Genotype-Phenotype Correlation of *SCN5A* Mutation for the Clinical and Electrocardiographic Characteristics of Probands With Brugada Syndrome

A Japanese Multicenter Registry

BACKGROUND: The genotype-phenotype correlation of *SCN5A* mutations as a predictor of cardiac events in Brugada syndrome remains controversial. We aimed to establish a registry limited to probands, with a long follow-up period, so that the genotype-phenotype correlation of *SCN5A* mutations in Brugada syndrome can be examined without patient selection bias.

METHODS: This multicenter registry enrolled 415 probands (n=403; men, 97%; age, 46±14 years) diagnosed with Brugada syndrome whose *SCN5A* gene was analyzed for mutations.

RESULTS: During a mean follow-up period of 72 months, the overall cardiac event rate was 2.5%/y. In comparison with probands without mutations (*SCN5A* (–), n=355), probands with *SCN5A* mutations (*SCN5A* (+), n=60) experienced their first cardiac event at a younger age (34 versus 42 years, *P*=0.013), had a higher positive rate of late potentials (89% versus 73%, *P*=0.016), exhibited longer P-wave, PQ, and QRS durations, and had a higher rate of cardiac events (*P*=0.017 by log-rank). Multivariate analysis indicated that only *SCN5A* mutation and history of aborted cardiac arrest were significant predictors of cardiac events (*SCN5A* (+) versus *SCN5A* (–): hazard ratio, 2.0 and *P*=0.045; history of aborted cardiac arrest versus no such history: hazard ratio, 6.5 and *P*<0.001).

CONCLUSIONS: Brugada syndrome patients with *SCN5A* mutations exhibit more conduction abnormalities on ECG and have higher risk for cardiac events.

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Sources of Funding, see page 2267

Key Words: Brugada syndrome ■ computer similation ■ death, sudden ■ genetic association studies ■ NAV1.5 voltage-gated sodium channel ■ risk assessment

Clinical Perspective

What Is New?

- The current study included 415 Japanese Brugada syndrome probands to assess the association between *SCN5A* mutations and clinical outcomes.
- Including only probands avoided patient selection bias typically associated with including affected family members with similar clinical severity.
- Probands with *SCN5A* mutations were more prone to cardiac events, especially when the mutations were located in the pore region of the encoded protein.

What Are the Clinical Implications?

- Genetic screening for *SCN5A* mutations among Brugada syndrome probands can be useful for stratifying such patients regarding the risk of subsequent cardiac events.
- In silico analyses may provide additional information for further risk stratification of *SCN5A* mutations.

Brugada syndrome (BrS) is a rare heritable arrhythmia syndrome that increases an individual's risk for sudden cardiac death (SCD) attributable to ventricular fibrillation (VF). BrS is characterized by an elevated ST segment in the right precordial leads (V_1 through V_3) in the absence of structural heart disease.[1,2] The *SCN5A*-encoded α-subunit of the NaV1.5 cardiac sodium channel has been linked to BrS,[3] and mutations in *SCN5A* are identified in 14% to 26% of BrS cases.[4–9] In comparison with patients without *SCN5A* mutations, those with *SCN5A*-mediated BrS have a higher incidence of bradyarrhythmia events, are more likely to exhibit conduction abnormalities (longer PQ interval, longer QRS duration, frequent fragmentation) on ECG, and typically have a longer HV interval during the electrophysiology study (EPS).[2,7,10–12] Histologically, *SCN5A*-mediated BrS is characterized by myocyte apoptosis in the ventricular myocardium.[13]

Despite the more severe clinical presentation of patients with *SCN5A* mutations, previous multicenter BrS registries have not indicated *SCN5A* mutation status as a significant predictor of subsequent cardiac events.[6,14] We hypothesized that patient selection bias might be responsible for this inability to quantify the role of *SCN5A* mutations, because previous studies involved not only probands, but also family members with a clinical presentation similar to that of the probands. Therefore, we performed a multicenter registry study including only probands with BrS, and compared the ECG parameters, patient characteristics, and rate of cardiac events between BrS probands with *SCN5A* mutations and those without *SCN5A* mutations. We also investigated the correlation between the location of the *SCN5A* mutation and the clinical severity of BrS, to clarify whether it could be useful to distinguish high-risk probands. Because *SCN5A* hosts a nonnegligible number of benign, nonsynonymous single-nucleotide variants, further subgroup analyses were performed after in silico reclassification of mutations into pathological and benign variants.

METHODS

Patients

The study population consisted of 415 probands with BrS, who underwent genetic testing for *SCN5A* mutation and were followed up between 1988 and 2013. These probands were registered from the following participating institutions in Japan: National Cerebral and Cardiovascular Center, Okayama University Hospital, Hiroshima University Hospital, Osaka City University Hospital, Kyoto University Hospital, St. Marianna University School of Medicine Hospital, Keio University Hospital, Tokyo Metropolitan Hiroo Hospital, Hokkaido University Hospital, Yamaguchi University Hospital, Shiga University of Medical Science Hospital, Tokyo Women's Medical University Hospital, Niigata University Medical & Dental Hospital, and Kanazawa University Hospital. All probands received the diagnosis based on the type 1 Brugada ECG pattern, noted either spontaneously or after administration of a sodium channel blocker. The BrS diagnosis was established according to the criteria of the consensus report on the diagnosis and management of patients with inherited primary arrhythmia syndromes.[15] The present study was performed under the ethical code approved by the Health, Labor and Welfare Ministry of Japan, and informed consent for participation was obtained in written form from all probands.

Clinical Characteristics and Follow-Up

Clinical characteristics such as the age at the time of the diagnosis, sex, family history of SCD, time from the first visit to the index cardiac event, implantation of an implantable cardioverter defibrillator (ICD), and several ECG parameters were collected for all probands. A family history of SCD was defined as any unexplained death before 45 years of age. Structural heart disease was excluded based on findings of the physical examination, chest roentgenograms, echocardiography, and coronary angiography if needed.

An ICD was implanted according to the guidelines for the diagnosis and management of patients with inherited primary arrhythmia syndromes.[15] ICD indication and programming were decided at baseline and were not changed during follow-up unless the patient experienced a cardiac event.

The ECG parameters measured were the RR interval (lead II), P-wave duration (lead II), PQ interval (lead II), QRS duration (leads II, V_2, V_5), and QT interval (leads II, V_2, V_5). The corrected QT interval was calculated by the Bazett formula ($QTc=QT/\sqrt{RR}$). For the sodium channel blocker challenge test, pilsicainide, procainamide, flecainide, or disopyramide were used, at the discretion of the treating institution.

An EPS was performed in 339 patients (82%). A maximum of 3 ventricular extrastimuli with a minimum coupling interval of 200 ms were delivered from the right or left ventricular apex and right ventricular outflow tract, and considered positive when VF

or polymorphic ventricular tachycardia (VT) were triggered and resolved spontaneously within 30 seconds, or caused syncope and had to be terminated via direct cardioversion.

Late potentials on the signal-averaged ECG were defined as positive when 2 of the 3 following criteria were satisfied: (1) filtered QRS duration >135 ms; (2) root mean square voltage <18 μV for the terminal filtered QRS; and (3) low-amplitude signals of <40 μV for a terminal filtered QRS duration of >38 ms.

Each patient was followed up at the treating institution, and cardiac events were defined as the earliest (index) appropriate ICD shock, aborted cardiac arrest (ACA), or SCD. During follow-up, no data were collected on syncope as a cardiac event, because it is difficult to differentiate neurally mediated syncope from truly arrhythmic syncope.

SCN5A Genetic Analyses

DNA analyses were conducted on genomic DNA extracted from leukocytes, and consisted of a combination of polymerase chain reaction, denaturing high-performance liquid chromatography, single-stranded conformation polymorphism analysis, and DNA sequencing. Samples were analyzed at the following participating institutions: National Cerebral and Cardiovascular Center, Okayama University Hospital, Hiroshima University Hospital, Kyoto University Hospital, Hokkaido University Hospital, Shiga University of Medical Science Hospital, and Kanazawa University Hospital. Samples with either the first or the last 2 nucleotides of a particular exon were included, because the substitution of such portions could alter mRNA splicing.

We first evaluated the frequency of the detected variants by referring to the National Center for Biotechnology Information dbSNP database,[16] the Human Genetic Variation Database of single-nucleotide polymorphism data in Japanese populations,[17] the 1000 Genomes Project,[18] and our in-house database comprising samples from 1000 Japanese individuals.[19] We then evaluated the *SCN5A* mutation status by using an in silico phenotype prediction algorithm, which was described in detail elsewhere.[20] The initial classifications were updated based on available functional data, and their frequency noted among >60 000 exomes from the Exome Aggregation Consortium,[21] as well.

All mutations were denoted using known and accepted nomenclature based on the full length of the splice variant with 2016 amino acids (aa) (PubMed Accession No. NM 198056). The location of each domain (D), with particular focus on the pore region and the selectivity filter of NaV1.5, was predicted using Swissprot[22] as follows: the N terminus (aa 1–126), 4 transmembrane regions (DI S1-S4/S5, aa 127–252; DII S1-S4/S5, aa 712–841; DIII S1-S4/S5, aa 1201–1336; and DIV S1-S4/S5, aa 1524–1659), 4 pore and channel selectivity filter regions (DI S5-S6, aa 253–415; DII S5-S6, aa 842–939; DIII S5-S6, aa 1337–1470; and DIV S5-S6, aa 1660–1772), 3 interdomain linkers (IDL I-II, aa 416–711; IDL II-III, aa 940–1200; and IDL III-IV, aa 1471–1523) and the C terminus (aa 1773–2016).

Statistical Analyses

Quantitative variables are presented as mean±standard deviation. The means of the continuous variables with normal distribution were compared using the Student *t* test for independent samples. Fisher exact test or the χ^2 test was used for categorical variables. Continuous variables that did not show a normal distribution were compared using nonparametric statistics (Mann-Whitney test). The cumulative probability of an index cardiac event over the course of the follow-up was determined by using Kaplan-Meier methods for the entire population and for each subgroup, and the difference in survival rates was analyzed using a log-rank test. A Cox proportional hazards model was used to determine factors associated with the time to the first cardiac event (time-to-event analysis). In each analysis, a *P* value of <0.05 was considered to indicate statistical significance. Variables were included in the multivariate analysis if they had a *P* value of <0.05 in the univariate analysis. In the univariate analysis, the P-wave and QRS durations were included as categorical values using a cutoff of 120 ms, because this value is generally accepted as the threshold for abnormality. Among the ECG parameters, QRS (V_2) duration was chosen for the multivariate analysis, because it has been reported to reflect cardiac depolarization and to be related to prior cardiac events.[23] Because ICD implantation may have a strong correlation with the clinical status such as history of ACA, we built and evaluated multivariate models with and without ICD implantation at baseline. We performed interaction testing for the relationship between the *SCN5A* mutation and documented atrial fibrillation, QRS (V_2) ≥120 ms, history of ACA, and ICD implantation at baseline, but found no interaction. Because neither ICD indication nor programming was changed during the follow-up period unless the patient experienced a cardiac event, a time-updated analysis for ICD implantation during follow-up was not performed in this study. Clustering was assessed by including the institution as a random effect in the Cox proportional hazards model, but no significant difference was noted. All analyses were performed using the SPSS 17.0 statistical package (SPSS Inc.) and STATA 14.1 software (StataCorp LP).

RESULTS

Clinical Characteristics

The clinical characteristics of the 415 probands included in our registry are summarized in Table 1. The age among the study sample was 46±14 years (range, 4–86 years), and almost all patients were male (n=403, 97%). A total of 55 different *SCN5A* mutations were identified in 60 probands (14%) (*SCN5A* (+) group) (Table 2), whereas the remaining 355 probands (86%) exhibited no *SCN5A* mutations (*SCN5A* (–) group. Among the 60 *SCN5A* (+) probands, 25 (42%) had a mutation in the pore region (pore-*SCN5A* (+) group) and 35 (58%) in the nonpore region (nonpore-*SCN5A* (+) group). When comparing *SCN5A* (+) probands with *SCN5A* (–) probands, no significant difference was observed with regard to the age at inclusion, sex, or history of syncope or ACA. *SCN5A* (+) probands were younger at the time of the first syncopal event or ACA (34 versus 42 years; *P*=0.013), and had a higher rate of positive late potentials (89% versus 73%; *P*=0.016). An ICD was implanted in 241 probands (58%), with no significant difference in the proportion of

Table 1. Comparison of the Clinical and Electrocardiographic Characteristics Between *SCN5A* (+) and *SCN5A* (–)

	All	*SCN5A* (+)	*SCN5A* (–)	*P* Value
Clinical characteristics				
n	415	60	355	
Age, y	46±14	44±16	47±13	0.210
Male, n (%)	403 (97)	58 (97)	345 (97)	0.687
History of syncope (without aborted cardiac arrest), n (%)	99 (24)	15 (25)	84 (24)	0.822
History of aborted cardiac arrest, n (%)	88 (21)	15 (25)	73 (21)	0.437
Age of first syncopal episode or aborted cardiac arrest, y	41±16	34±17	42±15	0.013
Pore mutation of *SCN5A*, n (%)		25 (42)		
Family history of sudden cardiac death, n (%)*	64 (15)	11 (18)	53 (15)	0.500
Implantable cardioverter defibrillator implantation, n (%)	241 (58)	36 (60)	205 (58)	0.744
Electrocardiographic characteristics				
Spontaneous type 1 ECG, n (%)	299 (72)	48 (80)	251 (71)	0.138
Documented atrial fibrillation, n (%)	64 (15)	11 (18)	53 (15)	0.500
Late potential				
n	338	46	292	
Positive, n (%)	253 (75)	41 (89)	212 (73)	0.016
Ventricular fibrillation/ventricular tachycardia inducibility during the electrophysiology study†				
n	339	49	290	
Positive, n (%)	191 (56)	22 (45)	169 (58)	0.081

*Prevalence at ≤45 years old.
†Induction rate of ventricular fibrillation or polymorphic ventricular tachycardia by ventricular stimulation.

probands with ICDs between the *SCN5A* (+) and *SCN5A* (–) groups (60% versus 58%, respectively; *P*=0.744). Neither the frequency of a spontaneous type 1 Brugada ECG pattern (80% versus 71%; *P*=0.138) nor the incidence of documented atrial fibrillation (18% versus 15%; *P*=0.500) differed significantly between the *SCN5A* (+) and *SCN5A* (–) probands.

EPS was conducted in 339 probands, of whom 49 were in the *SCN5A* (+) group (82%) and 290 were in the *SCN5A* (–) group (82%). VF/VT was induced in 191 of 339 probands (56%). There was a trend toward a lower rate of VF/VT inducibility in the *SCN5A* (+) group (45% versus 58%; *P*=0.081).

A total of 17 of 415 probands (4%) were treated with quinidine, and all were *SCN5A* (–).

ECG Parameters

The RR interval did not significantly differ between the *SCN5A* (+) and *SCN5A* (–) probands (Table 3). However, the *SCN5A* (+) group had a longer P-wave duration (lead II), PQ interval (lead II), QRS duration (leads II, V_2, V_5), and QTc interval (leads V_2, V_5) (Table 3). When comparing the ECG parameters between the 25 pore-*SCN5A* (+) probands and 35 nonpore-*SCN5A* (+) probands, the QRS duration (lead V_5) was found to be significantly longer in the pore-*SCN5A* (+) probands (Table 4). Both the P-wave duration and PQ interval were longer in the pore-*SCN5A* (+) probands, but this trend was not statistically significant.

Index Cardiac Event During Follow-Up

The mean follow-up period was 72 months (range, 1–249 months). A Kaplan-Meier analysis of the cardiac events in all 415 probands is shown in Figure 1A. The overall cardiac event rate was 2.5%/y. During the follow-up period, there were 13 cardiac events in the 60 *SCN5A* (+) probands (22%) and 49 events in the 355 *SCN5A* (–) probands (14%) (Table 5, Figure 1A).

An ICD was implanted at baseline in 36 of the 60 *SCN5A* (+) probands (60%), with 13 in probands with prior ACA, 11 in probands with prior syncope, and 12 in asymptomatic probands. An ICD was implanted in 205 of the 355 *SCN5A* (–) probands (58%), with 68 in probands with prior ACA, 58 in probands with prior syncope, and 79 in asymptomatic probands. Ventricular arrhythmias were successfully terminated via an appropriate ICD shock in all 12 *SCN5A* (+) probands with an ICD implanted at baseline who presented with cardiac events

Table 2. Included *SCN5A* Mutations and Variants

Nucleotide Change	Coding Effect	Region	History of Aborted Cardiac Arrest	Cardiac Events (f/u)	Predicted as Benign
163C>T	Q55X	N-terminal			
311G>A	R104Q	N-terminal			
407T>C	L136P (2)	DI-S1			
535C>T	R179X	DI-S2/S3	Y	Y	
827T>A	L276Q	DI-S5	Y		
845G>A	R282H (3)	DI-S5/S6	Y		
870delC	N291TfsTer52	NA	Y		
1100G>A	R367H	DI-S5/S6		Y	
1127G>A	R376H	DI-S5/S6	Y	Y	
1217A>G	N406S	DI-S6			
1247A>G	Y416C	IDL I-II			
1282G>A	E428K	IDL I-II			Y
1537delC	R513VfsTer8	NA			
1595G>T	F532C	IDL I-II			Y
2066G>A	R689H	IDL I-II			Y
2077C>T	R693C	IDL I-II	Y	Y	Y
2204C>T	A735V	DII-S1	Y	Y	
2335C>A	Q779K	DII-S2/S3			
2441G>A	R814Q	DII-S4	Y		
2537T>G	L846R	DII-S5/S6	Y		
2632C>T	R878C	DII-S5/S6			
2677C>T	R893C	DII-S5/S6		Y	
2678G>A	R893H	DII-S5/S6			
2711G>A	W904X	DII-S5/S6			
2963G>A	R988Q	IDL II-III			Y
3285G>A	W1095X	IDL II-III			
3584G>A	R1195H	IDL II-III			
3598C>T	H1200Y	IDL II-III			
3673G>A	E1225K (2)	DIII-S1/S2			
3740C>T	T1247I	DIII-S2	Y		Y
3982G>A	V1328M	DIII-S4/S5			
4140_4142 delCAA	del1380N	NA			
4213G>A	V1405M	DIII-S5/S6			
4222G>A	G1408R	DIII-S5/S6			
4227C>G	Y1409X	DIII-S5/S6			
4258G>C	G1420R	DIII-S5/S6			
4282G>T	A1428S	DIII-S5/S6	Y	Y	
4295G>C	R1432S	DIII-S5/S6			
4389_4396delCCTCTTTA	L1464WfsTer5	NA			
4580A>G	K1527R	DIV-S1			

(*Continued*)

Table 2. Continued

Nucleotide Change	Coding Effect	Region	History of Aborted Cardiac Arrest	Cardiac Events (f/u)	Predicted as Benign
4732_4733dupAA	L1579SfsTer53	NA			
4867C>T	R1623X	DIV-S4	Y		
4930C>T	R1644C	DIV-S4			
4931G>A	R1644H	DIV-S4			
5126C>T	T1709M	DIV-S5/S6		Y	
5157delC	I1720SfsTer67	NA		Y	
5227G>A	G1743R	DIV-S5/S6	Y		
5290delG	V1764SfsTer23	NA			
5350G>A	E1784K (2)	C-Terminal	Y(1)	Y(1)	
5737C>T	R1913C	C-Terminal			
5756G>A	R1919H	C-Terminal			
5767C>G	H1923D	C-Terminal			
IVS21+1 G>A			Y	Y	
IVS23+1 G>A					
IVS24+1 delG					

Number of patients are indicated as (n). Cardiac Events (f/u) indicates aborted cardiac arrest, sudden cardiac death, or appropriate implantable cardioverter defibrillator therapy during the follow up; NA, not applicable; and Y, yes.

during the follow-up (7 probands with prior ACA, 2 with prior syncope, and 3 asymptomatic probands) (Table 5). Among the 49 *SCN5A* (–) probands who presented with cardiac events during the follow-up (30 probands with prior ACA, 8 with prior syncope, and 11 asymptomatic probands), 45 had been implanted with an ICD at baseline; ventricular arrhythmias were terminated by an appropriate ICD shock (44 cases) or resolved spontaneously without therapy (1 case). Over the course of the follow-up, 1 proband with a history of ACA and 1 asymptomatic *SCN5A* (–) proband without ICD implantation at baseline presented with ACA, and sudden death occurred in 3 asymptomatic *SCN5A* (–) probands without ICD implantation at baseline (Table 5). In summary, among the 240 probands with ICD implantation at baseline, 12 of 36 (33%) of *SCN5A* (+) probands and 44 of 205 (21%) of *SCN5A* (–) probands had an appropriate ICD shock for ventricular arrhythmias.

According to the results of the log-rank test, *SCN5A* (+) probands had a higher rate of cardiac events than that noted for *SCN5A* (–) probands (P=0.017). Although pore-*SCN5A* (+) probands (n=25) had a higher rate of cardiac events than the rate noted for *SCN5A* (–) probands (n=355) (P=0.002), there was no significant difference between pore-*SCN5A* (+) probands (n=25) and nonpore-*SCN5A* (+) probands (n=35) regarding the rate of cardiac events (P=0.110) (Figure 1B).

Among the 88 probands presenting with prior ACA, the cardiac event rate over the course of the follow-up was 8.0%/y. *SCN5A* (+) probands (n=15) had a higher rate of cardiac events than the rate noted in *SCN5A* (–) probands (n=73) (P=0.044) (Figure 2A). There was no significant difference between pore-*SCN5A* (+) probands (n=7) and either *SCN5A* (–) probands (73) or nonpore-*SCN5A* (+) probands (n=8) (P=0.316 and P=0.681, respectively) (Figure 2B).

Among the 99 probands with prior syncope, the cardiac event rate over the course of the follow-up was 2.1%/y. There was no significant difference between

Table 3. Electrocardiographic Parameters Comparing *SCN5A* (+) and *SCN5A* (–) Patients

	SCN5A (+) n=60	*SCN5A* (–) n=355	*P* Value
RR II (ms)	908±192	897±154	0.665
P II (ms)	117±27	93±15	<0.001
PQ II (ms)	214±46	176±25	<0.001
QRS II (ms)	102±25	86±17	<0.001
QRS V2 (ms)	109±21	94±15	<0.001
QRS V5 (ms)	103±24	87±17	<0.001
QTc II (ms)	394±35	386±29	0.076
QTc V2 (ms)	406±46	389±36	0.007
QTc V5 (ms)	394±34	384±30	0.014

Values indicate mean±standard. QTc indicates corrected QT interval.

Table 4. Electrocardiographic Parameters Comparing Pore-*SCN5A* (+) and Nonpore-*SCN5A* (+) Patients

	Pore-*SCN5A* (+)	Nonpore-*SCN5A* (+)	
	n=25	n=35	*P* Value
RR II (ms)	917±194	902±194	0.761
P II (ms)	123±29	113±25	0.144
PQ II (ms)	223±59	207±33	0.159
QRS II (ms)	107±27	98±23	0.138
QRS V2 (ms)	115±23	105±19	0.068
QRS V5 (ms)	113±28	96±18	0.010
QTc II (ms)	393±39	395±33	0.824
QTc V2 (ms)	416±55	400±37	0.202
QTc V5 (ms)	394±40	394±29	0.987

QTc indicates corrected QT interval.

SCN5A (+) (n=15) and *SCN5A* (–) (n=84) probands (*P*=0.486) (Figure 3A). However, pore-*SCN5A* (+) probands (n=5) had a higher cardiac event rate than the rate noted for *SCN5A* (–) probands (n=84) or nonpore-*SCN5A* (+) probands (n=10) (*P*=0.017 and *P*=0.039, respectively) (Figure 3B). There were no cardiac events in the 10 nonpore-*SCN5A* (+) probands with prior syncope.

Among the 228 probands who were asymptomatic at baseline, the cardiac event rate noted over the course of the follow-up was 0.9%/y. There was no significant difference between *SCN5A* (+) (n=30) and *SCN5A* (–) probands (n=198) (*P*=0.187) (Figure 4A). However, pore-*SCN5A* (+) probands (n=13) had a higher rate of cardiac events than the rate noted for *SCN5A* (–) probands (n=198) (*P*=0.005) (Figure 4B). A total of 17 asymptomatic nonpore-*SCN5A* (+) probands had no cardiac events. The difference in cardiac event rate between pore-*SCN5A* (+) and nonpore-*SCN5A* (+) asymptomatic probands was not statistically significant (*P*=0.064).

Predictors of Cardiac Events

The univariate analysis showed that a history of ACA, *SCN5A* mutation–positive status, ICD implantation at baseline, documented atrial fibrillation, P-wave duration (lead II) ≥120 ms, and QRS duration (lead V_2) ≥120 ms predicted an increased likelihood for future cardiac events (Table 6). Importantly, other variables such as a history of syncope alone, family history of SCD, spontaneous type 1 ECG pattern, VF/VT inducibility during EPS, and positive late potentials were not significant predictors. In the multivariate analysis that did not include ICD implantation at baseline as a covariate, only a history of ACA and *SCN5A* mutation–positive status persisted as significant predictors (hazard ratio [HR], 6.5 and 2.0, respectively) (Table 7). When including ICD implantation at baseline into this model, only history of ACA remained a significant predictor, although ICD implantation at baseline and *SCN5A* mutation–positive status also had high HRs (HR, 1.9; Table 8).

In Silico Prediction

The in silico algorithm predicted that 6 of the 55 identified *SCN5A* mutations could be attributed to benign variants (Table 2, online-only Data Supplement Table I). After reclassifying the probands with benign variants from the *SCN5A* (+) to the *SCN5A* (–) group, there was still a significant difference between the *SCN5A* (+) (n=54) and *SCN5A* (–) probands (n=361, *P*=0.011) in terms of cardiac event rate (online-only Data Supplement Figure IA). None of the 6 probands predicted to have benign

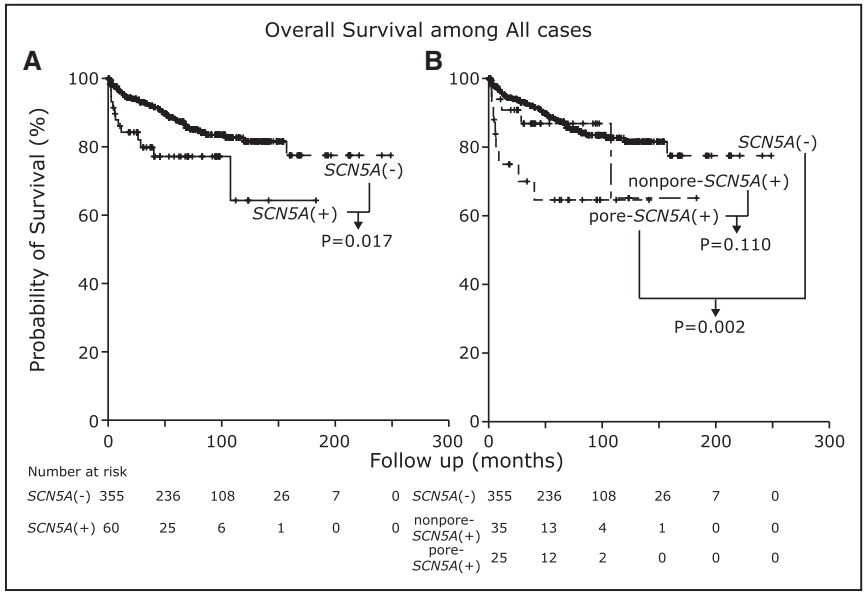

Figure 1. Kaplan-Meier analysis of the cardiac event–free survival in the entire registry.
A, Analyses according to with (= *SNC5A* (+)) and without (= *SCN5A* (–)) *SCN5A* mutations. **B,** Analyses with pore region mutations (= pore-*SCN5A* (+)) and nonpore region mutations (= nonpore-*SCN5A* (–)), and without mutations (= *SCN5A* (–)) in the *SCN5A* probands.

Table 5. Clinical Characteristics and Event Types in 62 Probands Who Had Cardiac Events During the Follow-Up

Patient ID	Sex	FH of SCD	History of ACA	History of Syncope	Spontaneous Type 1 ECG	QRS V2≥120 ms	Documented Atrial Fibrillation	EPS Positive	*SCN5A* Mutation	ICD Implantation	Event Type
5	M	+	-	–	–	–	–	–	–	+	ICD shock
10	M	–	–	+	+	–	–	NA	–	+	ICD shock
33	M	–	+		–	+	–	+	+ (pore)	+	ICD shock
38	M	–	+		+	–	+	+	–	+	ICD shock
42	M	–	+		+	–	–	+	–	+	ICD shock
44	M	–	+		+	–	+	–	+	+	ICD shock
45	M	+	+		+	–	–	–	–	+	ICD shock
48	M	–	+		–	–	+	+	–	+	ICD shock
52	M	–	+		–	–	–	+	–	+	ICD shock
55	M	–	+		+	–	–	–	+	+	ICD shock
60	M	–	+		–	–	–	–	–	+	ICD shock
64	M	–	+		+	–	+	+	–	+	ICD shock
65	M	–	+		+	+	–	+	–	+	ICD shock
68	M	–	+		+	–	–	+	–	+	ICD shock
70	M	–	+		–	–	–	+	–	+	ICD shock
71	M	–	+		+	–	+	+	–	+	ICD shock
73	M	–	+		+	–	–	–	–	+	ICD shock
75	M	–	+		+	–	–	–	+	+	ICD shock
76	M	–	–	+	+	+	+	+	+ (pore)	+	ICD shock
87	M	+	+		+	+	–	+	+	+	ICD shock
88	M	+	+		+	–	–	+	–	+	ICD shock
91	M	–	+		–	–	–	NA	–	+	ICD shock
118	M	–	–	–	+	–	+	+	–	–	SCD
129	M	–	–	–	+	–	–	–	–	–	SCD
131	M	+	–	–	+	–	–	–	–	–	SCD
139	M	–	–	–	+	–	–	+	+ (pore)	+	ICD shock
163	M	–	–	–	+	–	–	+	–	+	ICD shock
172	M	+	–	–	+	+	–	–	+ (pore)	+	ICD shock
186	M	–	+		+	–	–	+	–	+	ICD shock
229	M	–	+		+	–	+	+	–	+	ICD shock
232	M	–	–	+	+	–	+	+	–	+	ICD shock
238	M	–	–	+	+	+	–	+	+ (pore)	+	ICD shock
240	M	–	+		+	–	–	+	–	+	ICD shock
250	M	–	+		+	+	–	–	+ (pore)	+	ACA
253	M	–	–	–	–	–	–	–	–	–	ACA
257	M	–	+		–	–	–	NA	–	+	ICD shock
259	M	–	+		+	–	+	+	–	+	ICD shock
266	M	–	–	+	+	–	–	+	–	+	Self-termination
267	M	–	–	+	–	+	–	+	–	+	ICD shock

(*Continued*)

Table 5. Continued

Patient ID	Sex	FH of SCD	History of ACA	History of Syncope	Spontaneous Type 1 ECG	QRS V2≥120 ms	Documented Atrial Fibrillation	EPS Positive	*SCN5A* Mutation	ICD Implantation	Event Type
270	M	−	+		+	+	−	−	+ (pore)	+	ICD shock
280	M	−	+		+	−	+	−	−	+	ICD shock
281	M	−	+		+	−	−	+	−	+	ICD shock
282	M	−	+		−	−	+	+	−	+	ICD shock
285	M	−	−	+	+	−	−	+	−	+	ICD shock
286	M	−	−	+	+	+	−	+	−	+	ICD shock
287	F	−	+		+	−	−	+	−	+	ICD shock
288	M	−	−	−	+	−	−	+	−	+	ICD shock
290	M	−	+		+	−	−	+	−	+	ICD shock
319	M	−	−	−	+	−	−	NA	−	+	ICD shock
332	M	−	+		+	+	−	−	+	+	ICD shock
354	M	−	+		+	−	−	+	−	+	ICD shock
355	M	−	−	−	+	−	−	+	−	+	ICD shock
362	M	+	+		+	+	+	−	−	+	ICD shock
371	M	−	−	+	−	−	−	NA	−	+	ICD shock
380	M	+	+		+	−	+	−	−	+	ICD shock
395	M	−	+		−	+	−	+	−	+	ICD shock
399	M	−	−	+	+	+	−	+	−	+	ICD shock
400	M	−	−	−	+	−	−	+	−	+	ICD shock
403	M	+	+		+	−	−	−	−	+	ICD shock
410	M	−	−	−	+	−	−	+	−	+	ICD shock
411	M	+	+		−	−	+	+	−	+	ICD shock
412	M	−	−	−	+	−	−	+	+ (pore)	+	ICD shock

ACA indicates aborted cardiac arrest; EPS, electrophysiology study; F, female; M, male; ICD, implantable cardioverter defibrillator; ICD shock, appropriate implantable cardioverter defibrillator intervention; NA, not available; and SCD, sudden cardiac death.

mutations experienced cardiac events during the follow-up period (Table 2). The trends observed before reclassification were similar to those noted after reclassification among probands with prior ACA (online-only Data Supplement Figure II), those with prior syncope (online-only Data Supplement Figure III), and asymptomatic probands (online-only Data Supplement Figure IV).

DISCUSSION

Characteristics of the Study Population and *SCN5A* Mutations

The aim of this study was to investigate the correlation of *SCN5A* mutations with ECG characteristics and future cardiac events among 415 BrS probands recruited from several participating institutions in Japan. The average follow-up period was 72 months, which represents one of the longest follow-up durations reported for BrS registries.[4,6,14,24–28] The cardiac event rate was 2.5%/y among all probands, and 1.2%/y among probands without prior ACA, which is comparable to values reported in previous studies (1.1%–4.1%/y and 1.3%–1.5%/y, respectively). In the present study, the proportion of male patients was higher than that noted in previous reports regarding non-Japanese populations (97% versus ≈80%). However, the values of parameters such as the age at diagnosis, a history of syncope, and family history of SCD were almost equivalent to those reported in previous studies. Additionally, the incidence of *SCN5A* mutations (14%) and the prevalence of pore-region mutations among all *SCN5A* mutations (42%) were similar to those noted in the largest international compendium on BrS patients, which reports 21% and 42%, respectively.[8] Cardiac event rates were 4.1%/y among probands with an ICD and 0.36%/y among probands without an ICD implanted at baseline, which are comparable to the values noted in a recent cumulative analysis (3.1%/y and 0.65%/y, respectively).[29]

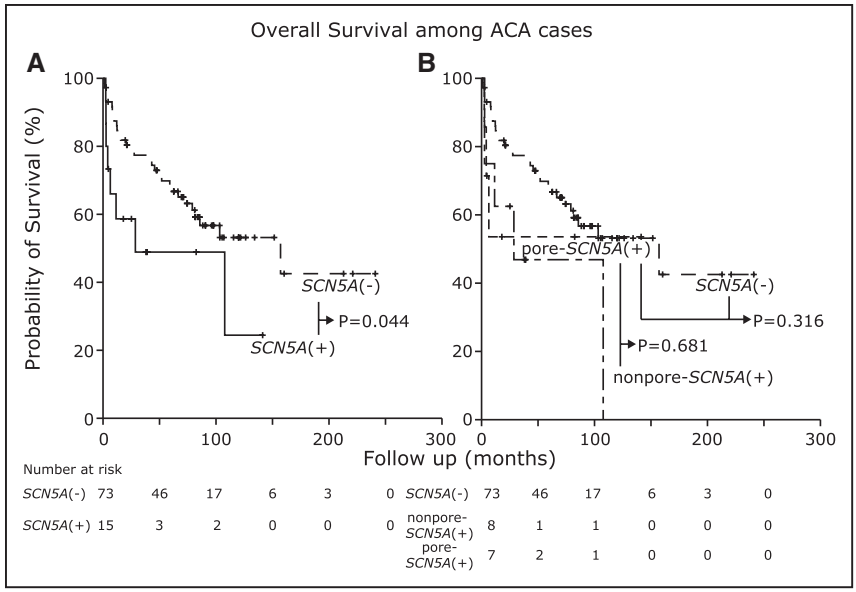

Figure 2. Kaplan-Meier analysis of the cardiac event–free survival in the prior ACA subgroup.
A, Analyses according to with (= *SNC5A* (+)) and without (= *SCN5A* (–)) *SCN5A* mutations. **B,** Analyses with pore region mutations (= pore-*SCN5A* (+)) and nonpore region mutations (= nonpore-*SCN5A* (–)), and without mutations (= *SCN5A* (–)) in the *SCN5A* probands. ACA indicates aborted cardiac arrest.

In summary, the characteristics of the study population and the *SCN5A* mutation rate were comparable to those noted in previous studies; the only exception was the prevalence of male patients, which was higher in our study sample.

Comparison of the ECG Parameters

The ECG parameters indicating depolarization, such as P-wave duration, PQ interval, and QRS duration, had significantly higher values in the *SCN5A* (+) group than in the *SCN5A* (–) group. These findings confirmed the conclusions of previous reports by Smits et al[2] and Yokokawa et al,[10] and were compatible with the fact that *SCN5A* mutations in BrS causes loss of function of the sodium channel, resulting in delayed conduction.[30] In the present study, P-wave duration was also significantly longer in the *SCN5A* (+) group than in the *SCN5A* (–) group, implying that not only the ventricle, but also the atrium is involved in probands with *SCN5A*-mediated BrS.[10]

Prognosis and Risk Stratification

In the present study, a history of ACA was the strongest predictor of future cardiac events, which is in agreement with the findings of previous studies.[4,6,14,25,26,31] The present study also showed that the *SCN5A* mutation–positive status was the only other independent predictor of cardiac events among all probands. One hypothesis to explain this finding involves the mechanism of VF in BrS, which has been proposed to implicate conduction abnormalities.[32,33] Because probands with *SCN5A*-mediated BrS exhibit a reduction in the inward sodium current attributable to a trafficking defect and increased rate of

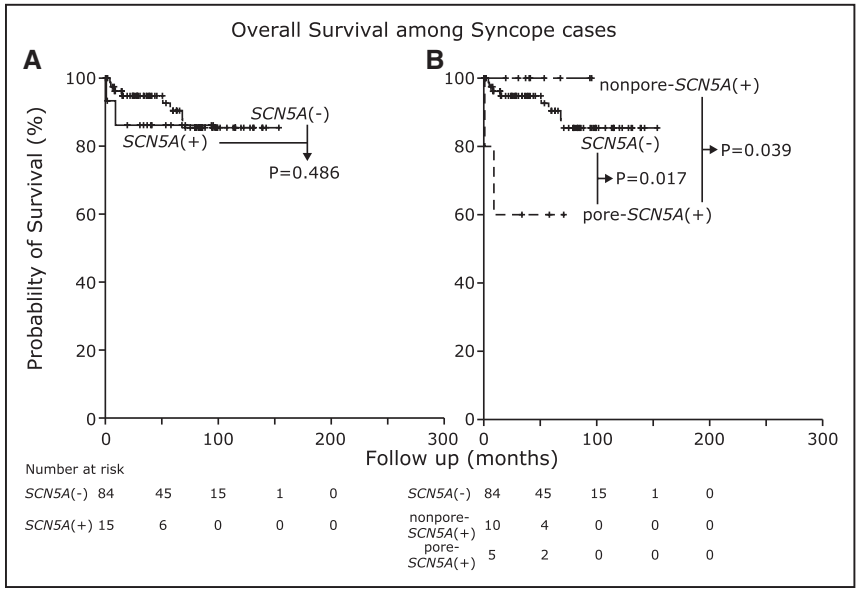

Figure 3. Kaplan-Meier analysis of the cardiac event–free survival in the prior syncope subgroup.
A, Analyses according to with (= *SNC5A* (+)) and without (= *SCN5A* (–)) *SCN5A* mutations. **B,** Analyses with pore region mutations (= pore-*SCN5A* (+)) and nonpore region mutations (= nonpore-*SCN5A* (–)), and without mutations (= *SCN5A* (–)) in the *SCN5A* probands.

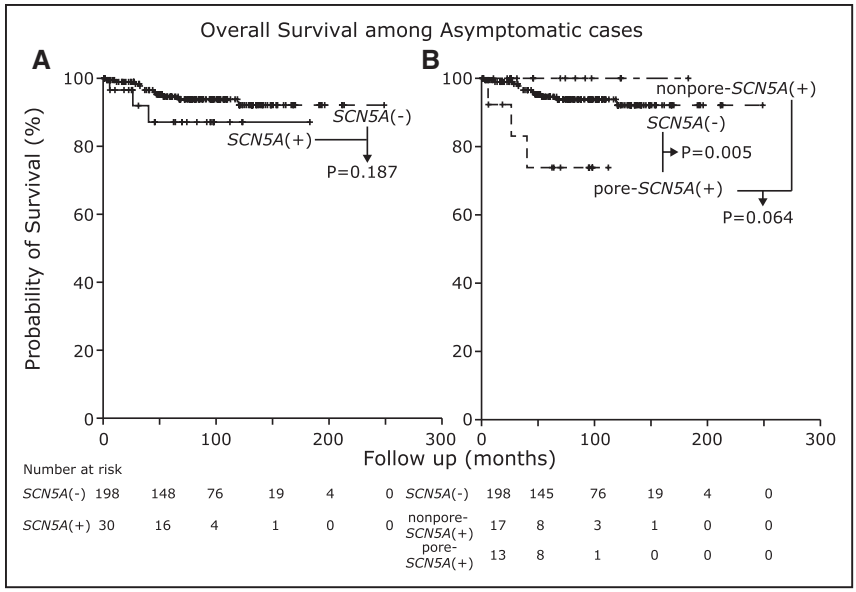

Figure 4. Kaplan-Meier analysis of the cardiac event–free survival in the asymptomatic subgroup.
A, Analyses according to with (= *SNC5A* (+)) and without (= *SCN5A* (–)) *SCN5A* mutations. **B,** Analyses with pore region mutations (= pore-*SCN5A* (+)) and nonpore region mutations (= nonpore-*SCN5A* (–)), and without mutations (= *SCN5A* (–)) in the *SCN5A* probands.

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myocyte apoptosis, there is adequate background for the development of a conduction abnormality, resulting in a higher chance for cardiac events.[13,34] *SCN5A* mutations in the pore region were associated with significantly poorer outcomes than those of *SCN5A* mutation–negative genotypes among probands with prior syncope, among asymptomatic probands, and in the entire study sample, as well (Figures 1B, 3B, and 4B). Moreover, pore-*SCN5A* mutations were associated with significantly poorer outcomes than those of nonpore-*SCN5A* mutations among probands with prior syncope (Figure 3B), and tended to be associated with a higher rate of cardiac events among asymptomatic probands (Figure 4B). Among asymptomatic probands or probands with prior syncope, *SCN5A* mutations in the nonpore region were not associated with cardiac events (Figures 3B and 4B). Several case studies have reported that pore *SCN5A* mutations cause a stronger reduction in the sodium current, and that the location of the *SCN5A* mutation may be related to the severity of the disease.[35,36] Our data suggest that, among all *SCN5A* (+) probands, those with mutations located in the pore region may indeed be at a higher risk for cardiac events. This hypothesis is also supported by our ECG data, which demonstrate that the depolarization parameters (P-wave duration, PQ interval, and QRS duration) had higher values in the probands with pore-*SCN5A* (+) than in those with nonpore-*SCN5A* (+).

The rate of cardiac events was higher in probands with ICD implantation at baseline than in those without (event rates: 4.0%/y versus 0.46%/y, respectively), which had also been seen in previous studies.[6,24,27] Because the ICD has no arrhythmogenic effect in BrS, our findings can be explained in the context of 2 speculations. First, the indication for ICD implantation is strongly influenced by the patient's clinical status such as history of ACA, which is fully described in the guidelines for the diagnosis and management of patients with inherited primary arrhythmia syndromes.[15] Hence, we can infer that the current guideline is established to differentiate high-

Table 6. Univariate Analysis for Arrhythmic Events During the Follow-Up in the Entire Registry

	Univariate Analysis		
	Hazard Ratio	95% Confidence Interval	*P* Value
History of aborted cardiac arrest	6.6	3.9–11.0	<0.001
SCN5A mutation (+)	2.1	1.1–3.8	0.020
Pore-*SCN5A* (+) (vs *SCN5A* (–))	3.2	1.5–6.7	0.003
History of syncope (without aborted cardiac arrest)	2.1	0.9–4.7	0.080
Male	1.4	0.2–10.3	0.722
Implantable cardioverter defibrillator implantation	8.5	3.4–21.2	<0.001
Ventricular fibrillation/ ventricular tachycardia induced at electrophysiology study†	1.7	1.0–3.0	0.058
Family history of sudden cardiac death*	1.1	0.6–2.2	0.769
Spontaneous type 1 electrophysiology study	1.3	0.7–2.4	0.332
Documented atrial fibrillation	1.8	1.0–3.3	0.043
Late potential positive	1.5	0.8–3.0	0.249
P II ≥120 ms	2.7	1.5–4.8	0.001
QRS V2≥120 ms	2.4	1.3–4.3	0.005

*Prevalence at ≤45 years old.
†Induction rate of ventricular fibrillation or polymorphic ventricular tachycardia by ventricular stimulation.

Table 7. Multivariate Analysis for Arrhythmic Events During the Follow-Up in the Entire Registry (Analysis Without Implantable Cardioverter Defibrillator Implantation)

	Multivariate Analysis		
	Hazard Ratio	95% Confidence Interval	*P* Value
History of aborted cardiac arrest	6.5	3.8–11.0	<0.001
SCN5A mutation	2.0	1.0–3.8	0.045
QRS V2≥120 ms	1.4	0.8–2.7	0.268
Documented atrial fibrillation	1.0	0.5–1.8	0.895

risk patients. On the contrary, it is possible that the ICD detected nonlethal arrhythmias that may have self-terminated after the observation period, resulting in overestimation of cardiac event rates in probands with ICD at baseline, because probands without ICD may have no symptoms during these events. Therefore, we also analyzed a multivariate model that included ICD implantation at baseline as a covariate (Table 8) and found that *SCN5A* mutation–positive status showed a similar HR for cardiac events as in the multivariate model that did not include ICD implantation at baseline as a covariate. Interestingly, a retrospective study involving a moderate-risk group reported no ventricular events even though the patients showed syncope.[37] This previous finding can be considered collateral evidence that, when ventricular arrhythmias are defined as a cardiac event, as in the current study, the results can be interpreted to indicate a higher possibility for lethal arrhythmias. Nevertheless, it remains unclear whether all ICD-detected arrhythmias can be considered SCD events, so that our findings may

Table 8. Multivariate Analysis for Arrhythmic Events During the Follow-Up in the Entire Registry (Analysis With Implantable Cardioverter Defibrillator Implantation)

	Multivariate Analysis		
	Hazard Ratio	95% Confidence Interval	*P* Value
History of aborted cardiac arrest	4.2	2.4–7.3	<0.001
SCN5A mutation	1.9	1.0–3.7	0.050
Implantable cardioverter defibrillator implantation	4.5	1.7–11.9	0.002
QRS V2≥120 ms	1.4	0.8–2.7	0.293
Documented atrial fibrillation	1.0	0.5–1.8	0.895

be directly compared against those of previous major studies, which mainly used this definition of cardiac events as a clinical end point.[6,24,26,27,38]

Comparison With Previous Studies

Several previous studies have described genotype-phenotype correlations in channelopathies. Kanai et al[39] reported that mutations in the pore region of the *SCN1A*, which is an analog of *SCN5A* expressed in neurons, correlated with the severity of epilepsy. Mutation site–specific severity in the clinical phenotype, and a genotype-phenotype correlation, as well, have been reported in congenital long-QT syndrome with mutations in *KCNQ1* (LQT1)[40] and *KCNH2* (LQT2).[41] However, only 1 single-center study demonstrated that the *SCN5A* genotype was a significant predictor of recurrent cardiac events in patients with BrS,[42] even though several studies have been undertaken on this topic.[6,14,43,44] However, the 2 large BrS registries included *SCN5A*-positive family members of probands, in a proportion as high as 38% and 67%.[6,14] In the present study, there was no significant difference in the cardiac event rate between the *SCN5A* (+) and *SCN5A* (–) groups of asymptomatic probands (Figure 4A). This observation suggests that including a higher proportion of *SCN5A*-positive family members, who are often asymptomatic, likely dilutes the relevance of the *SCN5A* genotype as a predictor of cardiac events in a given population. The present study included only probands with BrS, which likely explains the discrepancies between the observations of previous investigations and those of the present study in terms of the relevance of *SCN5A* mutation status.

In our study, prior syncope may have included neurally mediated syncope, because the condition was observed in >30% of Japanese BrS probands.[45] This fact may have influenced our conclusion that prior syncope was not a significant risk factor for cardiac events. Nevertheless, we did note a tendency for higher HR in the univariate analysis (HR, 2.1; *P*=0.080).

A spontaneous type 1 ECG pattern was observed in 72% of probands, which is among the highest incidences reported to date (45%–71%).[6,24,26,27,31] It is possible that this high incidence of spontaneous type 1 ECG pattern is related to the long-term follow-up period included in the present study, which may have resulted in a higher chance to record such ECG features. These probands are considered to have lower risk of cardiac events,[46,47] and may explain our conclusion that spontaneous type 1 ECG pattern does not represent a significant predictor of cardiac events.

During the EPS, we used aggressive triple extrastimuli performed at 2 ventricular sites, with a coupling interval of up to 200 ms, which may have influenced our conclusion that EPS parameters did not represent significant predictors. Nevertheless, this finding is concordant

with the conclusion of a recent study showing a negative predictive value for EPS parameters.[24,27]

Another factor that may account for the difference between the findings of previous investigations and those of the present study is related to the *SCN5A* promoter haplotype B, which is only recognized in Asians (including Japanese populations).[48] Because these polymorphisms are absent in white and black individuals, it is possible that there are population-specific differences in the rate of cardiac events resulting from the combination of such polymorphisms and pathological *SCN5A* mutations. Furthermore, the mutation spectrum varies between registries, and the severity of each sodium current deficiency depends on which mutation the probands possess. However, because the incidence of *SCN5A* mutations and the rate of pore-localizing mutations noted in this study were similar to those reported in other registries, we believe that the effect of the variability in the mutation spectrum was negligible.[6,8,14]

Utility of an In Silico Prediction Tool

Differentiating a pathogenic mutation from a variant of the *SCN5A* gene is important, because 2% to 5% of ostensibly healthy individuals host a rare, and most likely innocuous, nonsynonymous single-nucleotide variant.[8,49] In an effort to account for this potential confounding factor, we used an in silico prediction algorithm to assign a pathogenic/benign status. The algorithm suggested that 6 of the 55 mutations (10.9%) could be attributed to benign variants. Performing the same analyses after the in silico reclassification of the identified mutations, we found the same trends regarding the risk for cardiac events; only the values for patients with a history of ACA differed, but there was still a tendency for an increased rate of cardiac events in the *SCN5A* (+) probands (Figures 1 through 4 and online-only Data Supplement Figures I through IV). Currently, it is difficult to estimate the severity of the biochemical deficiency entailed by each mutation, and thus the exact utility of in silico tools for risk stratification among BrS patients remains unknown. However, because there were no cardiac events in patients with mutations attributed to benign variants, as predicted by the in silico algorithm, we believe that such an algorithm can contribute to improving risk stratification efforts. In the future, a tailored risk stratification approach may be developed by using mutation analyses involving induced pluripotent stem cells.[50]

Study Limitations

The present study focused only on mutations in the coding region of *SCN5A*, and the possibility of a mutation occurring in other regions could not be excluded. The effect of such genetic mutations represents a topic of research to be addressed in future studies.

CONCLUSIONS

Patients with *SCN5A*-mediated BrS exhibit stronger conduction abnormalities and have higher risk for future cardiac events. Worse outcomes are noted when the *SCN5A* mutation lies in the pore region.

ACKNOWLEDGMENTS

The authors express gratitude for the timely assistance provided by Dr Yoko M. Nakao from the Department of Preventive Medicine and Epidemiological informatics, National Cerebral and Cardiovascular Center, and by Dr Koko Asakura from the Office of Biostatistics and Data Management, National Cerebral and Cardiovascular Center, Osaka, in analyzing the large number of samples. The authors thank John Martin for his linguistic assistance.

SOURCES OF FUNDING

Drs Horie, Ogawa, Aizawa, Ohe, Yamagishi, Makita, Tanaka, Hagiwara, Kishi, Nakano, Makiyama, Fukuda, Watanabe, Morita, Ohno, Kusano, Hayashi, Aiba, Kamakura, Miyamoto, and Shimizu were supported in part by grants from the Ministry of Health, Labor and Welfare of Japan for Clinical Research on Intractable Diseases (H18-002, H24-033, H26-040). Drs Ackerman and Kapplinger were supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program (to Dr Ackerman).

DISCLOSURES

Dr Ackerman is a consultant for Boston Scientific, Gilead Sciences, Invitae, Medtronic, and St. Jude Medical. The Mayo Clinic and Dr Ackerman have received sales-based royalties from Transgenomic for FAMILION-LQTS and FAMILION-CPVT genetic tests. However, none of those companies provided any support for this study.

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FOOTNOTES

Received July 21, 2016; accepted March 13, 2017.

The online-only Data Supplement is available with this article at http://circ.ahajournals.org/lookup/suppl/doi:10.1161/CIRCULATIONAHA.117.027983/-/DC1.

Circulation is available at http://circ.ahajournals.org.

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