ECG pattern did not fulfill Type 1 ECG, 1 mg/kg pilsicainide (a pure sodium channel blocker) was intravenously administered for 10 minutes with continuous monitoring in the intensive care unit and it was confirmed that ECG had changed to a Type 1 pattern as previously described.[10] Genetic analysis of the *SCN5A* gene was performed in all patients.

All of the studies were approved by the Ethics Committee of Okayama University Graduate School of Medicine, Density, and Pharmaceutical Sciences, and written informed consent was obtained from all patients before the procedure. The investigation also conforms to the principles outlined in the Declaration of Helsinki.

Protocol of Treatment With Bepridil

Low-dose bepridil (100 mg/day) was administered to the patients who had repeated episodes of VF. All patients treated with bepridil had undergone ICD implantation, so we could check accurate frequencies of VF episodes using its episodic memory function in this study. If the effect was insufficient 1 month after the start of administration of low-dose bepridil, the dose was increased to 200 mg/day. Twelve-lead ECG and SAECG were recorded before bepridil administration and 1 month after the start of administration. QTc and ST levels at J points in leads V1 and V2 and additional leads V1 and V2 at the third intercostal space (ICS) were evaluated in 12-lead ECG.

Genetic Analysis

Genetic analysis was performed in compliance with the guidelines for human genome studies of the Ethics Committee of Okayama University. Informed consent was obtained from all subjects. Analysis of *SCN5A* mutation was performed as reported previously.[10–13] Genomic DNA was extracted from peripheral blood leukocytes by using a DNA extraction kit (Gentra, Minneapolis, MN) and was stored at –30°C until use. Twenty-seven exons of the *SCN5A* gene were amplified with previously reported intronic primers.[10] We did not analyze *SCN5A* gene exon 1 in this study because it is a noncoding region. The mutations were confirmed three times by independent polymerase chain reaction amplification and sequencing.

Signal-Averaged Electrocardiogram

SAECG was examined with ART 1200EPX (noise level less than 0.3 μV, high-pass filtering of 40 Hz using a bidirectional four-pole Butterworth) as previously described.[14] The filtered QRS duration (F-QRS), root mean square voltage of the terminal 40 ms in the filtered QRS complex (RMS_{40}), and duration of low-amplitude signals less than 40 μV in the terminal filtered QRS complex (LAS_{40}) were measured.

Statistical Analysis

All data are expressed as mean ± standard deviation. Statistical significance for comparison before and after treatment with bepridil was determined using Student *t* test. Values of $P < 0.05$ were considered to be significant.

RESULTS

Study Population

The entire population of patients with Brugada-type ECG followed in our hospital includes 130 patients (Fig. 1).

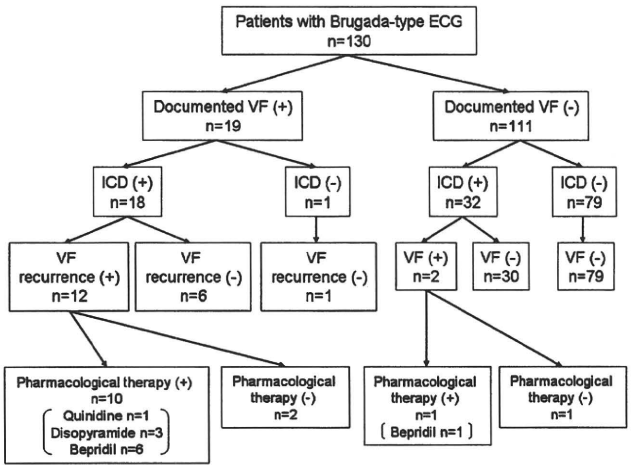

FIGURE 1. Study profile.

Nineteen (14.6%) of those patients had documented symptomatic VF at the initial visit. There was no documented VF in the 111 remaining patients (85.4%). The percentages of the patients are similar to those in a previous study (documented VF: 12.3%, no documented VF: 87.7%).[15] An ICD was implanted in 18 patients in the documented VF group and in 32 patients in the nondocumented VF group. One patient in the documented VF group refused ICD implantation. Indication of ICD implantation in the nondocumented VF group was defined by family history of sudden death, episodes of syncope, and result of VF inducibility during electrophysiological study according to the guidelines of the Japanese Circulation Society. There were 12 cases with recurrent episodes of VF in the documented VF group. In 10 of those cases, pharmacologic therapy was required to reduce the frequent episodes of VF and ICD discharges. Two patients with ICD implantation in the no VF documented group had new onset of VF after ICD implantation. One of those patients needed pharmacologic therapy because VFs and ICD discharges occurred twice a month. We obtained informed consent for bepridil treatment from the patients (six patients in the documented VF group and one patient in the nondocumented VF group) and we used bepridil in those seven patients.

Characteristics of Patients Treated With Bepridil

All seven patients were male and the mean age of the subjects was 47 ± 13 years (range, 33–74 years) (Table 1). Three patients (Case Nos. 1–3) had *SCN5A* mutations (IVS21+1 g>a, R282H, and V1951L) (Table 1). IVS21+1 g> a is an intronic mutation,[16] R282H is a trafficking defective mutant,[17] and V1951L is a disease-associated variant in Asians.[18,19] Six patients (Case Nos. 1–6) had documented VF before ICD implantation. In one asymptomatic patient (Case No. 7) with a family history of sudden death, VF was induced in electrophysiological study, and then an ICD was prophylactically implanted. Therefore, ICDs had been implanted in all patients. All patients had been admitted to our hospital because of frequent discharges from the ICD before

TABLE 1. Characteristics of Patients Treated With Bepridil

Case No.	Age (Years)	*SCN5A* Mutation	Family Hhistory of SCD	ICD
1	39	IVS21+1 g>a	(−)	(+)
2	51	R282H	(−)	(+)
3	74	V1951L	(−)	(+)
4	35	(−)	(+)	(+)
5	53	(−)	(−)	(+)
6	33	(−)	(−)	(+)
7	44	(−)	(+)	(+)

SCD, sudden cardiac death; ICD, implantable cardioverter–defibrillator.

administration of bepridil. There were no patients from the same family. Electrolyte levels, metabolic status, and results obtained by cardiac imaging techniques, including echocardiography and right ventriculography, were normal in all patients. Quinidine had been used for one patient (Case No. 1), but administration of quinidine was discontinued after a short time because of its side effect, digestive symptoms. The other six patients had no pharmacologic therapies before bepridil treatment.

Preventive Effects of Bepridil for Ventricular Fibrillation

Because ICDs had been implanted in all seven patients, we could check accurate frequencies of VF episodes using its episodic memory function. The average observation period before low-dose bepridil (100 mg/day) administration, from ICD implantation to initiation of low-dose bepridil (100 mg/day) treatment, was 32.4 ± 23.3 months (range, 2–72 months). Frequencies of VF episodes were significantly reduced after treatment with low-dose bepridil in three patients (Case Nos. 1–3) with *SCN5A* mutation (before: 0.33 versus after: 0.02 episodes/month, $P < 0.01$) (Table 2). Frequencies of VF episodes were not reduced after bepridil treatment in four patients without *SCN5A* mutation (before: 0.43 versus after: 2.94 episodes/month, P = nonsignificant). Also, patients without *SCN5A* mutation (Case Nos. 4–7) had relapses of VF in a shorter term after administration of low-dose bepridil. VF episodes apparently increased in one patient (Case No. 5) and cessation of bepridil treatment was needed. His QT interval was prolonged markedly after treatment with bepridil (QTc = 549 ms). In the other three patients (Case Nos. 4, 6, and 7), 200 mg/day of bepridil was administered. Bepridil treatment was effective for preventing VF in one patient (Case No. 7) but not in the other two patients.

Effects of Low-Dose Bepridil on 12-Lead Electrocardiogram Parameters

In patients with *SCN5A* mutation, levels of ST elevation at J points in the third ICS V1 lead were significantly decreased after treatment with low-dose bepridil ($P < 0.05$) (Fig. 2A; Table 3). In Case No. 2, Type 1 ECG in the third ICS V2 lead converted to Type 2 ECG (Fig. 3). However, additional ST elevation at J points in the third ICS V1 lead was observed in two patients without *SCN5A* mutation (Case Nos. 6 and 7) (Fig. 2B; Table 3). QTc interval tended to be increased in all patients with *SCN5A* mutation (Fig. 2C). On the other hand, the QTc interval was shortened in two patients without *SCN5A* mutation (Case Nos. 4 and 6) (Fig. 2D). In Case No. 5, the QTc interval became markedly prolonged with frequent episodes of VF after low-dose bepridil treatment (Fig. 3). In Case No. 7, VF relapse occurred during treatment with low-dose bepridil (100 mg/day) and the dose of bepridil was increased to 200 mg/day. After that, the QTc interval was further prolonged (QTc 434 ms) and there has been no relapse of VF for 36 months.

Effects of Low-Dose Bepridil on Signal-Averaged Electrocardiogram Parameters

In patients with *SCN5A* mutation, LAS_{40} was significantly shortened ($P < 0.05$) and F-QRS tended to be shortened and RMS_{40} tended to be increased after low-dose bepridil treatment (Figs. 4A, C, E and 5A–B). On the other hand, there was no improvement in SAECG of patients without *SCN5A* mutation (Figs. 4B, D, F and 5C–D).

DISCUSSION

The major findings in the present study were as follows: 1) low-dose bepridil (100 mg/day) was effective for reducing frequency of VF events in patients with *SCN5A* mutation but

TABLE 2. Preventive Effects of Bepridil for VF

	Before			After		
Case No.	VF (Events)	Observation Period (Months)	Frequency of VF (Events/Month)	VF (Events)	Observation Period (Months)	Frequency of VF (Events/Month)
1	22	72	0.31	0	39	0.00
2	11	38	0.29	1	42	0.02
3	10	26	0.38	1	31	0.03
4	4	13	0.31	3	1	3.00
5	10	27	0.37	3	4	0.75
6	2	2	1.00	6	1	6.00
7	2	49	0.04	2	1	2.00

VF, ventricular fibrillation.

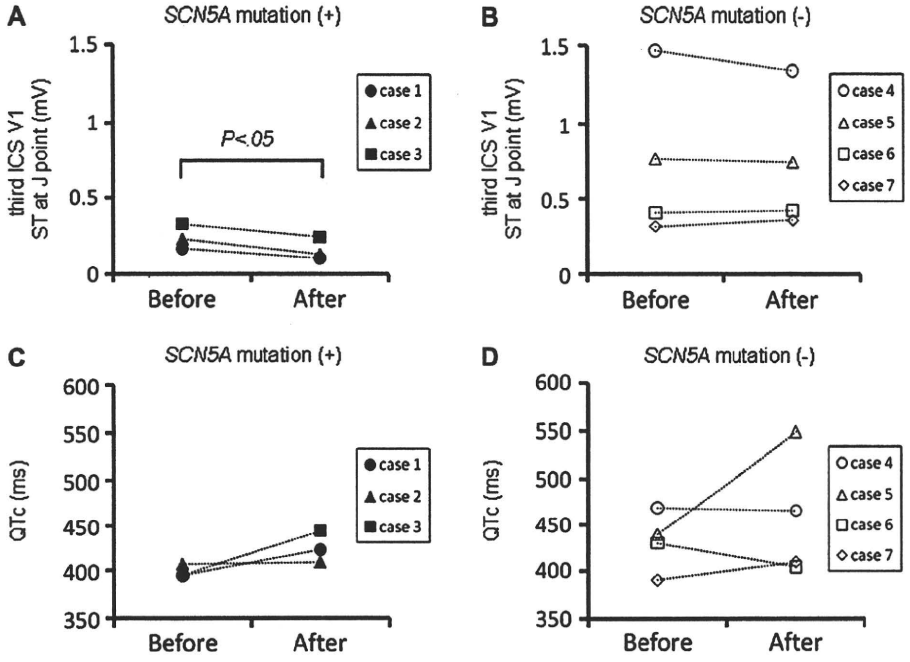

FIGURE 2. Effects of low-dose bepridil on electrocardiographic parameters. Levels of ST elevation at J points in the third intercostals space V1 lead before and after treatment with low-dose bepridil in patients with *SCN5A* mutation (A) and without *SCN5A* mutation (B). QTc before and after treatment with low-dose bepridil in patients with *SCN5A* mutation (C) and without *SCN5A* mutation (D).

was not effective in those without *SCN5A* mutation; 2) ST elevation at J points was significantly decreased by low-dose bepridil in patients with *SCN5A* mutation; and 3) the parameters of SAECG showed a trend to be normalized in patients with *SCN5A* mutation with significant improvement in LAS_{40}. This tendency was not seen in patients without *SCN5A* mutation.

An experimental model of BrS using arterially perfused canine right ventricular wedge preparations showed that the I_{to}-mediated action potential (AP) notch and loss of the AP dome in epicardial cells, but not in endocardial cells, of the right ventricle gives rise to a transmural voltage gradient, producing ST segment elevation in ECG, which leads to the development of Phase 2 re-entry, polymorphic ventricular tachycardia, and VF.[20–23] Additional Na^+ channel blockers such as pilsicainide, mimicking the *SCN5A* defect, produce further accentuation of the Phase 1 notch and a greater prolongation of epicardial AP, and that gives rise to coved-type ST segment elevation. Therefore, I_{to} and decreased I_{Na} are important to produce ST segment elevation in ECG. Quinidine[20,24] blocks I_{to} and consequently prevents excessive deepening of the Phase 1 notch of the AP and eliminates J-ST elevation and arrhythmogenesis. Bepridil is also able to inhibit I_{to}.[4] I_{to} was inhibited by 1 μmol/L bepridil,[4] and the concentration is similar to the levels observed in human plasma after oral 100 mg/day of bepridil (Daiichi Sankyo Company, Tokyo, Japan). Our study showed that ST elevation at J points was significantly decreased by low-dose bepridil in patients with *SCN5A* mutation and that low-dose bepridil was effective for reducing frequency of VF events in those patients. Therefore, the effect of blocking I_{to} by bepridil plays an important role in prevention of VF in patients with BrS. However, the reason why bepridil is more effective in patients with *SCN5A* mutation than in those without *SCN5A* mutation remains unknown. Further studies are needed to clarify this point.

Bepridil is a calcium antagonist affecting both L and T type calcium channels with a lidocaine-like fast kinetic block of

TABLE 3. ST Levels in Right Precordial Leads

	Before				After			
	V1	V2	Third ICS V1	Third ICS V2	V1	V2	Third ICS V1	Third ICS V2
Case No.	(mV)	(mV)	(mV)	(mV)	(mV)	(mV)	(mV)	(mV)
1	0.060	0.180	0.165	0.277	0.055	0.180	0.101	0.183
2	0.140	0.275	0.227	0.498	0.055	0.145	0.126	0.265
3	0.360	0.431	0.328	0.517	0.348	0.348	0.240	0.284
4	1.375	0.760	1.468	0.841	1.305	0.805	1.343	1.009
5	0.790	0.260	0.766	0.248	0.852	0.185	0.744	0.296
6	0.060	0.210	0.404	0.717	0.060	0.210	0.420	0.464
7	0.105	0.360	0.313	0.836	0.275	0.615	0.356	0.879

ICS, intercostal space.

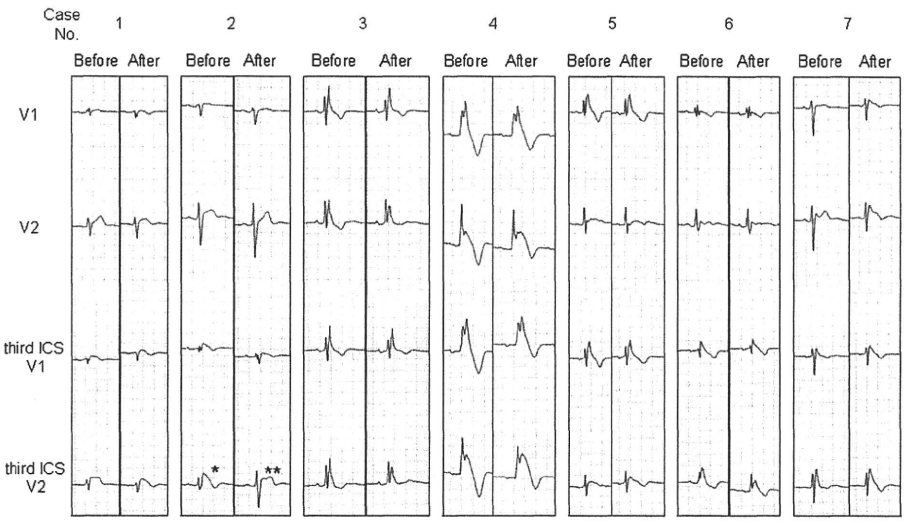

FIGURE 3. Electrocardiograms of patients with Brugada syndrome before and after treatment with low-dose bepridil. In Case No. 2, coved-type ST elevation of the third intercostals space V2 lead (*) changed to saddle-back type (**).

sodium current and has unique electrophysiological properties to inhibit outward currents including most types of K current (I_{Kr}, I_{Ks}, I_{Kur}, I_{K1}, I_{KAch} and I_{KATP} and I_{to}).[4,5,25] It is used for treatment of atrial fibrillation and ventricular arrhythmia in Japan.[5,26] QT prolongation occurs after bepridil therapy by its I_K blocking.[5] In our study, QT interval was prolonged in all patients with *SCN5A* mutation but only in half of the patients without *SCN5A* mutation. QT prolongation may be required to

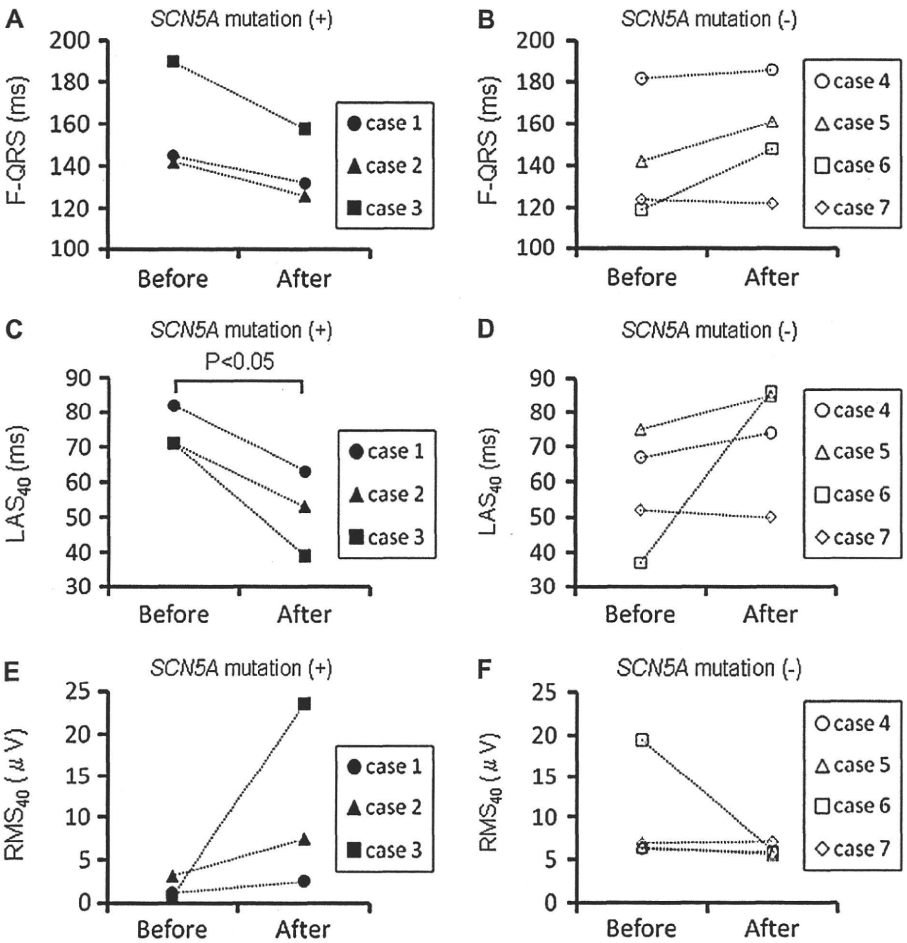

FIGURE 4. Effects of low-dose bepridil on signal-averaged electrocardiographic parameters. Filtered QRS before and after treatment with low-dose bepridil in patients with *SCN5A* mutation (A) and without *SCN5A* mutation (B). LAS_{40} before and after treatment with low-dose bepridil in patients with *SCN5A* mutation (C) and without *SCN5A* mutation (D). RMS_{40} before and after treatment with low-dose bepridil in patients with *SCN5A* mutation (E) and without *SCN5A* mutation (F).

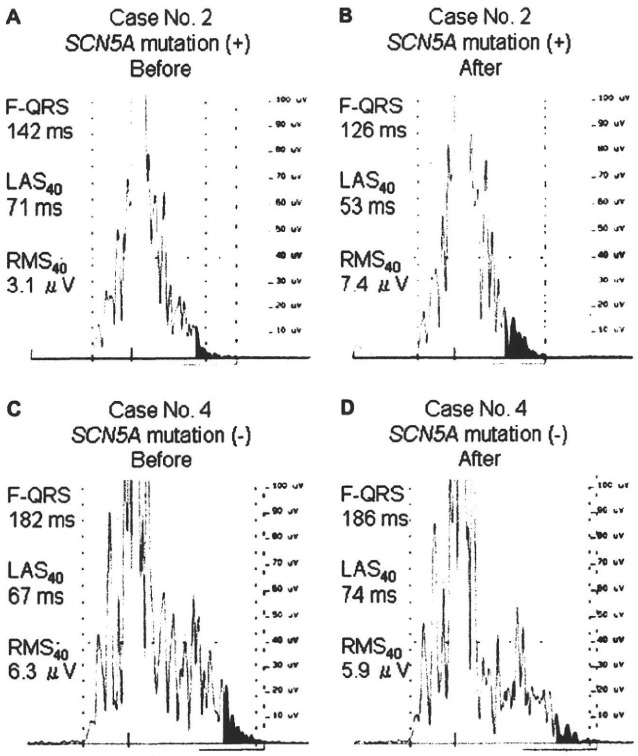

FIGURE 5. Representative signal-averaged electrocardiograms (SAECGs) of patients with Brugada syndrome before and after treatment with low-dose bepridil. SAECG before (A) and after (B) treatment with low-dose bepridil in a patient with *SCN5A* mutation (Case No. 2). SAECG before (C) and after (D) treatment with low-dose bepridil in a patient without *SCN5A* mutation (Case No. 4).

induce efficacy of bepridil. However, the QTc interval became markedly prolonged with frequent episodes of VF after low-dose bepridil in one case (Case No. 5). Monitoring of the QT interval is needed after administration of bepridil.

Abnormal depolarization and conduction have been observed in patients with BrS. [14,27,28] The incidence of late potential (LP) in patients with BrS was found to be significantly higher than that in the control group,[28] and the LP corresponded to the epicardial electrogram after termination of the QRS complex.[14] LP in BrS is considered to be related to delayed activation in the epicardial site of the right ventricular outflow tract or prolonged action potential with second upstroke and/or Phase 2 re-entry between the epicardial sites with different action potential duration in the right ventricular outflow tract.[29,30] Watanabe et al reported elimination of LP by quinidine in a patient with BrS.[30] They suggested that it might be responsible for restoration of the Phase 1 action potential notch and reappearance of the Phase 2 dome by the I_{to} blocking property of quinidine. In our study, LAS_{40} was improved after bepridil treatment in patients with *SCN5A* mutation. It might be responsible for the I_{to} blocking property of bepridil. However, further investigations are needed to clarify this point.

Limitations

The population of patients treated with bepridil was relatively small. Thus, definite interpretation of the results as to the real effectiveness of bepridil in the subjects is difficult. An investigation using a larger number of patients should be done in the future.

Because of the small number of subjects and uncertainty of the results, the possible mechanism of different responses to bepridil in patients with or without mutation is not clear. Further studies are needed to clarify this point.

It is well known that ECG of patients with BrS show day-to-day and circadian variance.[31] We evaluated ECG before and 1 month after bepridil treatment only once a day. Accordingly, the possibility that the changes of ECG in our study were the result of this characteristic cannot be ruled out.

In conclusion, treatment with bepridil prevents recurrence of VF along with improvement of ST elevation and LAS_{40} in patients with *SCN5A* mutation.

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ORIGINAL ARTICLE
Arrhythmia/Electrophysiology

SCN5A Mutation Is Associated With Early and Frequent Recurrence of Ventricular Fibrillation in Patients With Brugada Syndrome

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Background: Mutations in *SCN5A* are reportedly linked to Brugada syndrome (BS), but recent observations suggest that they are not necessarily associated with ventricular fibrillation (VF) in BS patients. Therefore, the clinical importance of *SCN5A* mutations in BS patients was examined in the present study.

Methods and Results: The 108 BS patients were examined for *SCN5A* mutations and various parameters were compared between patients with and without mutations. An implantable cardioverter defibrillator (ICD) was implanted in 49 patients and a predictor of appropriate ICD shock was investigated. The existence of a *SCN5A* mutation was not associated with initial VF episodes (21.7% vs 20.0%, P=0.373). In the secondary prevention group, appropriate shock-free survival rate was significantly lower in patients with spontaneous type 1 ECG than in those without (41.1% vs 85.7% at 2 years, P=0.014). The appropriate shock-free survival rate was also significantly lower in patients with *SCN5A* mutations than in those without (28.6% vs 83.3% at 1 year, P=0.040). Appropriate shock was more frequent in patients with *SCN5A* mutations than in those without (6.6±6.2 vs 1.7±3.0, P=0.007).

Conclusions: *SCN5A* mutations are associated with early and frequent VF recurrence, but not with initial VF episodes. This is the first report on the genotype–phenotype interaction and clinical significance of this mutation. (*Circ J* 2010; **74:** 2572–2578)

Key Words: Appropriate ICD shock; Brugada syndrome; Genotype–phenotype interaction; Implantable cardioverter defibrillator; *SCN5A* mutation

Brugada syndrome (BS) is characterized by ST-segment elevation in the right precordial leads and sudden death (SD) because of ventricular fibrillation (VF).[1,2] *SCN5A* encodes for the alpha subunit of the cardiac sodium channel gene and BS is associated with *SCN5A* mutations in approximately 15% of probands.[3–5]

Previous studies have suggested that *SCN5A* mutations are associated with depolarization abnormalities such as bradyarrhythmia, conduction delay and conduction block.[6–14]

However, it is unclear whether BS patients with *SCN5A* mutations have a greater risk of arrhythmic events or SD. Recent observations have also revealed that *SCN5A* mutations are not necessarily associated with VF or syncopal episodes in patients with BS.[15,16]

Thus, the clinical importance of *SCN5A* mutation for developing VF in BS patients is not clear.

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Methods

Patients

The subjects were 108 consecutive BS patients who were admitted to Okayama University Hospital, Fukuoka University Hospital, Cardiovascular Center Sakakibara Hospital, National Hospital Organization Okayama Medical Center and Fukuyama Cardiovascular Hospital during January 1997 to December 2009. All 108 BS patients were examined for *SCN5A* mutations and we divided them into 2 groups according to the presence or absence of a mutation. All patients underwent echocardiography and chest X-ray and no abnormalities were found. All of the tests that were performed were approved by the medical ethical review committees of each hospital and informed consent was given by all patients.

Clinical Examination

All BS patients had a type 1 ECG recorded spontaneously and after provocation with a class I antiarrhythmic drug[2] (pilsicainide 1 mg/kg body weight at 10 mg/min IV).[17] We evaluated the ECG before drug administration, immediately after, and at 5 and 10 min after drug administration.

An electrophysiological study was performed in 99 of the 108 patients. Induction of ventricular arrhythmia (VA) was attempted without using any antiarrhythmic drugs. The criterion for induction of VA was induction of sustained polymorphic ventricular tachycardia (VT) or VF by programmed electrical stimulation (PES) from the right ventricular apex or right ventricular outflow tract. The protocol of ventricular stimuli included up to 3 extrastimuli (2 basic cycle lengths of 600 and 400 ms) and rapid ventricular pacing, with the coupling interval of the extrastimuli not being less than 180 ms and the ventricular rate of rapid burst pacing not exceeding 270 beats/min. The electrophysiological study was performed as reported previously.[18] We also measured the His–ventricular (HV) interval.

The ECGs were measured by an investigator who was unaware of patient characteristics. QRS duration was analyzed in lead V_5 from the standard 12-lead ECG in all BS patients.

Signal-averaged electrocardiograms (SAECG) (ART 1200EPX; noise level <0.3 μV, high-pass filtering of 40 Hz using a bidirectional 4-pole Butterworth) were obtained in all BS patients. Filtered QRS duration, root mean square voltage of the terminal 40 ms in the filtered QRS complex (RMS40) and duration of low-amplitude signals <40 μV in the terminal filtered QRS complex (LAS40) were measured by SAECG. A late potential (LP) was considered to be positive when the 2 criteria (RMS40<20 μV and LAS40>40 ms) were met.[19]

In the absence of symptoms or device therapy (implantable cardioverter defibrillator: ICD), all BS patients were seen routinely every 3–12 months for clinical review and device interrogation, according to local practice. In the event of a shock, patients were seen at the ICD clinic within 48 h and the device was interrogated. Appropriate shocks were defined as shocks delivered for VT or VF.

None of the patients received antiarrhythmic drugs at the time of first admission. We started treatment with antiarrhythmic drugs in patients with VF if they had experienced recurrent VF episodes and appropriate ICD shocks.

Mutation Analysis of *SCN5A*

Genetic analysis was performed in compliance with the ethics committee guidelines for human genome studies of the Ethics Committee. Informed consent was given by all 108 subjects. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit (Gentra, Minneapolis, MN, USA) and was stored at –30°C until use.

In total, 27 exons of *SCN5A* were amplified with previously reported intronic primers.[20] *SCN5A* exon 1 is a non-coding region, and we did not analyze it in this study. Exons 6, 17-1 sense, 21, and 25 were unable to be amplified enough by the primers, and we therefore designed the following intronic primers as previously described.[21–22] The primers used in this study were: exon 6: sense, 5'-GTT ATC CCA GGT AAG ATG CCC-3'; anti-sense, 5'-TGG TGA CAG

Table 1. Characteristics of the Patients With BS

N	108
Male, (%)	105 (97.2)
Age (years)	46.8±11.6
Spontaneous type 1 ECG, (%)	71 (65.7)
Family history of SD, (%)	30 (27.8)
Documented VF, (%)	23 (21.3)
Syncopal episode, (%)	42 (38.9)
VF induced by programmed electrical stimulation, (%)	48/99 (48.5)
Late potential, (%)	71/105 (67.6)
SCN5A mutation, (%)	17 (15.7)
ICD implantation, (%)	49 (45.4)

BS, Brugada syndrome; SD, sudden death; VF, ventricular fibrillation; ICD, implantable cardioverter defibrillator.

Table 2. Characteristics of BS Patients With and Without Documented VF

	Documented VF (+)	Documented VF (–)	P value
N	23	85	
Male, (%)	22 (95.7)	83 (97.6)	0.666
Age (years), (%)	45.8±11.5	47.0±11.7	0.624
Spontaneous type 1 ECG, (%)	13 (56.5)	58 (68.2)	0.294
Family history of SD, (%)	5 (21.7)	25 (29.4)	0.466
VF induced by programmed electrical stimulation, (%)	13/23 (56.5)	35/76 (46.1)	0.175
Late potential, (%)	16/22 (72.7)	55/83 (66.3)	0.565
SCN5A mutation, (%)	5 (21.7)	17 (20.0)	0.373
QRS duration (ms)	107±23	97±14	0.015
HV interval (ms)*	45.3±7.4	41.3±7.9	0.042

*No. of patients with and without documented VF who were examined for HV interval was 16 and 78, respectively. HV, His-ventricular. Other abbreviations see in Table 1.

Table 3. Characteristics of BS Patients With and Without *SCN5A* Mutation

	SCN5A mutation (+)	*SCN5A* mutation (–)	P value
N	17	91	
Male, (%)	16 (94.1)	89 (97.8)	0.396
Age (years)	46.4±13.6	46.8±11.3	0.894
Spontaneous type 1 ECG, (%)	13 (76.5)	58 (63.7)	0.310
Family history of SD, (%)	3 (17.6)	27 (29.7)	0.310
Documented VF, (%)	5 (29.4)	18 (19.8)	0.373
Syncopal episode, (%)	9 (52.9)	34 (37.4)	0.452
VF induced by programmed electrical stimulation, (%)	4/15 (26.7)	44/84 (52.4)	0.066
Late potential, (%)	13/14 (92.9)	58/91 (63.7)	0.030
QRS duration (ms)	110±13	97±17	0.004
HV interval (ms)*	50.1±9.4	41.1±7.0	0.0001

*No. of patients with and without *SCN5A* mutation examined for HV interval was 12 and 82, respectively.
Abbreviations see in Tables 1,2.

Table 4. Characteristics of BS Patients With ICD Implantation for Secondary or Primary Prevention

	Secondary prevention	Primary prevention	P value
N	31	18	
Male, (%)	29 (93.5)	18 (100)	0.271
Age (years)	47.6±11.5	48.7±12.1	0.769
Spontaneous type 1 ECG, (%)	17 (54.8)	10 (55.6)	0.961
Family history of SD, (%)	5 (16.1)	10 (55.6)	0.004
VF induced by programmed electrical stimulation, (%)	18 (58.1)	14 (77.8)	0.162
Late potential, (%)	20/28 (71.4)	13/18 (72.2)	0.395
SCN5A mutation, (%)	7 (22.6)	1 (5.6)	0.120

Abbreviations see in Table 1.

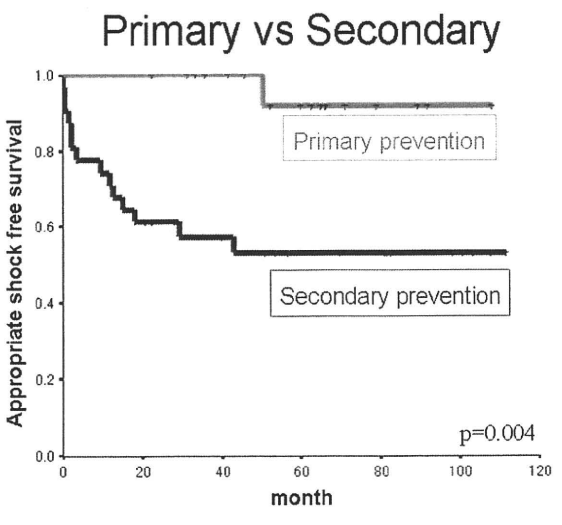

Figure 1. Appropriate-shock-free survival rate in Brugada syndrome (BS) patients with an implantable cardioverter defibrillator (ICD) for secondary or primary prevention. 14 (46.7%) of the 31 patients with ICD implantation for secondary prevention (secondary prevention group) and 1 (5.6%) of the 18 patients with ICD implantation for primary prevention (primary prevention group) received appropriate shocks. The appropriate shock-free survival rate was significantly lower in the secondary prevention group than in the primary prevention group (52.8% vs 91.7% at 5 years, P=0.004).

GCA CAT TCG AAG-3'; exon 17-1: sense, 5'-AAG CCT CGG AGC TGT TTG TCA CA-3'; exon 21: sense, 5'-TGC CTG GTG CAG GGT GGA AT-3'; anti-sense, 5'-ACT CAG ACT TAC GTC CTC CTT C-3'; exon 25: sense, 5'-TCT TTC CCA CAG AAT GGA CAC C-3'; anti-sense, 5'-AAG GTG AGA TGG GAC CTG GAG-3'. Polymerase chain reaction (PCR) was performed in a 20-μl reaction volume containing 50 ng of genomic DNA, 20 pmol of each primer, 0.8 mmol/L dNTPs, 1×reaction buffer, 1.5 mmol/L MgCl2, and 0.7 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) or TAKARA Taq (Takara Bio Inc, Otsu, Japan). All PCR products were treated with exonuclease I (New England BioLabs, Ipswich, MA, USA) and shrimp alkaline phosphatase (USB Corporation, Cleveland, OH, USA), reacted with a Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems), and analyzed on an ABI PRISM3130xl sequencer (Applied Biosystems). The mutations were confirmed 4 times by independent PCR amplification and sequencing.

Statistical Analysis

Data are expressed as mean values±standard deviation. Student's t-test was performed to test for statistical differences between 2 unpaired mean values, and categorical data and percentage frequencies were analyzed by the chi-square test. The event-rate curve was generated according to the Kaplan-Meier method (SPSS II for Windows, SPSS Inc, Chicago, IL, USA). A P value<0.05 was considered significant.

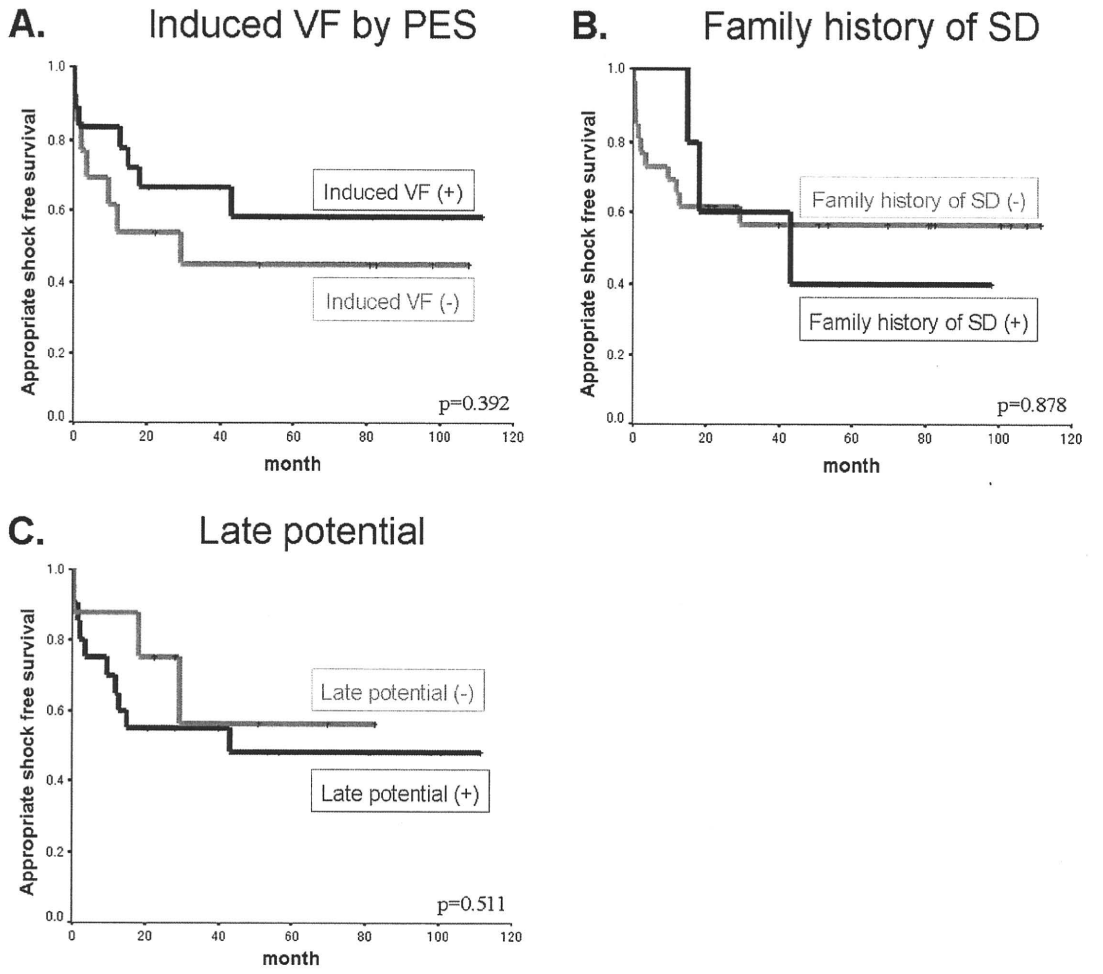

Figure 2. Appropriate-shock-free survival rate in Brugada syndrome (BS) patients with an implantable cardioverter defibrillator (ICD) for secondary prevention. There was no significant difference in the appropriate-shock-free survival rate between BS patients with and without (**A**) ventricular fibrillation (VF) induced by programmed electrical stimulation (PES), (**B**) family history of sudden death (SD) and (**C**) late potentials.

Results

Clinical Difference Between BS Patients With and Without Documented VF

Baseline characteristics of the patients are shown in Table 1. There were no significant differences in sex, age, family history of SD, VF induced by PES, LPs, spontaneous type 1 ECG and *SCN5A* mutation between patients with or without documented VF; however, QRS duration and HV interval were significantly longer in patients with documented VF (Table 2).

Clinical Difference Between BS Patients With and Without *SCN5A* Mutation

As shown in Table 3, there was no significant difference in documented VF between patients with or without a *SCN5A* mutation, nor were there significant differences in sex, age, family history of SD, syncopal episodes, VF induced by PES and spontaneous type 1 ECG between the 2 groups. However, patients with *SCN5A* mutations were significantly more likely to have LPs than were those without a mutation. QRS duration and HV interval were also significantly longer in patients with a *SCN5A* mutation.

Clinical Course of BS Patients With ICD

An ICD was implanted in 49 of the 108 patients, for secondary prevention in 31 patients (secondary prevention group, which included 14 patients with syncope only, and 17 patients with documented VF at the time of ICD implantation) and for primary prevention in 18 patients (primary prevention group) (Table 4). During a mean follow-up period of 71.9±41.3 months after ICD implantation, no cardiac deaths occurred. Of the 49 patients 15 (30.6%) had appropriate ICD shocks. All cases of spontaneous VF were successfully terminated by only 1 ICD shock. The patients with frequent VF occurrence were treated with drugs (mexiletine, n=1; disopyramide, n=2; bepridil, n=5; quinidine, n=4).

The appropriate shock-free survival rate in the secondary prevention group was significantly lower than that in the primary prevention group (Figure 1).

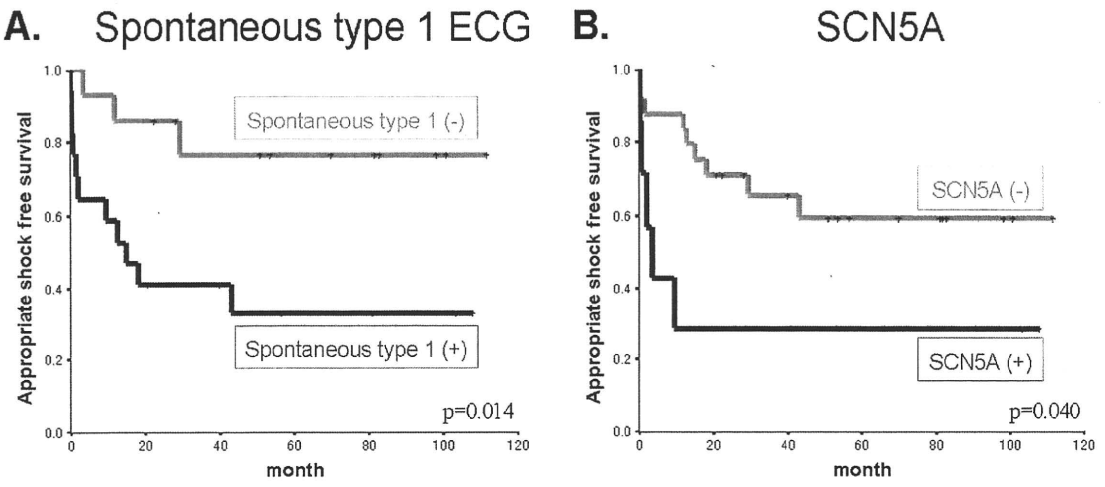

Figure 3. Appropriate-shock-free survival rate in Brugada syndrome (BS) patients with an implantable cardioverter defibrillator for secondary prevention. (**A**) 11 (64.7%) of 17 patients with spontaneous type 1 ECG and 3 (21.4%) of 14 patients without spontaneous type 1 ECG received appropriate shocks. The appropriate-shock-free survival rate was significantly lower in patients with spontaneous type 1 ECG than in BS patients without spontaneous type 1 ECG (41.1% vs 85.7% at 2 years, P=0.014). (B) 5 (71.4%) of 7 patients with a *SCN5A* mutation and 9 (37.5%) of 24 patients without *SCN5A* mutation received appropriate shocks. The appropriate-shock-free survival rate was significantly lower in patients with a *SCN5A* mutation (28.6% vs 83.3% at 1 year, P=0.040).

Table 5. Characteristics of BS Patients With and Without *SCN5A* Mutation and Implanted ICD for Secondary Prevention

	SCN5A mutation (+)	*SCN5A* mutation (–)	P value
N	7	24	
Male, (%)	6 (85.7)	23 (95.8)	0.338
Age (years)	47.0±14.6	47.8±10.8	0.440
No. of appropriate shock	6.6±6.2	1.7±3.0	0.007
Period from ICD implantation to drug administration or last follow-up	59.1±35.9	46.5±35.0	0.414
Drug administration, (%)	4 (57.1)	7 (29.2)	0.134
Spontaneous type 1 ECG, (%)	6 (85.7)	11 (45.8)	0.062
Family history of SD, (%)	0 (0)	5 (20.8)	0.187
Documented VF, (%)	5 (71.4)	17 (70.8)	0.976
VF induced by programmed electrical stimulation, (%)	1 (14.3)	17 (70.8)	0.008
Late potential, (%)	3/4 (75.0)	17/24 (70.8)	0.864
QRS duration (ms)	110±14	102±22	0.399
HV interval (ms)	52.2±12.8	43.5±6.5	0.204

Abbreviations see in Tables 1,2.

In the secondary prevention group, there were no significant differences in the appropriate ICD shock-free survival rate between patients with and without VF induced by PES, family history of SD and LPs (**Figure 2**). However, the appropriate shock-free survival rate in patients with spontaneous type 1 ECG (n=17) was significantly lower than that in patients without a spontaneous type 1 ECG (n=14) (**Figure 3A**). The appropriate shock-free survival rate in patients with *SCN5A* mutation (n=7) was also significantly loweer than that in patients without a mutation (n=24) (**Figure 3B**).

There was no significant difference in the number of appropriate ICD shocks between patients with and those without spontaneous type 1 ECG. However, appropriate ICD shock was more frequent in patients with a *SCN5A* mutation than in patients without during the period from ICD implantation to first drug administration (in patients with drugs) or last follow-up (in patients without drugs) (**Table 5**).

In the secondary prevention group, the odds ratio (OR) of spontaneous type 1 ECG for VF recurrence was 2.48 (P=0.248) at 1 year after ICD implantation, 5.24 (P=0.043) at 2 years after ICD implantation and 6.72 (P=0.021) at last follow-up. Also, the OR of *SCN5A* mutation for VF recurrence was 9.50 (P=0.021) at 1 year after ICD implantation, 5.00 (P=0.081) at 2 years after ICD implantation and 4.17 (P=0.128) at last follow-up.

Clinical Course of BS Patients Without ICD

None of the BS patients without an ICD suffered from VF or SD during the follow-up.

Discussion

New Findings

In the present study, we demonstrated that *SCN5A* mutations are not associated with the first onset of VF in BS patients. However, they are associated with early and frequent recurrence of VF in symptomatic BS patients. To our knowledge, this is the first report on the genotype–phenotype interaction and clinical significance of *SCN5A* mutations.

SCN5A Mutation Is Related to Depolarization Abnormality

In 1998, Chen et al[23] identified the first mutation in *SCN5A*, the gene encoding for the alpha subunit of the sodium channel (I_{Na}), that was linked to BS.[23] Mutations of *SCN5A* account for 10–30% of BS cases. Functional analysis using expression systems have revealed that mutations in *SCN5A* result in loss of function of I_{Na}. *SCN5A* has been reported as associated with depolarization abnormalities.[6,7,9–11] Previous studies showed that the P wave, QRS, PQ, and HV interval were significantly longer in BS patients with a *SCN5A* mutation than in those without.[7,9,10] Bradyarrhythmias, such as sick sinus syndrome and intraventricular conduction delay, are also more likely to occur in BS patients with *SCN5A* mutations.[10,11]

SCN5A Mutation Is Not Related to Initial Episodes of VF

Detection of a *SCN5A* mutation is a diagnostic tool for BS. However, no study has shown the impact of genetic analysis on risk stratification. Priori et al reported that *SCN5A* mutation is the most frequent mutation in known causative genes, but the incidence is only 10–30%.[24] Furthermore, the average penetrance based on ECG analysis is only 16%.[25] Several studies have also shown that *SCN5A* mutation is not associated with clinical significance, such as syncopal or VF episodes.[15,16] The present study also showed that *SCN5A* mutation was not associated with initial VF episodes in BS patients.

SCN5A Mutation Is Associated With Early and Frequent VF Recurrence

To our knowledge, only 1 study of mice with ischemic heart disease has reported an association of *SCN5A* mutation with recurrence of VF.[26]

In the present study, 5 (71.4%) of the 7 symptomatic BS patients with a *SCN5A* mutation had recurrence of VF within 1 year after ICD implantation. On the other hand, only 9 (37.5%) of the 24 symptomatic BS patients without *SCN5A* mutation had recurrence of VF up till the last follow-up. The recurrence of VF was significantly earlier and more frequent in symptomatic BS patients with *SCN5A* mutation than in symptomatic BS patients without a mutation.

The reasons why *SCN5A* mutation is associated with the clinical significance of symptomatic BS are speculative, based on some previous studies. Aiba et al reported that depolarization abnormalities in experimental BS models are associated with factors that predispose to VF maintenance.[27] Junttila et al also reported that prolonged QRS duration is associated with VF episodes.[16] Similar observations have been made in clinical settings where the occurrence of LPs in SAECG predicts adverse events in BS patients.[28] Yokokawa et al have also linked prolonged QRS duration and aging to *SCN5A* mutation carrier status in BS patients.[7] In the present study, SCN5A was also associated with prolonged QRS duration and HV interval. Thus, *SCN5A* mutation is associated with a prolonged and progressive conduction delay, which is strongly associated with VF. Therefore, *SCN5A* mutations might be the cause of early and frequent recurrence of VF.

Clinical Implications

Management of symptomatic BS patients is very important because frequent ICD shocks are likely to worsen quality of life and cause psychological damage. Therefore, risk stratification of symptomatic BS patients is important. In the present study, we showed that having a *SCN5A* mutation was a predictor of early and frequent VF recurrence. Therefore, symptomatic BS patients with spontaneous type 1 ECG or *SCN5A* mutation should be administered drugs to avoid early and frequent recurrence of VF.

Study Limitations

First, the mean follow-up period was not long, and it is necessary to evaluate the same data during a longer period. Second, the number of the BS patients with *SCN5A* was relatively low. If the number of BS patients increased, the results might change.

Conclusions

We demonstrated that *SCN5A* mutation is not associated with initial episodes of VF in BS, but is associated with early and frequent recurrence of VF in symptomatic BS patients. To our knowledge, this is the first report of a genotype–phenotype interaction and the clinical significance of *SCN5A* mutation.

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Disclosures

None.

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CORRESPONDENCE

Research Correspondence

Electroanatomical Correlation of Repolarization Abnormalities in Brugada Syndrome

Detection of Type 1 Electrocardiogram in the Right Ventricular Outflow Tract

To the Editor: ST-segment elevation is the most important characteristic for the diagnosis of Brugada syndrome (BrS). The Consensus Report of Brugada Syndrome defined type 1 electrocardiogram (ECG) (coved-type ST-segment elevation ≥0.2 mV with a negative T-wave in the right precordial lead) is diagnostic for BrS (1,2). Although the standard ECG recording of the right precordial leads at the fourth intercostal space (ICS) sometimes fails to reveal type 1 ECG, additional recording of leads V_1 and V_2 at high (third and second) ICS increases the sensitivity for detecting type 1 ECG (3,4).

ST-segment elevation in BrS is believed to represent abnormal repolarization at the right ventricular outflow tract (RVOT) (3). However, the relationship between ECG recording site (standard and high ICS recording) and the anatomical position of the RVOT is still unclear. Accordingly, we examined the relationship between the lead positions of type 1 ECG and the location of the RVOT in patients with BrS.

We examined 60 patients with BrS (59 men; 47 ± 12 years of age). We defined BrS based on the Second Consensus Report of Brugada Syndrome (2). Cardiac catheterization, electrophysiologic study, and genetic analysis were performed according to the protocol approved by the ethics committee of Okayama University. Written informed consent was obtained from all patients. Cardiac catheterization and ECG recording were performed simultaneously in all patients. The ECG was recorded at the additional third and standard fourth ICS in leads V_1 and V_2 with fluoroscopically visible electrodes. Anatomical location of the RVOT was determined under fluoroscopic images, with right ventriculography performed in the right anterior oblique (RAO) view. We determined the location of the RVOT as being below the pulmonary valve and above the anterior border of the tricuspid valve in the end-diastole and -expiration phase. We examined the relationships between the location of the RVOT and the position of leads manifesting type 1 ECG. For detailed analysis of lead position, we made an 8-segment model of the right ventricle in RAO view. We analyzed the lead position and appearance of type 1 ECG at baseline conditions. If patients did not have type 1 ECG in any leads at baseline, we used an intravenous injection of a pure sodium-channel blocker, pilsicainide (n = 39). Genetic screening revealed that SCN5A mutation was present in 5 of 39 patients (12.8%).

Anatomic correlation of the RVOT and the locations of the third and fourth ICS were variable in each patient (Figs. 1A and 1B). Figure 1A shows an example of 1 patient in whom the RVOT was located at the fourth ICS and type 1 ECG was recorded in leads V_1 and V_2 at both the third and fourth ICS. Figure 1B shows an example of another patient in whom the RVOT corresponded to the third ICS, and the fourth ICS coincided with inflow to the anterior free wall of the right ventricle. Type 1 ECG was recorded only by the third ICS electrode in this patient. Overall, type 1 ECG was recorded in leads that corresponded with the RVOT.

Figure 1C shows the distribution of location of ECG leads with and without type 1 ECG. The location of the RVOT corresponded to the V_1 and V_2 leads at the fourth ICS in 11 patients (18.3%) and at the third ICS in 49 patients (81.7%). In 9 of 11 patients (82%) in whom the fourth ICS represented the RVOT, type 1 ECG was obtained at the fourth ICS. However, 41 of 49 patients (84%) in whom the third ICS represented the RVOT did not show type 1 ECG at the fourth ICS, but it was shown at the third ICS instead. Accordingly, most of the ECG leads manifesting type 1 were distributed at the RVOT ($p < 0.0001$). Documented VF (n = 12), syncope (n = 10), family history of sudden cardiac death (n = 19), induced ventricular fibrillation with programmed electrical stimulation (n = 30), presence of SCN5A mutation, and spontaneous type 1 ECG (n = 21) were not related to the location of the ECG leads corresponding with the RVOT.

In the present study, we showed that the RVOT determines the manifestation of type 1 ECG in patients with BrS. And the relationship between the position of the leads and the RVOT was variable in individual cases. Abnormal repolarization is thought to be associated with the RVOT in BrS. However, the exact anatomical location of the formation of type 1 ECG has not been fully examined yet. Because the V_1 and V_2 leads at the standard fourth ICS were not associated with the RVOT in approximately 80% of patients with BrS, the recording of additional V_1 and V_2 leads at the third ICS improved the detection of type 1 ECG in BrS.

Previous basic studies have shown that the transient outward current (Ito) is most prominent in the RVOT region of the ventricular myocardium, giving rise to a more prominent action potential notch in this region, which is responsible for inscription of the accentuated J-wave or ST-segment elevation in this lead facing this region of the right ventricle (5).

In summary, our results show that positioning of ECG electrodes at the RVOT can detect type 1 ECG in patients with BrS. The ICS, which is associated with the RVOT, can be different in each patient.

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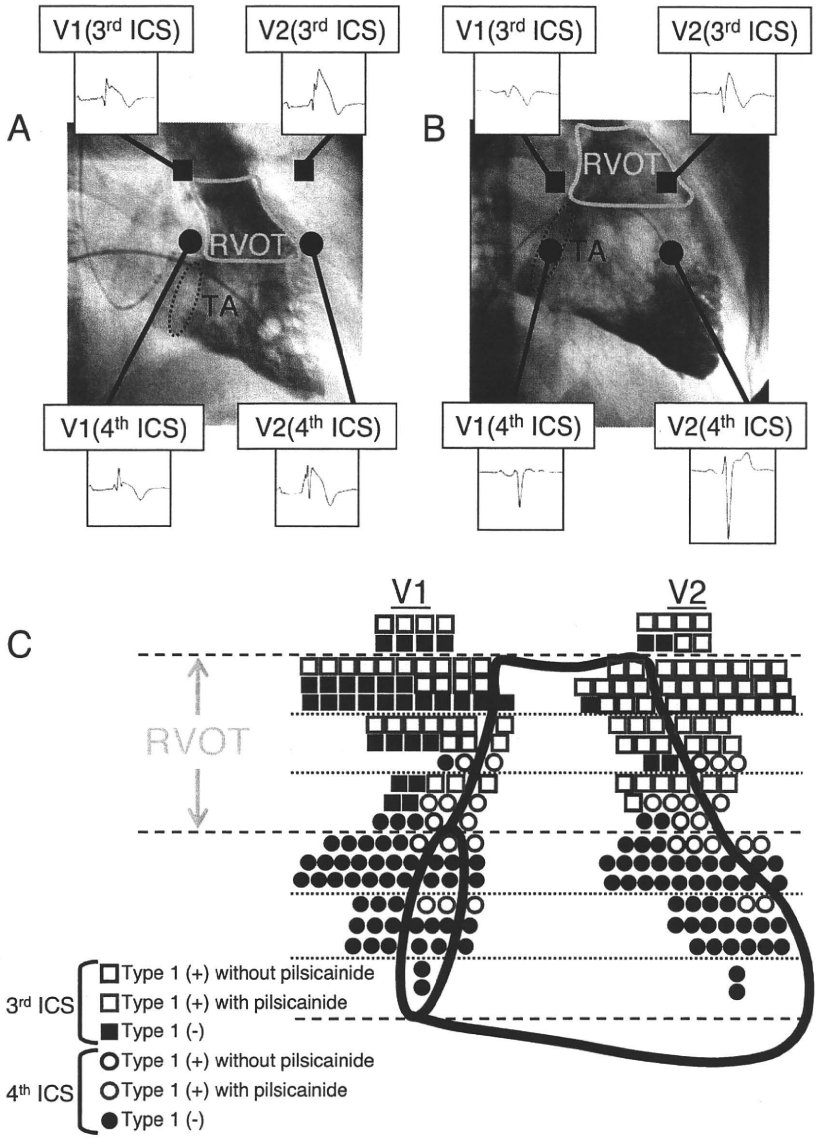

Figure 1 **Detection of Type 1 ECG in the RVOT**

(A) A 62-year-old male patient with recurrent unknown syncope attacks. **(B)** A 17-year-old male patient without any symptoms. **(C)** Position of electrocardiogram (ECG) leads in all patients. The lead location in front of the right ventricular outflow tract (RVOT) determined the manifestation of type 1 ECG. The lead position manifesting type 1 ECG coincided with the location of the RVOT in 34 of 44 (77.3%) V_1 leads and in 58 of 71 (81.7%) V_2 leads. ICS = intercostal space; TA = tricuspid annulus.

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Letters to the Editor

Exercise-Induced Troponin Elevation

Not Necessarily a Benign Phenomenon

In their comprehensive review of exercise-induced cardiac troponin elevation, Shave et al. (1) come to the conclusion that this is a benign phenomena most likely related to leakage of troponin from the cardiac myocyte membrane rather than myocyte necrosis—the usual cause of troponin elevation. In the absence of long-term follow-up of elite ultra-endurance athletes, this conclusion should be viewed with caution. There is increasing evidence that, in some athletes, participation in multiple extreme endurance events over a long period of years can lead to abnormal right ventricular (RV) enlargement, dysfunction, and—more ominously—potentially lethal arrhythmias (2–4). Such athletes are clinically and genetically distinct from those suffering from familial arrhythmogenic RV dysplasia/cardiomyopathy (5). An appropriate terminology for such individuals is "exercise-induced right ventricular dysplasia" (2–5). A plausible hypothesis for this syndrome is that extreme endurance exercise places a strain on the RV that on occasions leads to myocardial necrosis, albeit small, as reflected in post-exercise elevated troponin levels. The cumulative effect of repeated episodes of necrosis can eventually lead to sufficient fibrosis to result in RV dysfunction and to act as a substrate for potentially lethal arrhythmias. How prevalent exercise-induced RV dysplasia is and whether there is a genetic predisposition to the condition remains to be determined.

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Reply

We appreciate Dr. Harper's interest in our recent report (1). Dr. Harper suggests that without reassuring evidence from longitudinal studies it is possible that ultra-endurance exercise can produce a variant of right ventricular cardiomyopathy and suggests that the elevated cardiac troponin (cTn) observed after exercise are not benign. From a historical viewpoint, ultra-endurance events are not new. At the turn of the last century walking and running contests of several days duration were popular spectator events and raised concerns similar to those of Dr. Harper, but concerns about bicyclists', runners', and rowers' hearts were never documented (2). Right ventricular enlargement from endurance activity is not unusual but is rather one of the expected adaptations from exercise training. Indeed, all 4 cardiac chambers enlarge with exercise training, but there is little evidence of right ventricular dysfunction, except for reversible, transient changes after endurance events (3)—which might be related to a number of factors, such as elevations in heart rate, plasma volume alterations, or desensitization of beta-adrenoceptors. Furthermore, the observations that cTn is elevated after low-intensity exercise such as walking (4), early during a treadmill marathon (5), and after 30 min of high-intensity running (6) suggest that cTn elevations are common with exercise and not necessarily a product of prolonged effort. Recent studies have also shown no relationship between exercise-induced cTn release and late gadolinium enhancement with cardiac magnetic resonance imaging (7) and that, unlike acute coronary syndromes, cTn rapidly returns to baseline after exercise. In combination, such observations suggest a benign event. Finally, we would caution Dr. Harper as he has us: in the absence of longitudinal studies, it is provocative and premature to suggest that right ventricular problems observed in a case series of endurance athletes are produced by their athletic participation.

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Original article

What variables were associated with the inducibility of ventricular fibrillation during electrophysiologic stimulation test in patients without apparent organic heart disease?

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KEYWORDS
Electrophysiology;
Ventricular fibrillation;
Sudden death;
Syncope

Summary
Objective: The purpose of our study was to determine what variables were associated with ventricular fibrillation (VF) induced during electrophysiological stimulation test in patients without apparent organic heart disease.
Methods: Our study evaluated 77 patients (51 ± 15 years) who underwent electrophysiological stimulation test, signal averaging, and Na^+ channel-blocker challenge test (pilsicainide test). The subjects were divided into two groups, the Brugada group and non-Brugada group. Further, the patients were divided into three subgroups on the base of symptoms (8, 7 symptomatic; 9, 13 syncope; 28, 12 asymptomatic group; in the Brugada and non-Brugada groups, respectively). Multivariate analyses evaluated the association between baseline clinical factors and the induction of VF.
Results: The inducibility of VF was significantly ($p < 0.0001$) higher in the Brugada group ($n = 33$, 73%) than the non-Brugada group ($n = 4$, 13%). The multivariate analysis demonstrated that symptoms (odds ratio (OR) 31.6; 95% confidence interval (CI): 2.3–430.6; $p < 0.01$), type 1 electrocardiogram after pilsicainide test (OR 21.3; CI: 1.7–272.2; $p < 0.02$), and syncope (OR 13.5; CI: 1.2–158.8; $p < 0.05$) were strongly associated with the inducibility of VF, but not with family history, type 1 electrocardiogram in control, positive in late potential, maxΔST elevation ($\geqq$200 μV) after pilsicainide test.

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Conclusions: The symptoms, syncope, and type 1 electrocardiogram after pilsicainide test were independently associated with the electrophysiological substrate of VF in patients without apparent heart disease.

Introduction

Ventricular fibrillation (VF) is one of the important etiologies of sudden cardiac death and occurs commonly in patients with organic heart disease and left ventricular dysfunction. However, VF occurs even in patients without apparent heart disease, which is so-called idiopathic ventricular fibrillation. Recently, idiopathic VF has been classified as Brugada syndrome [1–7], long QT syndrome [8], short QT syndrome [9], and catecholaminergic polymorphic ventricular tachycardia [10], and others, based on their specific clinical characteristics.

Brugada syndrome is a relatively frequent disease associated with VF in patients without apparent heart disease in Japan and Asia [5]. It is also associated with electrocardiographic abnormality of the right bundle branch block with ST elevation in the right precordial leads [1–7]. The electrocardiogram (ECG) findings are the most useful parameters for the diagnosis of the Brugada type ECG [5]. Furthermore, the Na^+ channel-blockers challenge test is also useful because it can unmask the Brugada type ECG and induce the elevation of the ST-segment in patients with intermittent or concealed Brugada syndrome [5,6].

Although several parameters, such as the ECG findings, electrophysiological stimulation test (EPS), signal averaging, T-wave alternance (TWA), and abnormal genes have been reported to be predictive values for sudden cardiac death [7,11–15], the specificity of the induction test of VT (ventricular tachycardia)/VF during EPS remains unclear [13,14]. Since there is a wide variation in the incidence of events [7,16,17], the appropriate risk stratification for sudden cardiac death remains uncertain. A recently published second consensus report on the Brugada syndrome [18] from Europe and the USA implied that patients with either spontaneous or drug-induced type 1 Brugada ECG and a history of syncope or sudden cardiac death should have an implantable cardioverter defibrillator (ICD) implanted. Asymptomatic patients with a spontaneous type 1 Brugada ECG or asymptomatic patients with a drug-induced type 1 Brugada ECG and a family history of sudden cardiac death should undergo EPS to guide the selection of patients for ICD implantation.

In Japan, the inducibility of VF is one of the factors to make a decision of implantation of ICD according to the guidelines on ICD [19] or Brugada syndrome [20]. It is presumed that the inducibility of VF in patients without apparent organic heart disease indicates the existence of VF substrate. However, it is still unclear what variables are associated with VF induced during EPS in patients without apparent organic heart disease. Therefore, we performed a multivariate analysis to clarify what variables were strongly related to the inducibility of VF in patients without any apparent organic heart disease.

Methods

Subjects

Our study evaluated 77 patients who underwent EPS for the diagnosis of arrhythmia, risk stratification in Brugada syndrome, and/or sudden cardiac death in patients with no apparent heart disease and normal left ventricular function between January 2005 and August 2008. In addition, they underwent ECGs, Na^+ channel-blocker challenge tests, signal averaging, coronary angiography, and cardiac echocardiography during their hospitalization. Patients with idiopathic ventricular tachycardia (Ca antagonist sensitive) [21], long QT syndrome [8], short QT syndrome [9], and catecholaminergic polymorphic ventricular tachycardia [10] were excluded. Furthermore, those with spontaneous VF induced by ischemia, electrolyte disturbance, and/or hypothermia were also excluded.

A diagnosis of Brugada type ECG was made based on the following criteria: (1) J point amplitude over 0.2 mV with either spontaneous or drug-induced type 1 ECG (coved type ST elevation) in at least two of the three right precordial leads in the standard right precordial leads (V1–V3), or one or two intercostal spaces above the standard right precordial leads. Three types of repolarization patterns were defined as type 1, type 2, and type 3 according to the consensus report [5]. Type 1 is characterized by a prominent coved ST-segment elevation displaying J wave amplitude $\geq$2 mm. Type 2 also has a high take-off ST-segment elevation, but in this case J wave amplitude ($\geq$2 mm) gives rise to a gradually descending ST-segment elevation (remaining $\geq$1 mm above the baseline), followed by a positive or biphasic T-wave that results in a saddle back configuration. Type 3 has a J wave amplitude ($\geq$2 mm) that gives rise to (1) a gradually descending ST-segment elevation (remaining <1 mm above the baseline), followed by a positive T-wave that results in a saddle back configuration; (2) normal findings on physical examination; (3) no abnormality in either right or left ventricular morphology and/or function demonstrated by chest radiography and echocardiography; (4) absence of other factors, such as ischemia, electrolyte disturbance, or hypothermia that may have caused the ST-segment abnormality.

Since the criteria of ECG on Brugada syndrome in our study was that J point amplitude over 0.2 mV with either spontaneous or drug-induced type 1 ECG (coved type ST elevation) in at least two of the three right precordial leads (V1–V3) in the standard right precordial leads (V1–V3), or one or two intercostal spaces above the standard right precordial leads, we excluded the patients who had type 2 or type 3 ECG in control state and then showed still type 2 or type 3 ECG even after pilsicainide test. Therefore, the non-Brugada group included eight patients who had type 2

or type 3 ECG in control state and then showed still type 2 or type 3 ECG even after pilsicainide test, or many patients who had type 1, type 2, or type 3 repolarization pattern with J point amplitude less than 0.2 mV, because they were introduced to our hospital for the suspicion of Brugada syndrome with unknown etiology of palpitation attack.

The subjects were divided into two groups, the Brugada group and non-Brugada group. In addition, the subjects were divided into three subgroups: (1) the symptomatic group included patients that had a history of documented lethal VT or VF, or aborted sudden cardiac death; (2) the syncope group included patients who had episodes related to brain ischemia such as syncope or fainting, but no documented arrhythmias at that time; and (3) the asymptomatic group included patients who had no episodes of documented lethal ventricular arrhythmia and/or syncope.

All patients then underwent echocardiography while careful attention was paid to right ventricular enlargement and/or wall motion abnormalities. Therefore, patients with apparent organic heart disease or other factors that may have influenced ST-segment elevation were excluded. All patients provided their written informed consent to participate in the study, which was approved by the Institutional Clinical Research and Ethics Committee.

Na^+ channel-blocker challenge test (pilsicainide test)

Na^+ channel-blocker challenge tests were performed using pilsicainide, as previously reported [6]. Pilsicainide was administered intravenously at 1 mg/kg/over 10 min with continuous ECG and non-invasive blood pressure monitoring. During drug administration, 12-lead ECG was recorded and then the standard right precordial leads (V1–V3), and 3 leads at 1 intercostal space higher than the standard right precordial leads were recorded using the V4-6 electrodes. Drug administration was immediately stopped when ST-segment elevation (>5 mm), extensive QRS prolongation (>0.12 s), unfavorable symptoms, and/or frequent ventricular arrhythmias were observed. The test was considered positive if the coved type ECG pattern (type 1 ECG) appeared in more than one right precordial lead.

Electrocardiography

Standard 12-lead ECG (ECG-9322, Nihon Kohden Corp., Tokyo, Japan) was recorded before and after the administration of pilsicainide, as previously reported [6]. The J wave amplitude (μV) was analyzed by an organized computer algorithm (ECAPS 12C, Nihon Kohden). In the ECAPS 12C, the terminal point of the QRS (J point) was defined as the offset point of the QRS waveform determined from the averaged QRS waveforms from the 12 leads. After the drug test, the increase in the ST-segment (ΔST elevation) was calculated in each of the standard right precordial leads (V1–V3), one, or two intercostals space above the standard right precordial leads. The maximum ΔST elevation was defined as the maxΔST elevation.

Signal averaging

Signal averaging was performed using the signal-averaged ECG (Fukuda Denshi Co. Ltd., FDX-6531, Tokyo, Japan). A positive late potential was considered when two of three criteria (F-QRS >135 ms, or LAS40 >39 ms, or RMS40 <15 μV) were fulfilled.

Electrophysiological stimulation test

The EPS was performed in the fasting state, and all previous antiarrhythmic agents had been discontinued for at least five half-lives. An intravenous propofol infusion was used to achieve general anesthesia.

Recordings. A standard 5F decapolar catheter with ten 2-mm width electrodes and a 2-mm inter-electrode spacing was introduced via the right femoral vein or left or right subclavian veins. The catheters were positioned in the high lateral right atrium, His bundle region, coronary sinus, right ventricular (RV) apex and RV outflow tract. The 12 lead surface ECG leads were recorded simultaneously with the intracardiac electrograms. The bipolar endocardial electrograms were recorded from each of the five closely spaced bipolar pairs of electrodes filtered through a 30–400 Hz filter with a sampling interval of 1 kHz using a computed electric recorder (Cardio LAB v51D, GE Medical Systems, Milwaukee, WI, USA).

Stimulation protocol. Programmed electrical stimulation was delivered at twice the diastolic threshold at a 2-ms pulse width. The stimulation protocol for our study included up to 3 extrastimuli delivered during pacing at drive cycle lengths of 600 and 400 ms with a minimum coupling interval of S_2S_3 180 ms at two extrastimuli and of S_2S_3 and S_3S_4 200 ms at three extrastimuli, and rapid pacing down to a cycle length of 240 ms or 2:1 ventricular response from the RV apex and/or RV outflow tract.

When the induced VF lasted for several seconds, we started preparing for cardioversion. Then, the energy was discharged after the delivered energy was fulfilled. The results of the EPS were considered positive only when VF or sustained ventricular tachycardia lasting 30 s induced.

Statistical analysis

The data are presented as the mean ± SD. The ECG data were analyzed by paired *t*-test. The chi-square test for independence was used for comparisons of the prevalence. A tested analysis of variance (ANOVA) with the Bonferroni test was used to compare consecutive data among subgroups. The chi-square test and Student *t*-test for independent variables were used for comparisons among groups. The univariate analysis (Mantel-Haenszel method) and the multivariate analyses (linear model method) were performed the software (SPSS 16.0 Family for Windows, Mapinfo, Troy, NY, USA) to evaluate the association between clinical factors and the induction of VF. These analyses tested only main categories [symptoms, syncope, family history, type 1 ECG in control, positive in late potential, type 1 ECG after pilsicainide test, and maxΔST elevation (≧200 μV) after pilsicainide test]. A value of $p < 0.05$ was considered to be statistically significant.

Results

Clinical characteristics of the subjects

The study enrolled 77 patients (51 ± 15 years). The ratio of males to females was 8.6 (69/8) in all subjects (Table 1). There was no significant difference in the age (53 ± 14 years vs. 48 ± 15 years) or gender (male/female, 42/3 vs. 27/5) between the Brugada group and the non-Brugada group. A family history was observed in 22% (10/45) of the Brugada group and 9% (3/32) of the non-Brugada group, but it did not reach a significant difference ($p = 0.13$) between the two groups.

There were 45 subjects in the Brugada Group and 32 in the non-Brugada group. The Brugada group contained 8 in the symptomatic group, 9 in the syncope group and 28 in the asymptomatic group, and the non-Brugada group contained 7 in the symptomatic group, 13 in the syncope group and 12 in the asymptomatic group (Table 1).

The ratio of males to females in the comprised symptomatic patients (symptom/syncope group) of the Brugada group (17/0) was significantly higher than those of the non-Brugada group (16/4; $p = 0.05$).

There was no significant intra-group difference on the incidence of family history in either the Brugada or the non-Brugada group (Table 1).

One patient with symptoms in the non-Brugada group showed a type 2 ECG in control state and then still showed type 2 ECG even after pilsicainide test. Further, another patient with symptoms in the non-Brugada group had type 2 repolarization patterns ECG with J point amplitude less than 0.2 mV even after pilsicainide test (maxΔST elevation 140 μV). Interestingly, VF was induced during EPS in both patients. The other five patients with symptoms in the non-Brugada group had no induction of VF.

Electrophysiological findings of subjects

In the Brugada group, type 1 ECG was observed in 44% of the subjects (20/45) and types 1–3 ECG (the repolarization pattern of type 1, type 2, or type 3 was shown in ECG) was seen in 60% (27/45) in the control state. Type 1 ECG was observed in 100% of the patients (45/45) after pilsicainide test and types 1–3 ECG was also observed in 100%. In the non-Brugada group, five patients (1 symptoms, 1 syncope, and 3 asymptomatic) with type 2 or 3 ECG in the control did not show type 1 ECG after pilsicainide test. Thus, those five patients belonged to the non-Brugada group in our study.

The maxΔST elevation ($\geqq$200 μV) was seen in 84% (38/45) of the Brugada group, but 0% of the non-Brugada group. Positive of late potential was observed in 64% (29/45) of the Brugada group and 25% (8/32) of the non-Brugada group, and there was a significant difference ($p = 0.0005$).

There were no significant differences in the incidence of type 1 ECG and types 1–3 ECG in the control and those after pilsicainide test, the maxΔST elevation and positive in late potential among the subgroups of patients with Brugada syndrome (Table 2).

Table 1 Clinical characteristics of the subjects.

	Brugada group ($n = 45$)				Non-Brugada group ($n = 32$)			
	Symptom[a] n (%)	Syncope[a] n (%)	Asymptom[a] n (%)	Test for intra-group (p)	Symptom[a] (%)	Syncope[a] (%)	Asymptom[a] (%)	Test for intra-group (p)
Number of patients	8	9	28		7	13	12	–
Symptoms	+	–	–		+	–	–	
Syncope	–	+	–		–	+	–	
Age (years)	53 ± 17	51 ± 17	54 ± 14	0.89	43 ± 13	49 ± 14	51 ± 15	0.55
Gender (male/female)	8/0	9/0	25/3	0.38	5/2	11/2	11/1	0.50
Family history	4 (50)	2 (22)	4 (14)	0.10	0 (0)	1 (8)	2 (17)	0.47

Symptoms: documented VF and/or aborted sudden cardiac death; syncope: syncope from unknown etiology; VF, ventricular fibrillation.
[a] Subgroup.