defined at least >75% of the normal range) can be represented in a formula that needs to meet simultaneously six conditions: PR ≥ 150, QRS ≥80, QTd >10, QRSf ≥90, LAS ≥26, and RMS40 ≤29 to be positive (high probability of carrying a *SCN5A* mutation).[13] This formula was applied to 46 adult members of this family (including 25 mutation carriers), and it was positive in 22 of the 25 carriers with 2 false positives in 21 non-carriers (sensitivity of 88% and specificity of 90.5%). The accuracy to detect carriers in this family with this formula is much higher than the characteristic ECG repolarization pattern.

It was recently reported that it may be possible to find a type 1 ECG in both carriers and non-carriers of *SCN5A* mutations among

Table 3 Non-invasive and invasive risk markers measured and analysed among mutation carriers

Patient	Sex	Age (actual/ diagnostic ECG/ symptoms)	Time follow-up (ECG)	All spontaneous ECG (fourth IS)	Provocative test	EPS	Symptoms during follow-up	ICD (time)/ appropriate/ inappropriate shocks
II9	M	(62/60/61)	25	Type 1	–	–	NAB	Yes (20)/0/2
II10	F	(69/–/–)	12	PP SAF	–	Refused	No	No
II12	F	(50/–/–)	18	Normal	Negative	–	No	No
II14	M	(53/52/53)	15	PP SAF	–	–	Syn + VT	Yes (12)/0/0
II15	F	(46/45/–)	147	Normal	Type 1	Negative	No	No
II19	M	(39/37/–)	25	Normal	Type 1	Negative	No	No
II29	F	(51/49/–)	25	Type 1	–	Negative	No	No
II30	F	(49/47/–)	37	Type 1	–	Negative	No	No
II33	F	(64/63/–)	25	Type 2	Type 1	Refused	No	No
II34	M	(49/46/–)	24	Type 1 IsoL	–	VF	No	Yes (20)/0/0
III2	M	(20/18/–)	25	Type 1	–	Negative	No	No
III4	F	(31/30/–)	30	Type 1 IsoL	–	Negative	No	No
III5	F	(30/26/–)	47	Type 1	–	PolyM VT	No	Yes (44)/0/0
III6	F	(31/–/–)	25	Normal	Negative	Negative	No	No
III10	F	(33/31/–)	26	Type 1	–	Negative	No	Yes (23)/0/0
III12	F	(29/28/–)	19	Type 2	Type 1	Negative	No	No
III13	M	(28/26/–)	19	Type 1	–	Refused	No	No
III16	F	(43/43/–)	24	Type 3 IsoL	Type 1	Negative	No	No
III22	F	(32/29/–)	26	Type 1	–	Negative	No	No
III23	F	(38/–/–)	25	Normal	Negative	–	No	No
III24	F	(36/–/–)	25	Normal	Negative	–	No	No
III25	M	(36/34/34)	32	Type 1	–	–	Syn	Yes (27)/0/0
III26	M	(36/34/–)	29	Type 1	–	Negative	No	No
III27	F	(47/46/–)	25	Type 1	–	Negative	No	No
III30	M	(29/27/–)	25	Type 1	–	PolyM VT	No	Yes (16)/0/0
III48	M	(28/28/–)	1	Type 1 IsoL	–	Refused	No	No
III49	M	(24/22/–)	25	Normal	Type 1	Negative	No	No
III51	F	(27/25/–)	25	Normal	Type 1	Negative	No	No
III52	F	(20/18/16)	75	Type 1 IsoL	Type 1	–	Syn	Yes (20)/0/0
III53	M	(39/38/–)	17	Type 2 IsoL	Type 1	Negative	No	No
III58	M	(25/25/–)	25	Type 3 IsoL	Negative/ positive	–	No	No
IVI	F	(4/3/–)	27	Type 1 IsoL	–	–	No	No
IV6	F	(6/–/–)	15	Normal	–	–	No	No
IV7	M	(13/–/–)	12	Normal	–	–	No	No
IV8	M	(2/–/–)	7	Normal	–	–	No	No
IV9	M	(6/–/–)	26	Normal	–	–	No	No
IV15	F	(7/4/–)	26	Normal	–	–	No	No
IV17	M	(7/–/–)	15	Normal	–	–	No	No
IV18	F	(5/–/–)	26	Normal	–	–	No	No
IV22	M	(6/3/–)	26	Type 1 IsoL	–	–	No	No

IsoL, isolated lead; LPHB, left posterior hemiblock; LAHB + RBBB, left anterior hemiblock + right bundle branch block; PP, permanent pacemaker; SAF, slow atrial fibrillation; NAB, nocturnal agonic breathing; Syn, syncope; Syn + VT, syncope and sustained VT; VF, ventricular fibrillation; IS, intercostal space.

families affected with BrS.[14] This suggests that *SCN5A* mutations are not necessary for the occurrence of BrS and that the diagnosis is independent from the genetic results. Three individuals from this family were found to be affected by Brugada type 1 diagnostic ECG with a negative genotype. Among these three mutation-negative BrS individuals, two (III1 and III18, *Figure 1*) had a spontaneous

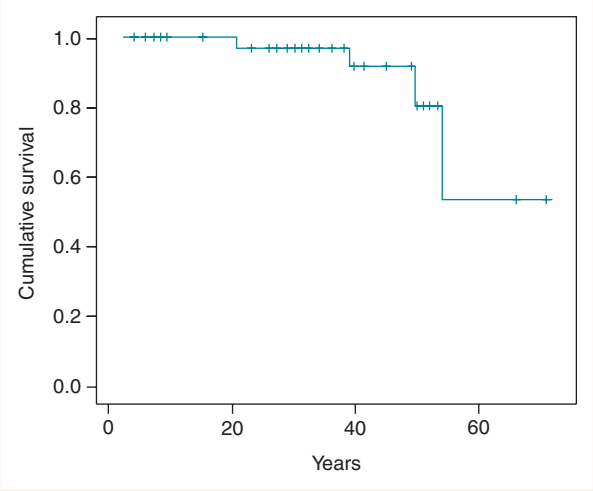

Figure 3 Kaplan–Meier curve representing events (symptomatic manifestation) on mutation carriers since birth.

type 1 ECG in the second IS, whereas one (III17, *Figure 1*) had a type 1 ECG only after the administration of sodium channel blockers. The proposed formula previously mentioned was negative in these three individuals. They are all asymptomatic, had a negative response to ventricular stimulation, and are on close clinical follow-up. Although these three individuals are considered Brugada patients, we must discuss the possibility that a false-positive result to unconventional position of the right precordial leads and/or to drug challenge can occur. The provocative test to unmask concealed forms of BrS is performed under the assumption that the number of false positives (patients developing a type 1 ECG without being affected by the disease) is extremely low, but the specificity of sodium channel blockage to identify unaffected individuals is uncertain.[7,15] As long as every single patient developing a type 1 ECG is defined as affected by BrS, it will be impossible to find anyone who is defined as false positive, thus perpetuating the perception that this manoeuvre and/or the pharmacological test are 100% accurate. If a type 1 ECG appears in the absence of a *SCN5A* mutation, should the diagnostic be definitive without further corroboration? At present, no data derived from systematic studies support or disprove the concept that upward displacement of the right precordial leads and/or sodium channel blockers provides an accurate diagnosis of the disease.

Conclusions

A novel Nav1.5 mutation (c.664C>T; p.Arg222X) related to BrS was described. The PC for BrS (type 1 ECG $\geq$1 right precordial lead) should be implemented, so revision of the consensus criteria should be considered. We suggest that conduction parameters in the Nav1.5-dependent areas (atrial muscle, Purkinje fibres, and ventricular muscle) can yield diagnostic information in differentiating between *SCN5A* mutation carriers whenever the genetic test is not available. Three individuals from this family were found to be affected by Brugada type 1 diagnostic ECG with a negative genotype. They are the evidence that there is much to know about the genotype–phenotype relationship in BrS, but we should consider that they also can represent a false-positive response to unconventional lead position and/or to drug challenge.

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