gation effect of antipsychotics more clearly, we sampled the patients who were administrated high-dose antipsychotics as the antipsychotic administration group [the mean chlorpromazine-equivalent dose of prescribed antipsychotic was 1799.7 mg (SD: 1454.5)]. Almost all of the patients were administered first-generation antipsychotics, and some of these medications have a relatively high anticholinergic effect (chlorpromazine and levomepromazine). First generation antipsychotics were administered to 76 patients [haloperidol: 53 patients [mean dose (SD) = 20.2 (12.2) mg], chlorpromazine: 32 patients [mean dose (SD) = 224.2 (208.0) mg]. levomepromazine: 31 patients [mean dose (SD) = 90.2 (68.5) mg]. On the other hand, only 3 patients were treated by monotherapy with a second generation antipsychotic (olanzapine 20 mg or risperidon 3 mg or 5 mg). The third group comprised 85 healthy volunteers who had undergone routine health check-ups between 2010 and 2012 at Dokkyo Medical University Hospital, Japan. These participants had no physical abnormalities or abnormal laboratory data.

Patients were diagnosed according to the DSM-IV-TR criteria. The demographic data for each group are shown in Table 1. One-way analysis of variance (ANOVA) revealed no significant differences in age or sex between the groups, although significant differences were observed for heart rate and smoking status. Namely, the groups were matched on age and the proportion of sex which are known to affect QT interval; however, the number of smokers with potential to affect the QT interval was not controlled.

This study is a retrospective cross-sectional study. The data we prepared were recorded for clinical use. All subjects for whom complete medical records were available were included in the study. From medical record, no participants were judged to use illicit substances. Based on physical examination and chest radiography, structural heart disease was considered unlikely in the included participants. We collected the data from clinical records after approval was given by the Institutional Review Boards of Dokkyo Medical University School of Medicine and the National Institute of Neurology and Psychiatry. The design of this study also follows the ethical norm from the Ministry of Health, Labor and Welfare of Japan.

In our approved method, written informed consent was not given by participants. To anonymize their clinical records which were used in this study, the information which can identify an individual (e.g. name and registration number) was not prepared. Furthermore, the clinical data were put together to one person and were managed. Another person who took charge of statistical analysis could not access original data of each individual. Because the all participated patients agreed with hospital treatment spontaneously and healthy controls underwent the examinations spontaneously, in the participants, there was no person who had a compromised capacity/ability to consent. The children were not included in the participants.

Evaluation of the QT interval

The QT interval was measured manually according to previous report [12]. The end of the T-wave was determined as the intersection between the tangent to the steepest downslope of the T-wave and the isoelectric line. The QT interval was corrected as a value that varies in relation to heart rate. A number of methods for correcting the QT interval by heart rate have been proposed, each with distinctive characteristics. In the current study, we used four correction methods, as follows: methods from Bazett [13], Fridericia [14], Framingham [15], and Hodges [16], with the following correction formulae:

Bazett: $QTcB = QT(HR/60)^{1/2} = QT(RR)^{-1/2}$

Fridericia: $QTcFri = QT(HR/60)^{1/3} = QT(RR)^{-1/3}$

Framingham:

$QTcFra = QT + 154(1 - 60/HR) = QT + 0.154(1000 - RR)$

Hodges: $QTcH = QT + 1.75(HR - 60) = QT + 105(1/RR - 1)$

where HR is heart rate and RR is R-R interval.

Datal analysis

Analysis of covariance (ANCOVA) was used to compare the QT interval corrected using the four correction methods described above (QTcB, QTcFri, QTcFra and QTcH) between drug-free patients with schizophrenia, medicated patients with schizophrenia and healthy controls. Different covariates were selected for the different comparisons and they are presented in the corresponding sections in the Results. Sex (male: 1, female: 2) and smoking status (smoking: 1, nonsmoking: 2) were also compared between the groups. Bonferroni's method for multiple comparisons was used to compare the simple main effect of the QT interval, and the level of significance was set at $P<0.05$. Statistical analysis was conducted using PASW statistics 18 (SPSS Inc., Chicago, IL).

Table 1. Demographic data of participants and comparison of QTc calculated with four types of formulae.

	Normal control	Schizophrenia (drug free)	Schizophrenia (medication)
N	85	85	85
Chlorpromazine equivalent daily dose (SD):mg/day	_	0	1799.7(1454.5)
Male (n, %)	38(45)	36(42)	40(47)
Age, years (SD)	42.5(5.6)	40.4(14.6)	40.2(14.0)
Smoking (n, %)*	19(22)	31(36)	33(39)
Pulse, beat per minute (SD)**	63.9(8.9)	81.0(19.8)	79.8(15.6)
QTcB(SD)$^{+}$:msec	390.8(21.2)	406.1(25.0)	423.3(33.6)
QTcFri (SD)$^{+}$:msec	387.2(18.4)	388.7(29.8)	405.3(36.2)
QTcFra (SD)$^{+}$:msec	387.2(18.6)	390.5(27.1)	406.4(32.7)
QTcH (SD)$^{+}$:msec	387.4(17.9)	394.6(25.4)	407.6(30.8)

Statistically Significant *p<.05; **p<.01 by one-way ANOVA
Statistically Significant $^{+}$p<.01 by ANCOVA.
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Results

Table 1 shows the demographic data and QT intervals corrected by the four methods for the drug-free patients, medicated patients, and controls.

The ANCOVA with age and heart rate as covariates showed a significant difference between the three groups regardless of the type of correction method used. Bonferroni's post hoc test revealed that the QT interval corrected using each of the four methods was significantly longer in medicated patients than in drug-free patients and controls. In addition, the corrected QT interval in drug-free patients was significantly longer compared to that of the controls (Table 2). These differences might be caused by differences in heart rate, as one-way ANOVA revealed a higher heart rate in the drug-free and medicated patients compared to normal controls and previous reports indicated that values corrected using the Bazett, Framingham and Fridericia formulae are more influenced by relatively higher heart rate than is the Hodges formula [17].

Taking this into account, we focused on subjects with heart rate <90 beats/min to minimize the influence of heart rate on the results. The demographic data for each of the three groups are shown in Table 3. The data revealed a significant difference between the groups regardless of the type of correction method used (i.e., QTcB, QTcFri, QTcFra, and QTcH) when ANCOVA was performed with only age as a covariate. Bonferroni's post hoc test also revealed that QTcB, QTcFri, QTcFra and QTcH were significantly longer in medicated patients than in drug-free patients and controls. Additionally, QTcB, QTcFri, QTcFra and QTcH were significantly longer in drug-free patients than in the controls (Table 4).

The QT interval is known to differ between the sexes, with females having longer QT intervals than males. In support of this, we observed that female controls had longer QTcB, QTcFri, QTcFra and QTcH intervals than did male controls. However, importantly, no sex difference in terms of QTc interval was observed in the drug-free or medicated patients. We observed no sex differences in heart rate between the drug-free patients, medicated patients, or controls (Table 5).

Discussion

In this study, after adjustments for age and heart rate, patients with schizophrenia displayed longer QT intervals than healthy volunteers even when they were not receiving antipsychotics, although antipsychotics were shown to further prolong the QT interval. The magnitude of the difference in the QT interval between controls and drug-free schizophrenic patients is small. Furthermore, patients with schizophrenia had a higher heart rate than did the healthy volunteers. Whereas a sex difference was observed in the QT interval in the normal controls, no such difference was found in the patients with schizophrenia. In schizophrenia, studies of the autonomic system, which affects the QT interval [10], have detected a number of abnormalities. For example, decreased heat rate variability, a marker of cardiac parasympathetic activity has been demonstrated in patients with psychosis [18–20]. Furthermore, increased variability in the QT interval has been reported in first episode-neuroleptic naïve psychosis [11] as well as in schizophrenic patients and their relatives [19], suggesting that schizophrenia itself is the risk factor for QT interval abnormality.

The KCNH2 channel is thought to be involved in the molecular biological mechanism of pre-existing prolonged QT intervals in schizophrenic patients for several reasons. First, the KCNH2 channel is expressed in myocardial cells and is related to the early components of the inward current necessary for repolarizing myocardial cells [1]. Second, some patients with familial long QT syndrome suffer from genetic KCNH2 abnormalities [2–3]. Third, blockage of the KCNH2 channel is a probable molecular mechanism for antipsychotic-induced QT interval prolongation [21]. Fourth, association studies have shown that the KCNH2 channel is associated with schizophrenia [6–9]. It was also recently discovered that KCNH2 channels are expressed in the brain, including the midbrain dopamine neurons, where they are expected to alter dopamine release [21].

Another genetic factor that is considered to be involved in pre-existing QT interval prolongation in schizophrenic patients is the neuregulin 1 gene (NRG1), which Stefansson et al. reported as potentially related to the onset of schizophrenia [22]. NRG1 plays various roles in the central nervous system, promotes the differentiation of myocardial cells of the cardiac conduction system [23], and is thought to be related to the control of cardiac autonomic nervous balance [24]. Therefore, NRG1 could be a candidate factor for explaining the prolongation of the QT interval observed in the patients with schizophrenia in the present study. In addition, a report recently indicated that a common missense variant in the NRG1 gene is associated with both schizophrenia and sudden cardiac death [25]. This report might indicate the association between NRG1 and QT interval prolongation, as QT interval prolongation is the most widely used surrogate marker for assessing the risk of torsades de pointes (TdP), one of the causes of sudden cardiac death, although the QT interval is considered somewhat imprecise as a surrogate marker for TdP [26]. Furthermore, the present finding that the heart rate of patients with schizophrenia was increased compared to controls is consistent with previous reports [19,27] and with the finding that the heart rate of mice with impaired NRG1 function was higher than that of control animals [28].

Healthy female volunteers have significantly longer QT intervals than healthy males [13]. However, some studies on QT intervals in patients with schizophrenia receiving antipsychotics have reported no significant sex difference [5,29–30]. For

Table 2. Difference of main effects between each group.

	Difference of estimated marginal means (msec)			
	QTcB (SD)	QTcFri (SD)	QTcFra (SD)	QTcH (SD)
Schizophrenia(drug free)-normal control	19.6(5.3)**	20.1(5.0)**	19.8(4.6)**	21.5(4.5)**
Schizophrenia(medication)-normal control	38.5(5.2)**	37.5(4.9)**	36.5(4.5)**	35.4(4.4)**
Schizophrenia(medication)-schizophrenia(drug free)	18.9(4.3)**	17.4(4.0)**	16.7(3.7)**	13.8(3.6)**

Note, SD: standard deviation.
Statistically significant **p<.01.
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Table 3. Demographic data of participants whose heart rate is less than 90 beats per minutes.

	Normal control	Schizophrenia (drug free)	Schizophrenia (medication)
N	83	55	65
Chlorpromazine equivalent daily dose (SD):mg/day	_	0	1654.2(1313.7)
Male(n, %)	37(45)	24(44)	31(48)
Age, years (SD)	42.4(5.5)	40.4(14.2)	40.3(14.3)
Smoking (n, %)*	19(45)	23(42)	28(43)
Pulse, beat per minute (SD)**	63.3(7.8)	68.9(11.9)	73.4(11.0)
QTcB (SD)$^{+}$:msec	390.5(21.3)	408.8(25.2)	427.1(28.9)
QTcFri (SD)$^{+}$:msec	387.5(18.5)	400.6(26.5)	414.0(29.8)
QTcFra (SD)$^{+}$:msec	387.5(18.8)	401.4(24.7)	414.7(27.5)
QTcH (SD)$^{+}$:msec	387.5(18.0)	401.4(26.1)	413.2(28.1)

Pulse<90.
Statistically Significant *p<.05; **p<.01 by one-way ANOVA
Statistically Significant $^{+}$p<.01 by ANCOVA.
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Table 4. Difference of main effects between each group.

	Difference of estimated marginal means (msec)			
	QTcB (SD)	QTcFri (SD)	QTcFra (SD)	QTcH (SD)
Schizophrenia(drug free)-normal control	19.1(5.1)**	19.7(4.9)**	19.4(4.7)**	21.4(4.6)**
Schizophrenia(medication)-normal control	38.0(5.0)**	38.0(4.8)**	36.7(4.6)**	38.5(4.6)**
Schizophrenia(medication)-schizophrenia(drug free)	18.9(4.8)**	18.3(4.6)**	17.3(4.4)**	17.1(4.3)*

(heart rate of participants is less than 90 beats per minutes).
Note, SD: standard deviation.
Statistically significant *p<.05; ** p<.01.
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Table 5. Comparison between gender in heart rate and QT interval by one-way ANOVA.

	Normal control	Schizophrenia (drug free)	Schizophrenia (medication)
Heart rate(beats/min.)			
Male	62.5(9.3)	81.1(21.5)	79.1(15.5)
Female	65.0(8.4)	80.8(18.6)	80.6(15.9)
QTcB(msec)			
Male	379.2(20.5)**	406.7(25.8)	424.7(36.6)
Female	400.2(16.6)	405.7(24.7)	409.1(26.2)
QTcFri(msec)			
Male	377.1(17.4)**	389.4(29.9)	391.6(31.5)
Female	395.3(15.0)	388.2(30.0)	395.5(28.9)
QTcFra(msec)			
Male	376.8(17.6)**	390.7(27.0)	392.0(29.3)
Female	395.7(14.9)	390.3(27.4)	396.9(26.7)
QTcH(msec)			
Male	377.9(17.4)**	396.2(27.6)	394.6(29.6)
Female	395.0(14.4)	393.4(23.9)	398.1(23.8)

Statistically significant **p<.01 by one-way ANOVA.
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example, Ramos-Rios et al. reported that the mean corrected QT (QTc) for male schizophrenic patients aged >50 years exceeded that for female patients of the same age, whereas no significant difference was found between the mean QTc of male and female patients aged <50 years [31]. The present study found a sex difference in the QTc interval of healthy controls but not in patients with schizophrenia. Although sex differences in the symptoms and prognosis of schizophrenia have been reported [32], it is conceivable that the influence of factors that are involved in the etiology of schizophrenia (e.g., KCNH2 or NRG1) on the QT interval might be sufficient to negate the sex difference observed in healthy controls.

In the present study, four types of QT interval correction formulae were applied, although the Bazett formula seems to give higher QTc values in patients with schizophrenia but not in controls, compared to the other corrections. The Bazett correction is widely used in clinical practice but may thus not be the best choice in schizophrenia.

This study has some limitations. The number of participants is relatively small because of the difficulty in preparing the ECG data of drug-free schizophrenic patients. Each participant underwent a single ECG examination. In addition, the measurement timing was not fixed, although it has some diurnal variation. Obesity and binge drinking have been indicated as factors affecting the QT interval [33–34], but we did not estimate such factors in this study. Structural heart disease, which is one of the most important variables responsible for QT prolongation, could not be excluded completely because echocardiography was not performed. However, all participants underwent physical examination and chest radiographic inspection. Therefore, we decided that in participants, the probability of suffering from structural heart disease was very low and should hardly affect the results.

In conclusion, this study found that patients with schizophrenia, even when not receiving antipsychotics, had a longer QT interval than healthy volunteers. Additionally, the QT interval was further prolonged by the administration of antipsychotics. Although the QTc interval is considered somewhat imprecise as a surrogate marker for assessing the risk of TdP, we speculate that the QT interval may be manifestation of a certain biological feature of schizophrenia.

Author Contributions

Conceived and designed the experiments: KF YO. Performed the experiments: KF YO HO YT TS HH. Analyzed the data: KF YO. Contributed reagents/materials/analysis tools: KF YO. Wrote the paper: KF YO. Aided in study design and edited the manuscript: MO MH HK KS.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

Risk Stratification in Patients With Brugada Syndrome Without Previous Cardiac Arrest

– Prognostic Value of Combined Risk Factors –

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Background: Risk stratification in patients with Brugada syndrome for primary prevention of sudden cardiac death is still an unsettled issue. A recent consensus statement suggested the indication of implantable cardioverter defibrillator (ICD) depending on the clinical risk factors present (spontaneous type 1 Brugada electrocardiogram (ECG) [Sp1], history of syncope [syncope], and ventricular fibrillation during programmed electrical stimulation [PES+]). The indication of ICD for the majority of patients, however, remains unclear.

Methods and Results: A total of 218 consecutive patients (211 male; aged 46±13 years) with a type 1 Brugada ECG without a history of cardiac arrest who underwent evaluation for ICD including electrophysiological testing were examined retrospectively. During a mean follow-up period of 78 months, 26 patients (12%) developed arrhythmic events. On Kaplan-Meier analysis patients with each of Sp1, syncope, or PES+ suffered arrhythmic events more frequently (P=0.018, P<0.001, and P=0.003, respectively). On multivariate analysis Sp1 and syncope were independent predictors of arrhythmic events. When dividing patients according to the number of these 3 risk factors present, patients with 2 or 3 risk factors experienced arrhythmic events more frequently than those with 0 or 1 risk factor (23/93 vs. 3/125; P<0.001).

Conclusions: Syncope, Sp1, and PES+ are important risk factors and the combination of these risks well stratify the risk of later arrhythmic events. (*Circ J* 2015; **79:** 310–317)

Key Words: Brugada syndrome; Electrophysiological study; Primary prevention; Risk stratification; Syncope

Brugada syndrome (BrS), characterized by ST-segment elevation in the right precordial leads (type 1 Brugada electrocardiogram [ECG]) and a high incidence of ventricular fibrillation (VF) leading to sudden cardiac death (SCD) in young and otherwise healthy adults, has been reported since the initial description of the disease.[1] It is estimated to be responsible for at least 4% of all sudden deaths and at least 20% of sudden deaths in patients with structurally normal hearts.[2] Clearly, a method for the risk stratification of patients with BrS is required. A number of studies have noted a high recurrence rate of VF in patients with BrS who had previously experienced cardiac arrest.[3–7] Further discussion regarding the indications for implantable cardioverter defibrillator (ICD) in these patients (secondary prevention) is unnecessary. The indications for ICD for primary prevention of SCD in patients with BrS without a history of VF or cardiac arrest, however, have not been well evaluated. A recent consensus statement suggested the indication of ICD for these patients based on the following risk factors: spontaneous diagnostic type 1 Brugada ECG (Sp1), history of syncope likely caused by ventricular

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Table 1. Patient Characteristics vs. Presence of SCD or VF

	SCD or VF (+) (n=26)	SCD or VF (–) (n=192)	P-value	Overall (n=218)
Age (years)	50±11	46±13	0.19	46±13
Male	25 (96)	186 (97)	0.84	211 (96)
Follow-up period (months)	51±54	81±49	0.0023	78±49
ICD implantation	24 (92)	98 (51)	<0.001	122 (56)
ICD period of implantation (months)	44±34	87±40	<0.001	78±43
Sp1	24 (92)	135 (70)	0.018	159 (73)
Syncope likely caused by arrhythmia	21 (81)	66 (34)	<0.001	87 (40)
Inducibility of VF (PES+)	13 (50)	48 (25)	0.0077	61 (28)
Family history of SCD	7 (27)	57 (30)	0.77	64 (29)

Data given as n (%) or mean±Standard Deviation. ICD, implantable cardioverter defibrillator; PES, programmed electrical stimulation; SCD, sudden cardiac death; Sp1, spontaneous type 1 Brugada electrocardiogram; VF, ventricular fibrillation.

arrhythmia (syncope), and development of VF during programmed electrical stimulation (PES+, ie, inducible patients).[8] This statement recommends ICD implantation for primary prevention of SCD as a class IIa indication for patients with Sp1 and syncope and as a class IIb indication for patients with PES+. The indication of ICD for the patients who do not meet these conditions, however, remains unclear. Here we hypothesized that these 3 proposed risk factors may be used to stratify the risk of SCD in patients with BrS and without previous cardiac arrest.

Methods

Patients and Baseline Data

Among 612 patients who had been given the diagnosis of BrS at 2 Japanese institutions, Okayama University Hospital or the National Cerebral and Cardiovascular Center between 1996 and 2012, we retrospectively enrolled 218 consecutive patients with type 1 Brugada ECG, without a history of VF or cardiac arrest, who were hospitalized and underwent electrophysiological testing to evaluate the suitability of ICD therapy. All patients were followed for at least 12 months from the start of the investigation. The primary endpoint was the occurrence of VF or SCD. Patients with structural cardiac abnormalities on transthoracic echocardiography were excluded. Written informed consent regarding the data acquisition was obtained from all individuals. The study conforms to the 1975 Declaration of Helsinki as reflected by approval by the Institutional Review Board in both institutions.

In accordance with the consensus report in 2005, type 1 Brugada ECG was defined as coved-type ST-segment elevation ≥0.2 mV followed by a negative T wave in more than 1 right precordial lead (V1–V3) in the presence or absence of a sodium channel-blocking agent.[5] Although ECG could change during follow-up, Sp1 was defined as spontaneous type 1 Brugada ECG in the absence of a sodium channel-blocking agent at the beginning of the study. ECG was recorded at least 4 different times in each patient. Placement of the right precordial leads in a superior position up to the second intercostal space was performed to increase sensitivity. A sodium channel-blocking agent (i.v. pilsicainide up to 1 mg/kg body weight) was used under careful monitoring to unmask ECG abnormalities when Sp1 was not observed. All patients underwent electrophysiological evaluation to assess the inducibility of VF by PES. The protocol involved up to 3 extrastimuli applied to the right ventricular apex and right ventricular outflow tract at a minimum coupling interval of 180 ms. PES+, however, was defined as VF or polymorphic ventricular tachycardia lasting >30 s or requiring direct current shock induced at a coupling interval ≥200 ms according to the consensus document.[5] Syncope was considered present when a patient had an episode of syncope judged as likely caused by ventricular arrhythmia. Syncope likely due to vasovagal events such as those occurring during abrupt postural changes, exposure to heat and dehydration, or emotional reactions were excluded. Data on family history of SCD prior to age 45 (FH) were also collected. Combinations of the 3 risk factors proposed in the consensus statement, that is, Sp1, syncope, and PES+, were examined for further risk stratification of arrhythmic events.

Follow-up and Arrhythmic Events

Patient treatment, including ICD implantation, was based on physician clinical judgment. All patients were followed up for at least 12 months after the start of the investigation. Patients with ICD were followed every 3 months while those without ICD were followed at least once a year. Arrhythmic events were defined as SCD or appropriate shock delivery by ICD against ventricular tachyarrhythmia.

Statistical Analysis

Data were analyzed using JMP (version 11.0.0, SAS, Cary, NC, USA). For continuous variables, comparisons among groups were made using Student's t-test. Pearson's chi-squared test was used for categorical variables. Event analysis over time was performed using the Cox proportional hazard regression model. Risk was quantified as a hazard ratio (HR) with 95% confidence interval. Survival curves were constructed using the Kaplan-Meier method and compared with the log-rank test. $P<0.05$ was considered statistically significant.

Results

Patient Characteristics and Overall Event Rate

A total of 218 consecutive patients were examined. As shown in Table 1, the mean patient age at diagnosis was 46±13 years and most patients were men (n=211, 96%). ICD were implanted in more than half of the patients (n=122, 56%). Sp1 was observed in 159 patients (73%), and 87 patients (40%) had experienced syncope. PES+ was observed in 61 patients (28%), and 64 patients (29%) had FH. During the mean follow-up period of 78±49 months (median, 76 months), 26 patients (12%) experienced at least 1 arrhythmic event. The pe-

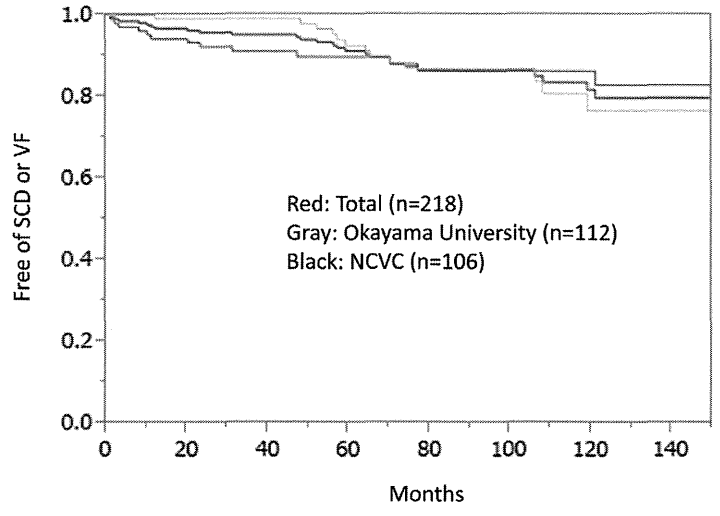

Figure 1. Kaplan-Meier analysis of lethal arrhythmic events during follow-up in 218 patients with Brugada type 1 electrocardiogram but without a history of cardiac arrest, both overall (red line) as well as separately at Okayama University (gray) and the National Cerebral and Cardiovascular Center (NCVC; black). SCD, sudden cardiac death; VF, ventricular fibrillation.

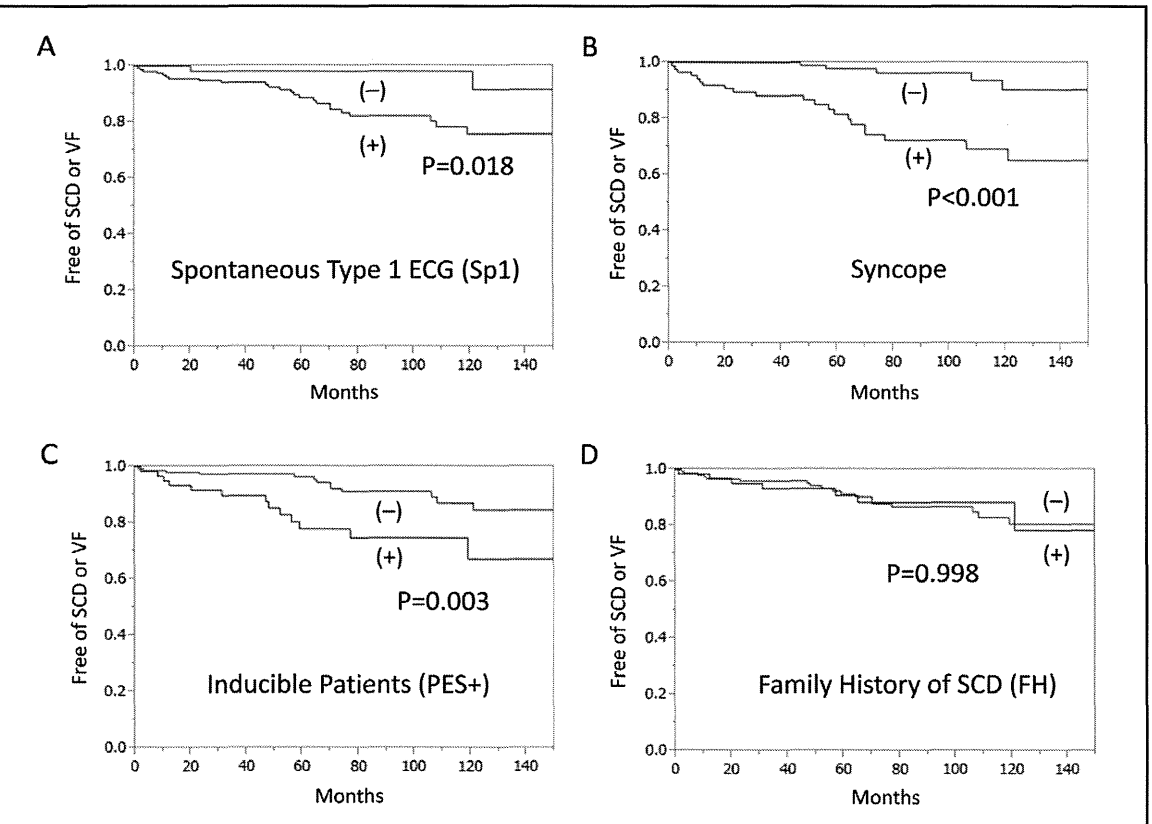

Figure 2. Kaplan-Meier analysis of lethal arrhythmic events during follow-up, categorized by risk factors present: (**A**) spontaneous type 1 Brugada ECG (Sp1– vs. Sp1+); (**B**) history of syncope judged to be likely caused by ventricular arrhythmias (syncope– vs. syncope+); (**C**) inducibility of ventricular fibrillation by programmed electrical stimulation (PES– vs. PES+); and (**D**) family history of SCD (FH– vs. FH+). ECG, electrocardiogram; SCD, sudden cardiac death; VF, ventricular fibrillation.

Table 2. Predictive Factors of SCD or VF

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age (per year)	1.02	0.98–1.05	0.38			
Male	0.72	0.15–12.94	0.72			
Sp1	4.81	1.43–29.92	0.0079	4.51	1.30–28.37	0.014
Syncope	6.87	2.80–20.59	<0.001	6.81	2.76–20.46	<0.001
Inducibility of VF (PES+)	3.01	1.38–6.56	0.0062	2.03	0.92–4.49	0.078
Family history of SCD	0.94	0.37–2.16	0.90			

CI, confidence interval; HR, hazard ratio. Other abbreviations as in Table 1.

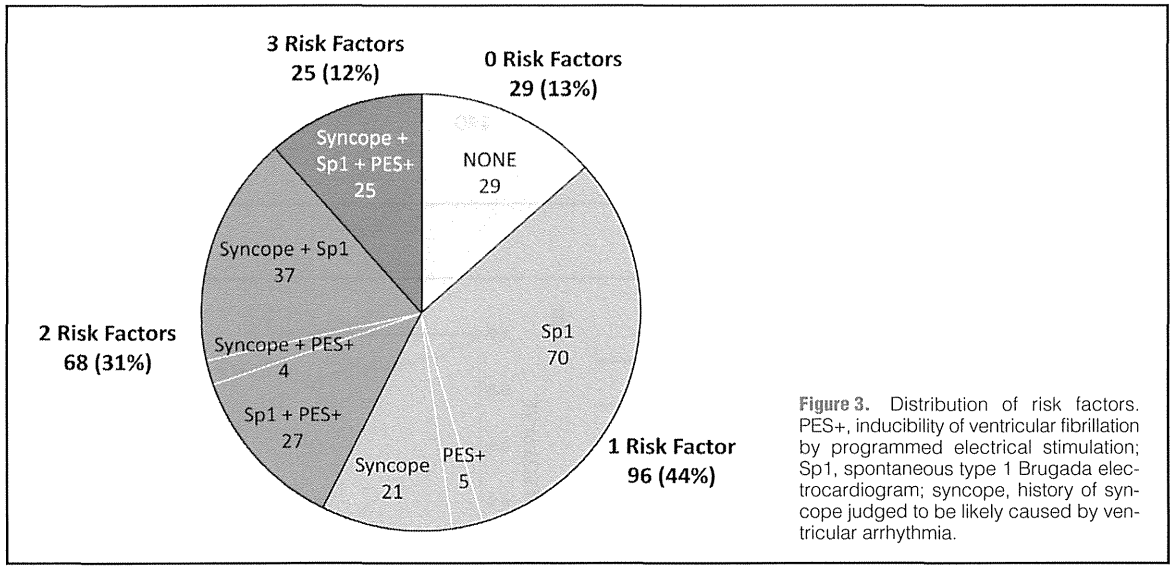

Figure 3. Distribution of risk factors. PES+, inducibility of ventricular fibrillation by programmed electrical stimulation; Sp1, spontaneous type 1 Brugada electrocardiogram; syncope, history of syncope judged to be likely caused by ventricular arrhythmia.

riod from the start of the investigation to the first arrhythmic event was 51±37 months (median, 54 months). Twenty-four patients received appropriate ICD shock delivery against VF with successful termination, while 2 patients who had refused ICD therapy died suddenly. Figure 1 shows the Kaplan-Meier event-free survival curve for the overall group as well as for each institution. Table 1 also compares the patient characteristics between 2 groups defined according to the presence (n=26) or absence (n=192) of an arrhythmic event during follow-up. The prevalence of ICD was higher and the follow-up period was shorter in patients with an arrhythmic event (92% vs. 51%, P<0.001; 51 months vs. 81 months, P=0.0023, respectively). The period of ICD implantation was also longer in patients without an arrhythmic event (44 months vs. 87 months, P<0.001). The prevalence of SP1, syncope, and PES+ were higher in patients with an arrhythmic event compared to those without (92% vs. 70%, P=0.018; 81% vs. 34%, P<0.001; and 50% vs. 25%, P<0.0077, respectively). There was no difference in FH between the 2 groups (27% vs. 30%, P=0.77).

Arrhythmic Events

Figure 2 shows the influence of each of the proposed risk factors (Sp1, syncope, and PES+) and FH on the incidence of arrhythmic events during the follow-up period. The prevalence of arrhythmic events was higher in patients with any of the proposed risk factors compared to those without it (Sp1, P=0.018, Figure 2A; syncope, P<0.001, Figure 2B; PES+, P=0.003, Figure 2C). FH, however, had no association with event rate (P=0.998, Figure 2D).

Next, univariate and multivariate analysis were used to investigate the possible clinical variables associated with SCD and VF. On multivariate analysis syncope and Sp1 were independent predictors of arrhythmic events during follow-up (HR, 6.81 and 4.51, respectively) as shown in Table 2. PES+, however, was not an independent predictor (HR, 2.03, P=0.078).

Risk Factor Combinations

Figure 3 shows the number of risk factors of Sp1, syncope and PES+ in each patient, from 0 to 3. The majority of patients had 1 (44%) or 2 (31%) risk factors. Using event-free survival curve according to the number of risk factors, the event-free rate decreased as the number of risk factors increased (Figure 4). The prevalence of arrhythmic events was higher in patients with 2 risk factors compared with patients with 1 risk factor (P<0.001). There was no statistically significant difference in the number of arrhythmic events between patients with 2 and 3 risk factors (P=0.089), and patients without any risk factors had no SCD or VF.

Among patients with either 1 or 2 risk factors, there were no statistical differences in event-free survival rates between

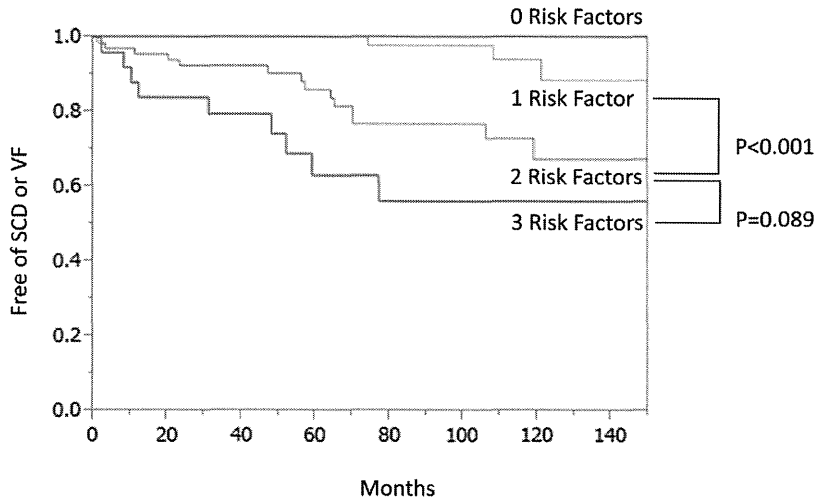

Figure 4. Kaplan-Meier analysis of lethal arrhythmic events during follow-up depending of the number of risks factors present, including syncope, inducibility of ventricular fibrillation, and spontaneous type 1 Brugada electrocardiogram. SCD, sudden cardiac death; VF, ventricular fibrillation.

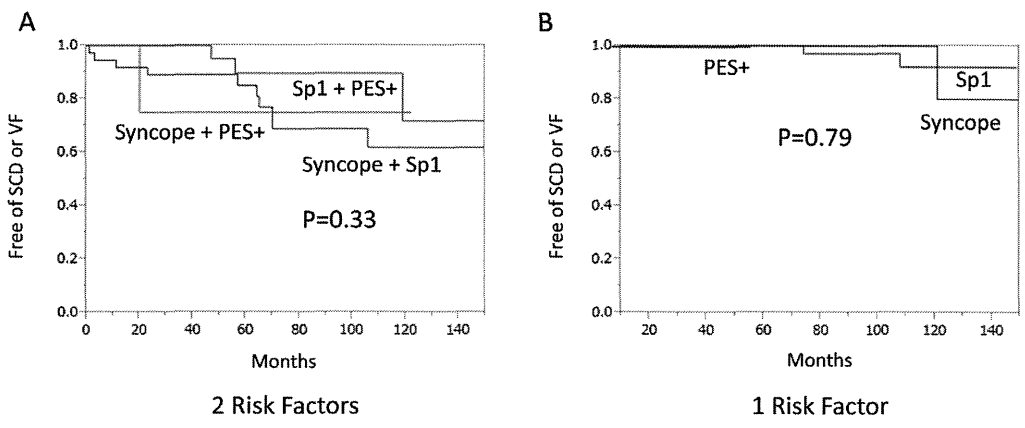

Figure 5. Kaplan-Meier analysis of arrhythmic events during follow-up depending on the presence of (A) 2 risks (syncope+Sp1 vs. Sp1+PES+ vs. syncope+PES+) or (B) 1 risk (Sp1 vs. syncope vs. PES+). PES+, inducibility of ventricular fibrillation by programmed electrical stimulation; Sp1, spontaneous type 1 Brugada electrocardiogram; syncope, history of syncope judged to be likely caused by ventricular arrhythmia.

subgroups (P=0.33 for 2 risk factors, syncope+Sp1/Sp1+PES+/syncope+PES+; and P=0.79 for 1 risk factor, Sp1/syncope/PES+; Figure 5).

Furthermore, there were significant differences in event-free survival rates between all subgroups with 2 risk factors and 1 risk factor (P<0.001 for syncope+Sp1 vs. 1 risk, P=0.03 for Sp1+PES+ vs. 1 risk and P=0.0036 for syncope+PES+ vs. 1 risk; Figure S1).

Discussion

Major Finding

This is one of the largest studies on this topic to date, with a subject group of 218 patients with BrS without cardiac arrest who were considered for ICD therapy for primary prevention of SCD. The major finding was that the proposed 3 risk factors, Sp1, syncope, and PES+, successfully stratified the risk of later arrhythmic events in Japanese patients with BrS with-

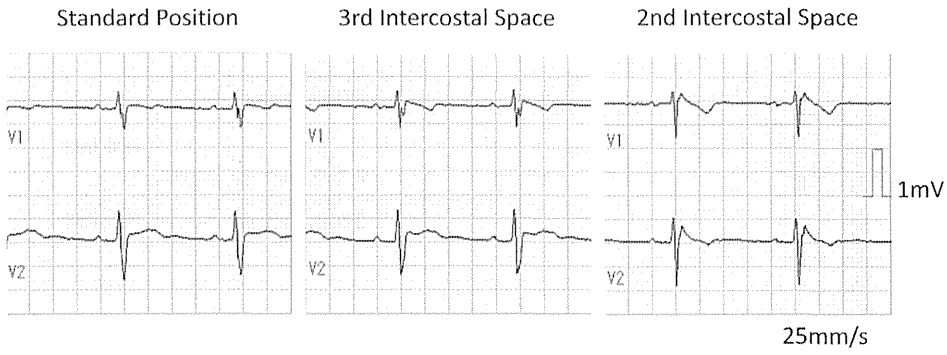

Figure 6. Electrocardiogram (V1 and V2) of a patient with a history of syncope who developed ventricular fibrillation during the follow-up. Spontaneous coved-type ST elevation was seen in the superior position (**Middle**: 3rd intercostal space; **Right**: 2nd intercostal space) not in the (**Left**) standard position.

out prior cardiac arrest. In particular, syncope and Sp1 were both independent risk factors. To our knowledge, this is the first study to show that the magnitude of the risk of SCD or VF was stratified by combining these 3 risk factors. When a patient had ≥2 risk factors, the risk of fatal arrhythmic events dramatically increased.

Combining Risk Factors for VF

The idea of combining risk factors arose from the guidelines for non-pharmacotherapy of cardiac arrhythmias published by the Japanese Circulation Society in 2011.[9] These guidelines recommended ICD for primary prevention of SCD in patients with BrS according to the number of the following 3 risks factors present: syncope, PES+, and FH. ICD was categorized as a class IIa therapy for patients with ≥2 risk factors and ICD was categorized as class IIb for patients with a single risk factor. Sp1 was not included as a risk factor in these guidelines. Delise et al reported the risk stratification in a similar patient type as in the present study, with a focus on risk combinations.[10] Although FH itself failed to predict arrhythmic events, the authors concluded that a multi-parametric approach that included syncope, FH, and PES+ helped to identify patients at high risk. They also reported that the patients at highest risk were those with at least 2 risk factors in addition to Sp1. In this study, we could stratify the risk of arrhythmic events clearly by combining 3 proposed risk factors according to the 2013 consensus report: Sp1, syncope, and PES+.

Japanese Guideline 2011

As noted, the 2011 Japanese guidelines recommended ICD for primary prevention of SCD in patients with BrS according to 3 risk factors: syncope, PES+, and FH. ICD was categorized as a class IIa treatment for patients with ≥2 risk factors and ICD was categorized as class IIb for patients with a single risk factor. Event-free survival curve according to this ICD indication class seemed to well stratify the risk of SCD, although there was a modest but not significant difference between class IIa and class IIb indication (P=0.07; **Figure S2A**). Among patients with class IIa or class IIb indications, however, there were statistically significant differences in event-free survival rate between subgroups (P=0.049 for class IIa indications, syncope+FH+PES+/syncope+FH/FH+PES+/syncope+PES+; and P=0.035 for class IIb indications, FH/syncope/PES+; **Figure S2B,C**). These differences seemed to arise from the lack of predictive value of FH and further discussion on this guideline is required.

Spontaneous Coved-Type ECG

The present results demonstrated the importance of Sp1 as a predictor of arrhythmic events, confirming similar findings in other studies.[3,10–14] To increase the sensitivity for detecting Sp1, ECG were recorded in the superior position up to the second intercostal space as well as in the normal position in all patients, which may have resulted in the higher incidence of Sp1 (73%) in this study than in other studies (around 55%).[6,10,12,15]

Figure 6 presents a typical ECG of a patient with a history of syncope who developed VF during the follow-up, and in whom ICD was implanted because Sp1 was recorded in the superior position, not in the normal position. It has been reported that Sp1 can evolve spontaneously within minutes or can be unmasked by fever, although fever-induced Brugada ECG was not observed in this study.[16–18] Also, seasonal and circadian distributions of arrhythmic events in patients with BrS have been reported.[19] In the present study, ECG were recorded during the day, and circadian variation of ECG pattern was not evaluated. ECG recording immediately after the meal or at various times may further increase the sensitivity for detecting Sp1. Interestingly, ECG recording on deep inspiration has been reported to be useful to identify Brugada ECG.[20] Accordingly, repeated recordings of 12-lead ECG should be performed to increase the detection of Sp1.

PES

The role of electrophysiological evaluation remains controversial.[5] In this study, PES+ did not serve as an independent predictor of SCD or VF, but patients with PES+ had a higher incidence of arrhythmic events than those without it. Although there are several reports on the predictive value of PES+ that suggest that PES+ plays an important role in risk stratification, several studies including the PRELUDE registry reported by Priori et al argued against its usefulness.[3,11,12,21] The PRELUDE registry was a prospective study in which patients were followed for an average of 34 months, a much shorter duration

than that used in the present study. Furthermore, the median period from the start of the study to the arrhythmic event was 54 months in the present study. Short study duration might be one of the reasons for the discrepancy in PES+ importance between the PRELUDE registry and the present findings. The role of PES was also reported in the meta-analysis by Fauchier et al, which suggested the usefulness of PES in asymptomatic patients and in patients with syncope of unknown origin, not in patients with a history of cardiac arrest.[21] The present result supports the finding of that meta-analysis. Also, the importance of the negative inducibility of VF by PES should be discussed. In the present study, some patients with negative inducibility of VF developed arrhythmic events as well. The previously mentioned meta-analysis by Fauchier et al also concluded that negative inducibility does not portend a good prognosis.[21] Delise et al, however, reported that no arrhythmic events occurred in patients with negative inducibility of VF.[10] Makimoto et al reported that the number of extrastimuli in PES using the minimum coupling interval of 180 ms and the inducibility of VF by up to 2 extrastimuli had significant predictive value for future cardiac events.[22] Further discussion of these unresolved issues should be continued.

Family History of SCD

Although we focused on Spl, syncope and PES in the present study according to the current consensus report published in 2013, we also analyzed the impact of FH on future arrhythmic events, because Kamakura et al reported that FH was one of the predictors of SCD in patients with Spl.[7] Although 45 patients in the Kamakura et al report were included in the present study, the Kamakura et al study included 274 patients without previous cardiac arrest (primary prevention) from 26 institutions across Japan. Also, the Kamakura et al study included patients with aborted SCD (secondary prevention), a patient type that differed from the present study. The FINGER registry, the largest cohort of patients with BrS, also concluded that FH was not a predictor of cardiac events.[3] Although there are limited data on FH as a risk factor for lethal arrhythmia, it should not be disregarded. BrS is considered to be an inherited disease to a certain degree and the importance of FH should be discussed on an individual basis. Also, genetic information may provide important information on the relationship between FH and the risk of SCD. Further study is required on this unsolved issue.

Clinical Implications

The HRS/EHRA/APHRS consensus document 2013 provided partial recommendations for ICD therapy for primary prevention of SCD in patients with BrS.[8] Recently, Takagi et al reported that class IIa indication in this statement could identify a group of patients with increased risk compared to class IIb indication.[23] The indication for ICD for the patients who do not meet the conditions stated in the consensus document, however, remains unclear. In contrast, the combination of the 3 risk factors as proposed in the present study, which was based on the consensus document, covers all patients who are examined for ICD treatment. Patients with ≥2 risk factors are at high risk of SCD, which emphasizes the importance of measuring these 3 risk factors cautiously and exactly. First, repeated 12-lead ECG in the superior position in addition to the normal position should be performed to increase the detection of Spl. Second, to increase the accuracy of risk estimation, syncope judged as likely caused by vasovagal reflex should be excluded. Third, the PES result is important especially when a patient has either Spl or syncope. In this situation, electrophysiological evaluation should be recommended to estimate the risk of VF accurately.

Study Limitations

The retrospective nature of this study is an important limitation. Patients were enrolled from only 2 Japanese institutions and the number of patients in this study was still small. Furthermore, all patients in this study underwent electrophysiological testing to assess suitability for ICD therapy, which was designed to evaluate the 3 proposed risk factors, syncope, Spl, and PES+. Undoubtedly a certain bias existed in physician decision regarding whether or not to perform PES in each patient. Further prospective studies are needed to reach definitive conclusions.

Conclusions

Syncope, Spl, and PES+ were important risk factors for SCD and VF in patients with BrS without previous cardiac arrest. The combination of these proposed risk factors according to the consensus report 2013 accurately stratified the risk of arrhythmic events, which could be of assistance when considering ICD therapy in these patients.

Acknowledgments

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Disclosures

None.

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Appendix

Number of patients included at each center: Okayama University Hospital, 112; National Cerebral and Cardiovascular Center, 106.

Supplementary Files

Supplementary File 1

Figure S1. Kaplan-Meier analysis of arrhythmic events during follow-up according to the presence of 2 risks (syncope+Sp1 vs. Sp1+PES+ vs. syncope+PES+) and 1 risk.

Figure S2. (A) Kaplan-Meier analysis of lethal arrhythmic events during follow-up depending on implantable cardioverter defibrillator (ICD) indication class defined in the Japanese guideline 2011.

Please find supplementary file(s);
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Sodium Channelopathy Underlying Familial Sick Sinus Syndrome With Early Onset and Predominantly Male Characteristics

Keisuke Abe, Taku Machida, Naokata Sumitomo, Hirokazu Yamamoto, Kimie Ohkubo, Ichiro Watanabe, Takeru Makiyama, Satoki Fukae, Masaki Kohno, Daniel T. Harrell, Taisuke Ishikawa, Yukiomi Tsuji, Akihiko Nogami, Taichi Watabe, Yasushi Oginosawa, Haruhiko Abe, Koji Maemura, Hideki Motomura and Naomasa Makita

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Original Article

Sodium Channelopathy Underlying Familial Sick Sinus Syndrome With Early Onset and Predominantly Male Characteristics

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Background—Sick sinus syndrome (SSS) is a common arrhythmia often associated with aging or organic heart diseases but may also occur in a familial form with a variable mode of inheritance. Despite the identification of causative genes, including cardiac Na channel (*SCN5A*), the pathogenesis and molecular epidemiology of familial SSS remain undetermined primarily because of its rarity.

Methods and Results—We genetically screened 48 members of 15 SSS families for mutations in several candidate genes and determined the functional properties of mutant Na channels using whole-cell patch clamping. We identified 6 *SCN5A* mutations including a compound heterozygous mutation. Heterologously expressed mutant Na channels showed loss-of-function properties of reduced or no Na current density in conjunction with gating modulations. Among 19 family members with *SCN5A* mutations, QT prolongation and Brugada syndrome were associated in 4 and 2 individuals, respectively. Age of onset in probands carrying *SCN5A* mutations was significantly less (mean±SE, 12.4±4.6 years; n=5) than in *SCN5A*-negative probands (47.0±4.6 years; n=10; $P<0.001$) or nonfamilial SSS (74.3±0.4 years; n=538; $P<0.001$). Meta-analysis of SSS probands carrying *SCN5A* mutations (n=29) indicated profound male predominance (79.3%) resembling Brugada syndrome but with a considerably earlier age of onset (20.9±3.4 years).

Conclusions—The notable pathophysiological overlap between familial SSS and Na channelopathy indicates that familial SSS with *SCN5A* mutations may represent a subset of cardiac Na channelopathy with strong male predominance and early clinical manifestations. **(*Circ Arrhythm Electrophysiol.* 2014;7:511-517.)**

Key Words: mutation ■ $Na_v1.5$ voltage-gated sodium channel ■ sex ■ sick sinus syndrome

Sick sinus syndrome (SSS), or sinus node dysfunction (SND), is a common clinical disorder that was first described in 1967,[1,2] and is characterized by pathological sinus bradycardia, sinus arrest, chronotropic incompetence, and susceptibility to atrial tachycardia, especially atrial fibrillation. The syndrome comprises a variety of electrophysiological abnormalities in sinus node impulse formation and propagation and represents the most frequent indication of pacemaker implantation.[3] SSS may be associated with underlying structural heart diseases but most commonly occurs in the elderly in the absence of apparent accompanying heart disease. In 3 independent major trials of pacing in symptomatic SSS, the median or mean age was shown to be 73 to 76 years with both sexes affected approximately equally.[4–6] Although less common, SSS also occurs in young adults and children.

Clinical Perspective on p 517

Recent studies including our own have linked several genetic defects with familial SSS, both with and without other concomitant cardiac conditions, mainly through candidate gene approaches. Implicated genes include the pore-forming α-subunit of the cardiac Na^+ channel (*SCN5A*),[7–11]

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hyperpolarization-activated cyclic nucleotide gated channel generating pacemaker current (*HCN4*),[12] and membrane adaptor protein ankyrin-B (*ANK2*).[13] Most recently, a genome-wide association study identified a rare missense variant of *MYH6*, the gene encoding the α-myosin heavy chain, which predisposes affected individuals to SSS.[14] Additionally, several other ion channels and gap junctions have been implicated in the SSS phenotype by knockout mice studies.[15] The majority of familial SSS cases exhibit autosomal dominant inheritance (OMIM_163800),[8–12] but an autosomal recessive disorder of compound heterozygous *SCN5A* mutations (OMIM_608567) also exists.[7,16] Probands carrying compound heterozygous mutations typically manifest severe clinical phenotypes including ECG abnormalities with early onset mostly during the first decade of life[7] and often require the implantation of a pacemaker during infancy. Because familial SSS is relatively rare, the prevalence and functional consequences of these mutations and the epidemiological characteristics have not been extensively studied.

In the present study, we investigated the clinical and genetic backgrounds of 15 families with SSS. We found that familial SSS with *SCN5A* mutations may represent a subset of cardiac Na channelopathy with strong male predominance and early clinical manifestations.

Methods

Clinical Studies

The study population included 48 individuals from 15 unrelated Japanese families diagnosed with SSS. Family members underwent a physical examination, ECG, an exercise stress test, and Holter recording. SSS or SND was considered if one of the following conditions was recorded at ≥1 occasions when inappropriate for the circumstances: (1) sinus bradycardia, (2) sinus arrest or exit block, and (3) combinations of sinoatrial and atrioventricular conduction disturbances in conjunction with paroxysmal atrial tachyarrhythmias.[17] Long QT syndrome (LQTS) and Brugada syndrome (BrS) associated with SSS were diagnosed using the most recently available respective criteria.[18,19] Epidemiological data of nonfamilial SSS (n=538) were obtained from the most recent databases of 4 Japanese institutions, in which SSS cases with a family history of pacemaker implantation, sudden death, or underlying structural heart diseases were excluded. This study was approved by a review committee of each institution, and the subjects gave informed consent.

Genetic Screening

All probands and family members who participated in the study gave their written informed consent in accordance with the Declaration of Helsinki and local ethics committees. Genetic analysis was performed on genomic DNA extracted from peripheral white blood cells using standard methods. Coding regions of *SCN5A*, *HCN4*, *KCNQ1*, *KCNH2*, *GJA5*, *KCNJ3*, *MYH6*, *IRX3*, and *LMNA* were amplified by polymerase chain reaction using exon-flanking intronic primers. Primer information for *KCNJ3*, *MYH6*, *IRX3*, and *LMNA* is available in Table I in the Data Supplement. Direct DNA sequencing was performed using an ABI 3130 genetic analyzer (Life Technologies, Carlsbad, CA). Mutations were validated by screening DNA samples from 200 healthy Japanese volunteers and using public databases (dbSNP and 1000 Genomes).

Biophysical Analysis of *SCN5A* Mutants

Site-directed mutagenesis was performed using human heart Na channel α-subunit $Na_v1.5$. The human cell line tsA-201 was transiently transfected with wild-type or mutant *SCN5A* plasmids, and Na currents were recorded using the whole-cell patch clamp technique as described previously.[20] Further details are available in the Methods in the Data Supplement.

Statistics

Results are presented as means±SE, and statistical comparisons were made using the Student *t* test to evaluate the significance of differences between means followed by a Bonferroni adjustment for the total number of comparisons. Statistical significance was assumed for $P<0.05$.

Results

Case Presentations

We genetically screened 48 members of 15 families with SSS (A1–A5 and B1–B10) and identified 6 *SCN5A* mutations in 5 families (A1–A5; Figures 1 and 2). The clinical and genetic information of 15 probands and mutation-positive family members (n=14) is shown in Table II in the Data Supplement.

Family A1

A 4-year-old boy (III:2) visited a pediatric clinic to investigate the bradycardia identified during a physical checkup at kindergarten. He had no perinatal problems. Despite a prescription of denopamine, he experienced multiple syncopal episodes and visited a cardiology hospital at the age of 5 years. Holter ECG revealed SSS with a maximum R-R interval of 5.9 s (Figure 2A), so an epicardial pacemaker was

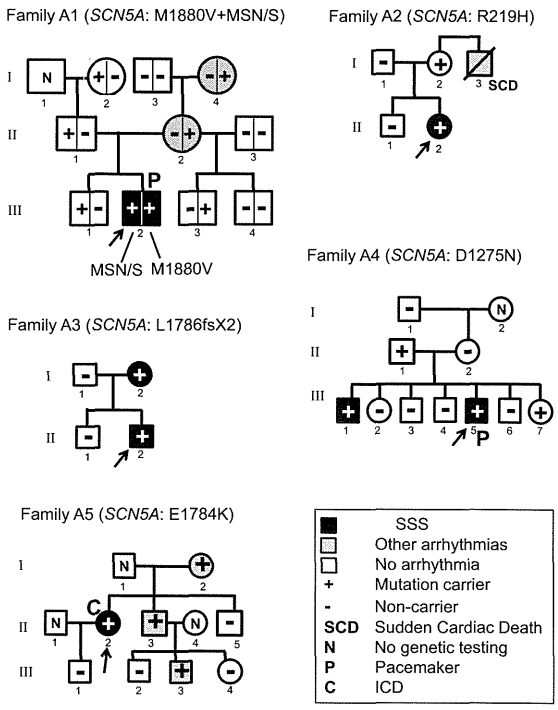

Figure 1. Familial sick sinus syndrome (SSS) pedigrees with *SCN5A* mutations. Probands are arrowed. In family A1, the proband (III:2) had compound heterozygous mutations of MSN/S (p.801_803delMSN/ins) and M1880V, while I:2, II:1, and III:1 had MSN/S; I:4, II:2, and II:3 had M1880V. Of 19 mutation carriers, 7 individuals were asymptomatic (penetrance, 63%). ICD indicates implantable cardioverter defibrillator.

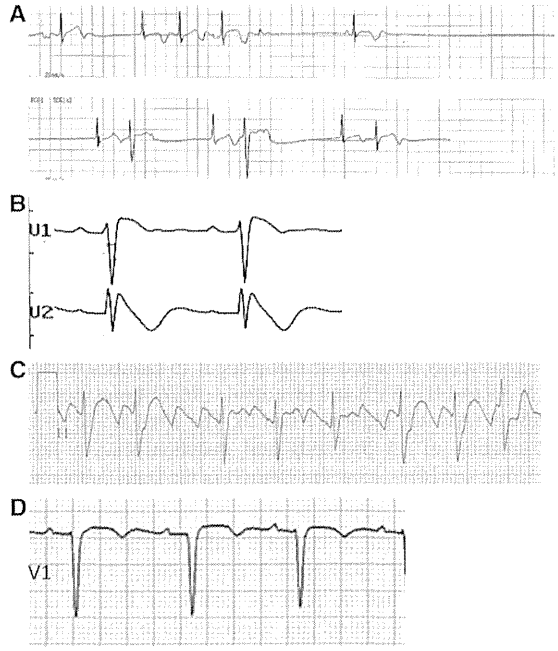

Figure 2. Electrocardiographic phenotypes. **A**, Consecutive strips of Holter ECG recording from proband A1-III:2 carrying compound heterozygous *SCN5A* mutations showed sinus arrest for 5.9 s (at the age of 5). **B**, His mother A1-II:2 showed coved-type ST-segment elevation in V1 through V2 leads during the flecainide challenge test. **C**, Paroxysmal atrial flutter (AFL) recorded in the proband A3-II:2. **D**, QT prolongation (QTc, 522 ms) remains evident in the proband A5-II:2 even after thyroid hormone supplemental therapy.

implanted. P wave amplitudes progressively diminished and had disappeared by the age of 12 when the pacemaker generator was replaced. However, atrial pacing could not be achieved even with the use of high voltages ≤6 V, compatible with atrial standstill. Genetic screening revealed 2 novel *SCN5A* mutations: an in-frame indel mutation 801_803delMSN/insS (c.2401_2409delinsTCC) in exon 15, referred to as MSN/S, and a missense mutation M1880V (c.5638A>G) in exon 28 (Figure 3). Heterozygous MSN/S was also demonstrated in paternal family members (II:1 and III:1) and heterozygous M1880V was observed in maternal family members (I:2, II:2, and III:3), demonstrating that the proband is a compound carrier of 2 distinct *SCN5A* mutations (Figure 1). There was no family history of SSS or pacemaker implantation, but his mother (II:2) was diagnosed with BrS from the observation of typical type-I ST-segment elevation provoked by the Na channel blocker flecainide (Figure 2B). The remaining affected members were asymptomatic and had no sign of cardiovascular diseases.

Family A2

An 18-year-old woman (II:2) was admitted to hospital because of dizziness on standing. Holter ECG recording revealed frequent episodes of sinus arrest with a maximum R-R interval of 7.7 s, so a diagnosis of SSS was made and a pacemaker was implanted. Echocardiography was normal. Her maternal uncle (II:3) died suddenly during running at the age of 35. Genetic screening revealed a missense mutation R219H (c.656G>A) in *SCN5A* exon 6 of the proband and her asymptomatic mother (II:2; Figure 3). This mutation was previously reported in an individual with familial dilated cardiomyopathy (DCM) associated with a third-degree atrioventricular block, ventricular tachycardia, and atrial flutter (AFL).[21]

Family A3

A 3-year-old boy (II:2), admitted to hospital because of fever, showed AFL and ventricular tachycardia (Figure 2C). Sinus arrest of 5.2 s was evident by Holter ECG recording, and he was diagnosed with SSS. Structural heart diseases were excluded by echocardiography. His mother also showed SSS and AFL. Genetic screening showed that they shared a novel heterozygous 2-bp deletion (c.5355_5354delCT) resulting in a frame shift mutation, L1786fsX2, located in exon 28 of *SCN5A* (Figure 3).

Family A4

A 15-year-old boy (III:5) with bradycardia lost consciousness after a collision during a soccer game. ECG displayed junctional bradycardia (heart rate, 38 beats per minute; maximum R-R interval=5 s) with left axis deviation. Echocardiography revealed dilatation of the left ventricle (left ventricular end-diastolic diameter, 59 mm), but the left ventricular systolic function was normal (ejection fraction, 64%). His brother (III:1), a pacemaker recipient because of SSS, has a dilated right ventricle and has experienced episodes of AFL and ventricular tachycardia. Genetic screening revealed a missense mutation D1275N (c.3823G>A) in *SCN5A* exon 21, previously linked to DCM with conduction disorder (Figure 3).[22]

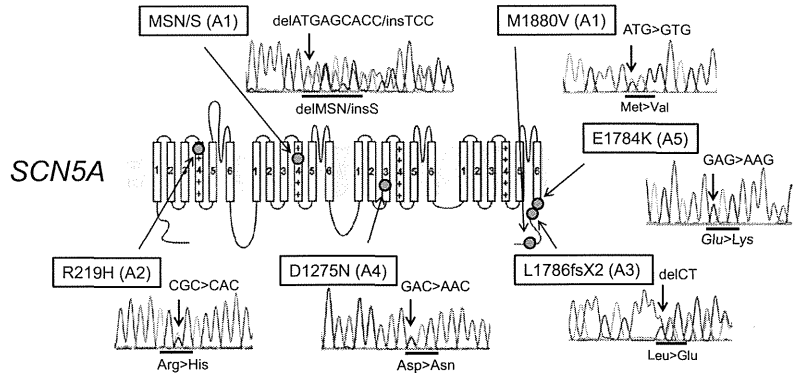

Figure 3. Sequencing of *SCN5A*. Schematic of the transmembrane topology of *SCN5A* representing the location and sequencing electropherogram of each mutation. Three mutations are located in the transmembrane domains, and 3 are in the cytoplasmic C-terminal.

The mutation was identified in his brother (III:1), asymptomatic father (II:1), and younger brother (III:7).

Family A5

Sinus bradycardia and QT prolongation with day-to-day variation (QTc, 450–530 ms) were observed when the proband (II:2) was 22 years old, which were exacerbated after thyroidectomy as a treatment of hyperthyroidism at the age of 36. An electrophysiological study revealed SND (sinus node recovery time, 5.08 s), atrioventricular block (His ventricular, 68 ms), and atrial standstill in addition to QT prolongation (QTc, 522 ms; Figure 2D), whereas the thyroid function was normally controlled. Her mother (I:2) and son (III:3) showed QT prolongation, while her brother (II:3) had both LQTS and BrS. Genetic screening revealed that the proband and these 3 family members carried an *SCN5A* missense mutation in exon 28, E1784K (c.5350C>A; Figure 3), which is the most common *SCN5A* mutation in type-3 LQTS (LQT3) associated with multiple clinical phenotypes of LQTS, BrS, and SSS.[20] The proband prophylactically received an implantable cardioverter defibrillator at the age of 36, which discharged appropriately 1 year later during an episode of spontaneous ventricular fibrillation.

Mutation Analysis of Probands and Family Members

Nineteen mutation carriers were identified within the 5 families with SSS (A1–A5; Table II in the Data Supplement). Seven individuals (37%) exhibited SSS, while 7 carriers (37%) were asymptomatic; other carriers showed variable arrhythmias including LQTS, BrS, and AFL without SSS, suggesting that SSS has a considerably reduced penetrance in these families. No mutations were identified in *HCN4*, *KCNQ1*, *KCNH2*, *KCNJ3*, *MYH6*, *GJA5*, and *IRX3*. Among the *SCN5A* mutations we found, D1275N[22,23] and E1784K[20] have previously been well characterized; therefore, we analyzed the functional properties of other mutants.

Functional Characterization of *SCN5A* Mutations

As shown in Figure 4A, all plasmids, except for L1786fsX2, elicited a robust Na current but the noninactivating late current, which characterizes LQT3 mutations,[24] was not evident. Peak current density measured 24 hours after transfection was significantly reduced in MSN/S, M1880V+MSN/S, R219H, and L1786fsX2 compared with wild type (Figure 4B and 4C). Because L1786fsX2 was nonfunctional, channel properties were further analyzed for M1880V+MSN/S and R219H (biophysical properties of other mutations are shown in Table III in the Data Supplement). The voltage dependence of activation was significantly shifted in the depolarizing direction (+7.5 mV; $P<0.01$) in M1880V+MSN/S, and the voltage dependence of steady-state inactivation was significantly shifted in the hyperpolarizing direction in R219H (−11.4 mV; $P<0.01$; Figure 4D). Recovery from inactivation was remarkably delayed in R219H (Figure 4E). The lower current density, depolarizing shift of the activation curve, hyperpolarizing shift of the inactivation curve, and delayed recovery from inactivation observed in M1880V+MNS/S, R219H, and the nonfunctional channel L1786fsX2 are typical loss-of-function properties of Na channels.

Epidemiological and Genetic Characteristics of Familial SSS

The average age of onset of the probands in our SSS cohort was 35.5±5.4 years (range, 3–65), which was substantially less than that of the 538 cases of sporadic SSS (74.3±0.4 years; $P<0.001$; Figure 5). When the cohort was classified by the presence or absence of *SCN5A* mutations, the *SCN5A*-positive subgroup showed an even earlier onset (12.4±4.6 years; n=5) than the negative subgroup (47.0±4.6 years; n=10; $P<0.001$). To confirm this observation, we searched the literature for descriptions of SSS probands with *SCN5A* mutations and a family history of SSS and identified 24 cases in addition to the 5 (A1–A5) in our cohort (Table IV in the Data Supplement). To our surprise, the 29 SSS probands with *SCN5A* mutations exhibited not only an early onset (20.9±3.4 years) but also a striking male predominance (male: 23/29; 79.3%). If we focus on the SSS subgroup without disease complications such as BrS or LQTS, the tendency of early onset was even more obvious. As shown in the histogram in Figure 5B, the SSS-only subgroup (filled boxes) had a young age of onset (mean age, 7.8±1.9 years; n=11) and a prominent male preponderance (10/11; 91%). These data indicate that the subset of familial SSS with *SCN5A* mutations has a strong male predominance resembling BrS but exhibits a considerably earlier clinical manifestation. Nevertheless, the same pathophysiological basis of loss-of-function of the cardiac Na channel is shared.

Discussion

We identified 6 *SCN5A* mutations in 15 familial SSS probands and demonstrated that familial SSS with *SCN5A* mutations may represent a distinct cardiac Na channelopathy with early onset and male predominance.

SCN5A is the cardiac Na channel gene responsible for the generation and rapid propagation of action potentials in the heart. Mutations in *SCN5A* have been linked to a wide range of inherited lethal arrhythmias, referred to as cardiac Na channelopathy, including LQT3,[24] BrS,[25] progressive cardiac conduction defect,[26] sudden infant death syndrome, and SSS. To date, ≥27 distinct *SCN5A* mutations that are causative of SSS have been reported, although some mutation carriers exhibit mixed clinical phenotypes in addition to SSS (Table IV in the Data Supplement).[8–10] Heterologously expressed mutant *SCN5A* commonly results in a loss of function with reduced (R219H, M1880V+MSN/S) or no (L1786fsX2) Na current density, in conjunction with alterations of biophysical properties (Figure 3).

The compound heterozygous mutation M1880V+MSN/S results in a channel behavior phenotype that is intermediate between that of M1880V and MSN/S, and functional analysis of the singular mutations suggests that M1880V may have more benign channel properties than MSN/S (Table III in the Data Supplement). However, the proband's mother (A1-II:2), carrying the M1880V allele but not MSN/N, showed BrS, suggesting that the in vivo consequences of M1880V may not be as benign as observed in the heterologous expression system. A similar discrepancy between in vivo and in vitro situations was previously reported in the *SCN5A* mutation D1275N, which was identified in the A4 family of our present cohort.

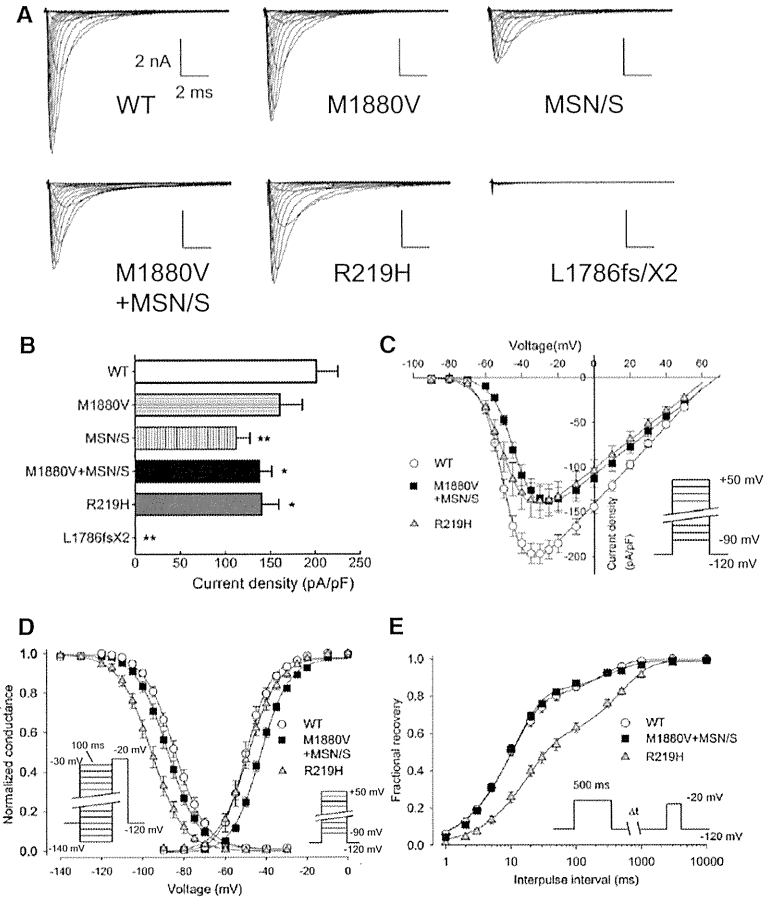

Figure 4. Whole-cell current recordings of wild-type (WT) and mutant Na channels. **A**, Representative whole-cell current traces obtained from tsA-201 cells transfected with WT or mutant Na channels. Currents were recorded from a holding potential of −120 mV and stepped to various membrane potentials from −90 to +50 mV for 20 ms. **B**, Current was normalized to cell capacitance to give a measure of Na current density. There were significant decreases in maximum current density in M1880V+MSN/S and R219H ($P<0.05$), and in MSN/S and L1786fsX2 ($P<0.01$) compared with WT. **C**, Current–voltage relationship of WT and 2 mutant channels (MSN/S+M1880V and R219H). **D**, Steady-state inactivation and conduction–voltage relationship in MSN/S+M1880V and R219H. **E**, Time course of recovery from inactivation at −120 mV. Detailed parameters are provided in Table III in the Data Supplement.

Despite the severe conduction disturbance and DCM, the heterologously expressed D1275N channel showed almost normal behavior.[27] Interestingly, cardiomyocytes from mice carrying the human D1275N *SCN5A* allele display both a decreased current density and late Na current, which is a hallmark of LQT3.[23] Such a mixed biophysical phenotype is observed in association with several *SCN5A* mutations including E1784K (identified in family A5), the most frequent *SCN5A* mutation causative of LQTS, BrS, and SSS.[20] Moreover, a negative shift of steady-state inactivation is a common biophysical mechanism underlying the phenotypic overlap of cardiac Na channelopathy.[20] Taken together, the most typical biophysical feature in *SCN5A* mutations causative of SSS is the loss-of-function property that reduces electric coupling between the sinus node and surrounding atria, resulting in conduction block (exit block). However, a subset of *SCN5A* mutations associated with SSS display a gain-of-function property characteristic of LQT3,[9,20] resulting in a reduction of the sinus rate by prolonging the sinus node action potential and disrupting its complete repolarization.[9]

Recently, Gosselin-Badaroudine et al[21] identified the R219H mutation in a family with DCM associated with ventricular tachycardia and a third-degree atrioventricular block. They found that the mutant R219H channel selectively permeates protons through the channel pore, which in turn results in severe left ventricular dysfunction and conduction disturbance. By contrast, clinical observations of our R219H carriers (A2-I:2, II:2) were rather benign, with electric abnormalities restricted to the sinus node with no left ventricular dysfunction. Moreover, we observed a reduced peak Na current, a hyperpolarizing shift of steady-state inactivation, and a slowed recovery from inactivation of the R219H mutant channel. However, we were unable to evaluate the proton permeation properties in our experimental system. Such loss-of-function properties are commonly observed in most *SCN5A* mutations responsible for familial SSS. The reasons for the discrepancy between our findings and those of Gosselin-Badaroudine et al[21] are not clear; however, additional genetic modifiers within *SCN5A* or other unidentified genes may contribute to the severe clinical and biophysical properties of mutant Na channels.

Autosomal dominant transmission is the most common mode of inheritance in familial SSS,[8,10,11] although autosomal recessive transmission has been reported in several severe juvenile cases of congenital SSS.[7,16] Consistent with previous reports,[4–6] the majority of patients with nonfamilial SSS in the present study are elderly, with both sexes nearly equally affected (Figure 5). By contrast, our familial SSS cases associated with *SCN5A*

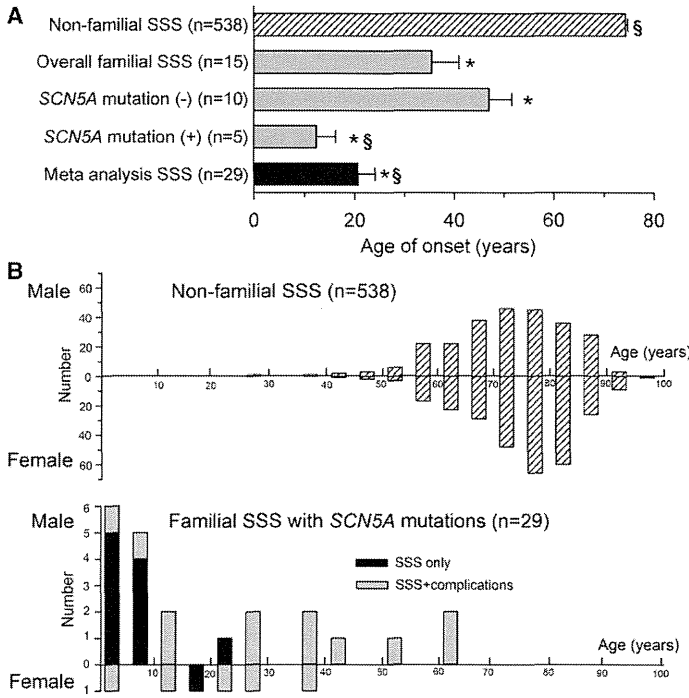

Figure 5. Age of onset and sex difference in probands with nonfamilial and familial sick sinus syndrome (SSS). **A**, Age of onset of nonfamilial SSS (n=538), SSS probands of our cohort (n=15) including *SCN5A* mutation negative (n=10) or positive (n=5), and meta-analysis of 29 cases with *SCN5A* mutations. § and * indicate $P<0.001$ vs mutation negative, and $P<0.001$ vs nonfamiliar SSS, respectively. **B**, Upper histogram shows age of onset in Japanese patients with nonfamilial SSS (n=538; 74.3±0.4 years): male (**upper**; n=241) and female (**lower**; n=257). There was no sex difference. Lower histogram shows the age of onset and sex difference in 29 probands with familial SSS. Filled and shaded columns show SSS only (n=11) and SSS with complications including BrS (n=18), respectively. Early onset and male predominance were more apparent in the SSS-only group.

mutations are characterized by early onset and a strong male predominance. Male predominance (80%–90%) and prevalence of *SCN5A* mutations (≈20%) are known features of BrS,[19] which often associates with SND or atrial arrhythmias.[28,29] Makiyama et al[11] genetically screened 38 BrS probands and identified 4 *SCN5A* mutation carriers (10.5%), all of which were complicated with bradyarrhythmias including SSS. These data suggest a close relationship between BrS and familial SSS, and our study further supports this notion by demonstrating the prominent male predominance in these 2 disorders.

Nonetheless, there is a clear difference between familial SSS and BrS regarding the age of manifestation. The mean age of the 29 probands of familial SSS with *SCN5A* mutations in our study were considerably less (20.9±3.4 years) than those affected with BrS, which typically manifests during adulthood at a mean age of around 40 years. Furthermore, it should be noted that only 2 of 24 (8.3%) of the family members of our cohort who were carriers of the mutations exhibited a BrS phenotype even later in their lives (mean age, 34.5±4.1 years), suggesting that penetrance of familial SSS in our cohort was incomplete (67%; 16/24). These data suggest that SND is the earliest electrophysiological manifestation of *SCN5A* mutation carriers, which may be associated with other arrhythmias such as LQT3, BrS, or DCM under the control of confounding factors including aging, hormones, other genetic variations, and undetermined environmental factors. We have followed up the SSS probands for 7.7±2.1 years (Table II in the Data Supplement), but longer term follow-up of the mutation carriers and further genetic studies of mutation-negative SSS probands may uncover crucial factors that determine the distinct age-dependent manifestations observed in familial SSS and BrS.

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Disclosures

None.

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