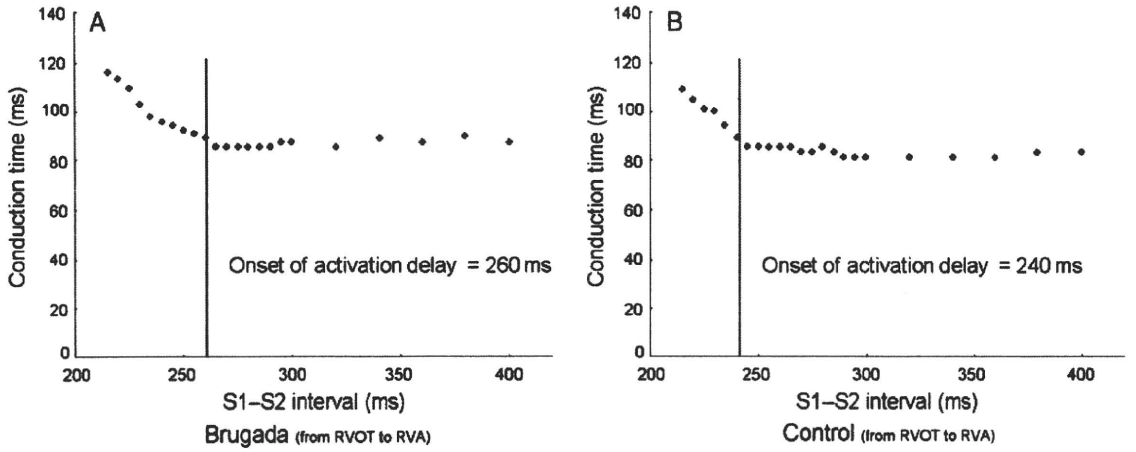

Figure 3 Onset of activation delay after the premature stimulation was defined as a marker of conduction delay from one right ventricular site to another right ventricular site or left ventricular lateral site. (*A*) A representative conduction time in a Brugada syndrome patient. The onset of activation delay was 260 ms. (*B*) A representative conduction time in a control subject. The onset of activation delay was 240 ms.

AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) or TAKARA *Taq* (Takara Bio Inc., Otsu, Shiga, 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 analysed on an ABI PRISM3130xl sequencer (Applied Biosystems). The mutations were confirmed four times by independent PCR amplification and sequencing.

Statistical analysis

Quantitative values are expressed as means ± standard deviation values. Differences in variables were assessed using unpaired Student's *t*-test or Mann–Whitney *U* test, as appropriate. Fisher's exact test was applied to comparisons of categorical variables (SPSS II for Windows, SPSS Inc., Chicago, IL, USA). A *P*-value of <0.05 was considered significant.

Table 1 Patient characteristics

	Brugada	Control	*P*-value
N	39	9	
Age (years)	47.2 ± 11.6	51.9 ± 16.6	ns
Men (%)	38 (97.4)	8 (88.9)	ns
Spontaneous Type 1 ECG	19 (48.7)	0	
Syncopal episodes	14 (35.9)	0	
Documented VF	9 (23.0)	0	
Induced VF	23 (59.0)	N/A	
Family history of sudden death	12 (30.8)	0	
Late potential	30 (76.9)	0	
SCN5A mutation	5/31 (16.2)	N/A	

Syncopal episodes included the events of documented VF.
VF, ventricular fibrillation; N/A, not assessed; ns, not significant.

Results

Patient characteristics

Patient characteristics are summarized in *Table 1*. Nineteen of the 39 BrS patients had spontaneous Type 1 ECG, and 20 of the 39 BrS patients had spontaneous Type 2 or 3 ECG, which was converted to Type 1 ECG by administration of a class IC antiarrhythmic drug. Fourteen patients had syncopal episode, and VF was documented in nine of those patients. Twenty-three patients had induced VF by PES, 12 patients had a family history of sudden death, and 30 patients had positive LP. Five of the 31 BrS patients who underwent genetic screening had SCN5A mutation.

Brugada syndrome patients vs. control subjects

There were no significant differences in APD_{90} during the basic cycle length of 500 ms at either the RVOT or RVA between BrS patients and control subjects. There were also no significant differences in effective refractory period (ERP) at both the RVOT and the RVA between BrS patients and control subjects (*Table 2*).

Maximum slope of the APDR curve was significantly steeper at the RVOT in BrS patients than in control subjects. Maximum slope of the APDR curve was also significantly steeper at the RVA in BrS patients than in control subjects. Dispersion of maximum slope of the APDR curve between the RVOT and the RVA was significantly larger in BrS patients than in control subjects (*Table 2*, *Figure 4*). The APD_{90} at shortest DI in BrS patients is significantly shorter than that in control subjects (*Table 2*).

The OAD after premature stimulation was significantly longer in BrS patients than in control subjects from the RVOT to RVA and from the RVA to RVOT. There was no significant difference in OAD from the RVOT or RVA to the LV between BrS patients and control subjects (*Table 2*, *Figure 5*).

Table 2 Brugada syndrome patients vs. control subjects

		Brugada	Control	*P*-value
N		39	9	
RVOT	APD_{90}	242 ± 14	242 ± 11	ns
	ERP (ms)	218 ± 11	216 ± 13	ns
	Maximum slope of restitution curve	0.77 ± 0.21	058 ± 0.14	0.009
	APD_{90} at shortest DI	192 ± 14	206 ± 17	0.02
	OAD from RVOT to RVA (ms)	256 ± 12	243 ± 7	0.003
	OAD from RVOT to LV (ms)	251 ± 14	246 ± 9	ns
RVA	APD_{90} (ms)	237 ± 15	232 ± 20	ns
	ERP (ms)	221 ± 14	220 ± 11	ns
	Maximum slope of restitution curve	0.98 ± 0.23	0.62 ± 0.16	0.001
	APD_{90} at shortest DI	181 ± 15	194 ± 17	0.02
	OAD from RVA to RVOT (ms)	252 ± 11	241 ± 9	0.01
	OAD from RVA to LV (ms)	250 ± 11	248 ± 12	ns
Dispersion of maximum slope of restitution curve		0.27 ± 0.19	0.11 ± 0.10	0.02
HV interval (ms)		43.3 ± 5.8	37.6 ± 4.4	0.008

APD_{90}, action potential duration at 90% repolarization; DI, diastolic interval; ERP, effective refractory period; LV, left ventricle; HV interval, His to ventricle interval; OAD, onset of activation delay; RVA, right ventricular apex; RVOT, right ventricular outflow tract.

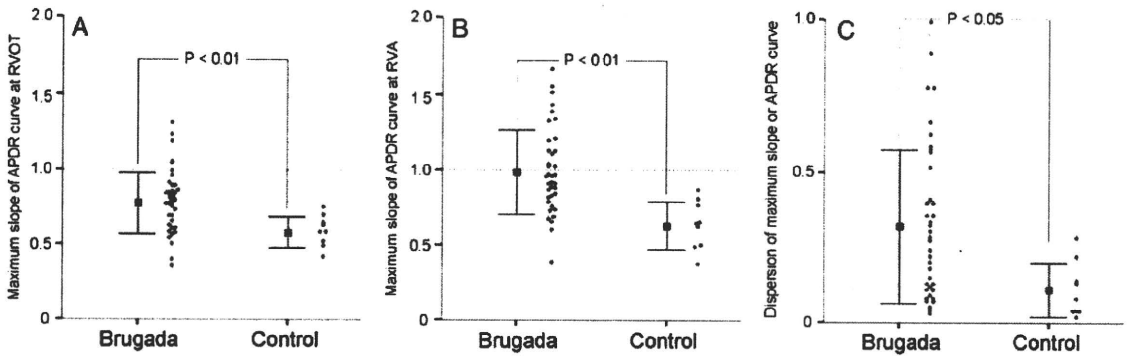

Figure 4 Maximum slope of the action potential duration restitution curve. (*A*) Maximum slope of the action potential duration restitution curve was significantly steeper at the right ventricular outflow tract in Brugada syndrome patients than in control subjects. (*B*) Maximum slope of the action potential duration restitution curve was also significantly steeper at the right ventricular apex in Brugada syndrome patients than in control subjects. (*C*) Dispersion of maximum slope of the action potential duration restitution curve between the right ventricular outflow tract and the right ventricular apex was significantly larger in Brugada syndrome patients than in control subjects.

HV interval in BrS patients was significantly longer than that in control subjects (*Table 2*).

Subgroups of Brugada syndrome patients

There was no significant difference in APD_{90}, ERP, and maximum slope of the APDR curve at the RVA between BrS patients with and without syncopal episodes. There was also no significant difference in OAD between BrS patients with and without syncopal episodes. However, the maximum slope of the APDR curve was steeper at the RVOT in BrS patients with syncopal episodes than in BrS patients without syncopal episodes (*Table 3*).

There was no significant difference in APD_{90}, ERP, maximum slope of the APDR curve, and OAD at both the RVOT and RVA between BrS patients with and without SCN5A mutation and induced VF.

There was no significant difference in APD_{90}, ERP, maximum slope of the APDR curve, and OAD at both the RVOT and RVA between BrS patients with and without spontaneous Type 1 ECG, family history of sudden death, and LP.

Discussion

New findings

We observed a steeply sloped APDR curve, larger dispersion of maximum slope of the APDR curve, longer OAD in conduction from the RVOT to RVA and from the RVA to RVOT, and longer HV interval in BrS patients than in control subjects. BrS patients who experienced syncopal episodes might have had steeper

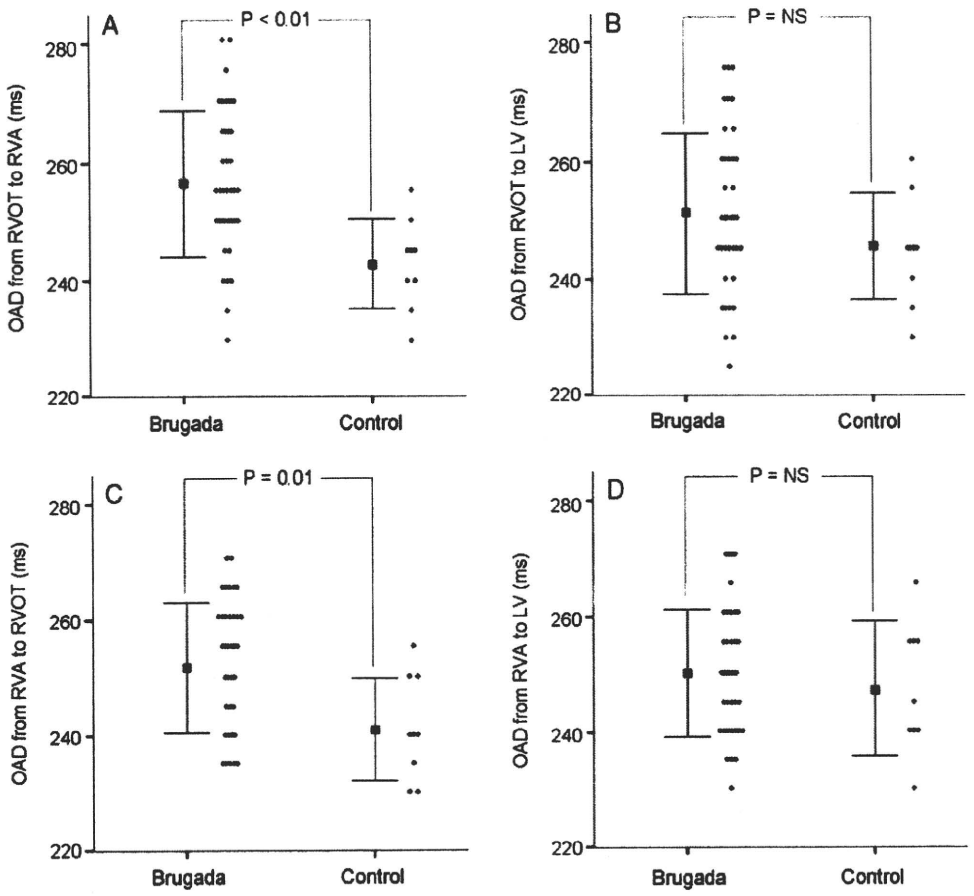

Figure 5 Onset of activation delays in Brugada syndrome patients and control subjects. (*A*) The onset of activation delay after premature stimulation was significantly longer in Brugada syndrome patients than in control subjects from the right ventricular outflow tract to right ventricular apex. (*B*) The onset of activation delay was longer in Brugada syndrome patients than in control subjects from the right ventricular apex to right ventricular outflow tract, but the difference was not significant. (*C*, *D*) There was no significant difference in the onset of activation delay from the right ventricular outflow tract or right ventricular apex to the left ventricle between Brugada syndrome patients and control subjects.

sloped APDR curves at the RVOT than those for BrS patients who did not experience syncopal episodes.

To our knowledge, this is the first report to disclose a steeply sloped APDR curve, large spatial and temporal dispersion of the APDR curve, and conduction delay in a large number of BrS patients by using MAP.

Repolarization and depolarization abnormalities

Results of animal experiments and computer modelling have shown that a steeply sloped APDR curve is associated with complex unstable electrophysiological dynamics, leading to conduction blocks and wave breakups.[10–14] Recently, it has been reported that the severity of ventricular arrhythmia was also associated with steeply sloped APD restitution in humans.[15,17–20,38] It has also been reported that not only steepness of APDR curves but also spatial and temporal heterogeneity are important for the development of VF.[15,17,39] In the present study, we found that BrS patients had a steeper APDR curve than did the control subjects at both the RVOT and the RVA. We also found that BrS patients had larger dispersion of maximum slope of the APDR curve between the RVOT and the RVA than did control subjects, which would mean large dispersion of APD. Thus, we observed repolarization abnormality and heterogeneity in BrS patients.

The heterogeneity of repolarization was also more facilitated under the condition of conduction delay. It has in fact been reported that conduction delay is also needed for explanation of deterioration in VF.[4,40] We demonstrated that BrS patients had longer OAD in conduction from the RVOT to RVA than did control subjects. We also demonstrated, as found in some previous studies,[1,41] that HV interval in BrS patients was longer than that in control subjects. As previously reported, the patients with BrS had subtle structural abnormalities and conduction delay;[25,26,31] our patients also had conduction delay. Although we could not detect massive structural abnormalities, conduction delay in our patients might be caused due to functional or subtle

Table 3 Brugada syndrome patients with or without syncopal episodes

		With syncopal episodes	Without syncopal episodes	*P*-value
N		14	25	
Age (years)		45.9 ± 13	47.8 ± 10.9	ns
RVOT	APD_{90}	238 ± 11	244 ± 15	ns
	ERP (ms)	213 ± 11	220 ± 11	ns
	Maximum slope of restitution curve	0.87 ± 0.19	0.71 ± 0.19	0.02
	APD_{90} at shortest DI	186 ± 16	195 ± 11	ns
	OAD from RVOT to RVA (ms)	253 ± 13	258 ± 12	ns
	OAD from RVOT to LV (ms)	254 ± 15	250 ± 13	ns
RVA	APD_{90} (ms)	233 ± 13	239 ± 16	ns
	ERP (ms)	224 ± 16	219 ± 12	ns
	Maximum slope of restitution curve	0.84 ± 0.24	0.84 ± 0.23	ns
	APD_{90} at shortest DI	182 ± 14	180 ± 15	ns
	OAD from RVA to RVOT (ms)	253 ± 13	251 ± 11	ns
	OAD from RVA to LV (ms)	255 ± 12	248 ± 9	ns
Dispersion of maximum slope of restitution curve		0.29 ± 0.23	0.33 ± 0.28	ns
HV interval (ms)		42.5 ± 6.3	43.8 ± 5.5	ns

Syncopal episodes included the events of documented VF.
APD_{90}, action potential duration at 90% repolarization; DI, diastolic interval; ERP, effective refractory period; LV, left ventricle; HV interval, His to ventricle interval; OAD, onset of activation delay; RVA, right ventricular apex; RVOT, right ventricular outflow tract.

structural abnormality. These abnormal markers of conduction delay can represent depolarization abnormality, and, like abnormal restitution property, may contribute to the large spatial and temporal heterogeneity of repolarization. In the state of the large heterogeneity, rapid VT which may be resulted from local re-entry may result in deterioration into VF.

The 'restitution hypothesis' states that a slope of the APDR curve >1 may lead to repolarization alternans, wavebreak, and transition from VT to VF even in a ventricle without pre-existing repolarization heterogeneity.[21,42] However, in the present study the mean maximum slope of the APDR curve in BrS patients was <1.0 at both the RVOT and the RVA. Xie *et al.*[43] reported that an APDR slope >1 might not always be necessary to produce wavebreak under the condition that a spatial heterogeneity of those slopes exists. In the present study, dispersion of maximum slope of the APDR curve between the RVOT and the RVA was significantly larger in BrS patients than in control subjects. Conduction velocity restitution may determine the spatial discordance of repolarization alternans and the genesis of a slower type of VF.[44–46] In the present study, as found in a previous study, conduction delay was recognized in BrS patients.

In accordance with the previous study,[44] the alternans of APD followed to wavebreak could be observed during burst pacing. However, in the present study, we could not observe polymorphic VT or VF during burst pacing, and we did not use the 7F MAP recording catheter during burst pacing. Therefore, we could not examine the relation between steeply sloped APDR curves and the alternans of APD followed to wavebreak during burst pacing.

Correspondence to previous reports

Narayan *et al.*[17] reported a case with BrS that had steeply restitution property and conduction delay. The result is similar to that in the present study. Recently, Hayashi *et al.*[18] reported that abnormal repolarization restitution property contributed to the induction of VF by PES. They found that patients with induced VF had steeper maximum slope of the activation recovery interval (ARI) restitution curve than did patients without induced VF.

We found that the BrS patients had a steep APDR curve, large dispersion of APDR, and conduction delay. Abnormal steep APDR was prominent in patients with syncope/spontaneous VF. We failed to reveal the difference between restitution properties in patients with and without induced VF.

We used a different method to evaluate repolarization kinetics than that used in a previous study.[18] We used MAP techniques and did not evaluate the ARI of unipolar local ECG. Differences in patient characteristics are also reasons for the different results of the two studies. The present study included patients with syncope (35.9%) or documented VF (23.0%), but the previous study[18] included few patients with syncope (9.5%) and no patients with documented VF.

We have no clear explanation for the fact that there was no significant difference in the maximum slope of the APDR curve between BrS patients with and without induced VF, but a possible explanation is as follows. It has been reported that reproducibility of the induction of VF by PES is not perfect and dose not correlate with clinical presentation in patients with BrS.[47] Programmed electrical stimulation fails to induce VF in some patients who have experienced aborted sudden death.[34] Patients also had day-by-day variations of both repolarization and depolarization parameters, and these variations can influence the induction of VF. The absence or presence of induced VF would therefore not have had any impact on the results of the present study.

Limitations

We used the dispersion of APDR curve in only two sites as the marker of APD dispersion, which may not represent the dispersion

in whole heart. And more, the steeper APDR curve had the shorter APD at the same coupling interval. Therefore, the dispersion of APDR curve would represent the dispersion of APD. However, we did not examine simultaneously APD at two sites. Therefore, we did not know precisely how the dispersion of APD was.

We examined from only endocardium but not epicardium, which is thought to be arrhythmogenesis in BrS patients. This study might not cover arrhythmogenic substrate in BrS patients.

We employed OAD as a marker of conduction delay. Because the endocardium structure is very complicated, the distance in the myocardium *in vivo* cannot be precisely measured. Moreover, the conduction velocity is different in the longitudinal and transverse direction in the myocardium *in vivo*. Therefore, precise measurement of conduction velocity in the myocardium *in vivo* is also very difficult.

We compared various parameters among BrS patients by using Fisher's exact test. However, because the sample size was relatively small, the result had a limitation.

Conclusions

Steeply sloped APDR properties, large dispersion of maximum slope of the APDR curve, long OAD after premature stimulation in the RV, and long HV interval were observed in BrS patients. These repolarization and depolarization abnormalities are thought to be related to the development of VF in BrS patients.

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Conflict of interest: none declared.

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CARDIOVASCULAR PATHOLOGY

Original Article

Elevated oxidative stress is associated with ventricular fibrillation episodes in patients with Brugada-type electrocardiogram without SCN5A mutation

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Abstract

Background: Brugada syndrome is a disease known to cause ventricular fibrillation with a structurally normal heart and is linked to SCN5A gene mutation. However, the mechanism by which ventricular fibrillation develops in cases of Brugada-type electrocardiogram without SCN5A mutation has remained unclear. Recently, oxidative stress has been implicated in the pathophysiology of cardiac arrhythmia. We also investigated oxidative stress levels in the myocardia of patients with Brugada-type electrocardiogram. **Methods:** Endomyocardial biopsy samples were obtained from 68 patients with Brugada-type electrocardiogram (66 males and two females). We performed histological and immunohistochemical analyses for CD45, CD68, and 4-hydroxy-2-nonenal-modified protein, which is a major lipid peroxidation product. **Results:** SCN5A mutation was detected in 14 patients. Ventricular fibrillation was documented in three patients with SCN5A mutation and in 11 without SCN5A mutation. In patients with SCN5A mutation, 4-hydroxy-2-nonenal-modified protein-positive area was not significantly different between the documented ventricular fibrillation (VF) group (VF+ group) and the group without documented VF (VF− group). However, in patients without SCN5A, the area was significantly larger in the VF+ group than that in the VF− group ($P<.05$). All other parameters (fibrosis area, CD45, and CD68) were not different between the VF+ and VF− group in both SCN5A+ and SCN5A− patients. **Conclusion:** Oxidative stress is elevated in the myocardium of patients with Brugada-type electrocardiogram who have VF episodes and do not have SCN5A gene mutations. Oxidative stress may be associated with the occurrence of VF in patients with Brugada-type electrocardiogram without SCN5A mutation.

Keywords: Oxidative stress; Ventricular fibrillation; Brugada syndrome

1. Introduction

Brugada syndrome (BS) is a disease characterized by ST-segment elevation in right precordial leads and episodes of ventricular fibrillation (VF) in the absence of structural heart disease [1]. About 20% of BS cases have been linked to mutations in the SCN5A gene, the gene encoding the alpha subunit of the cardiac sodium channel [2,3]. Functional analysis employing expression systems has revealed that mutations in SCN5A resulted in "loss of function" of I_{Na}, which reduces the inward sodium current, induces conduction delay, and predisposes the substrate for reentry. Other

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gene mutations such as CACNA1c [4], CACNB2b [4], GPD1-L [5], SCN1B [6], and KCNE3 [7] have also been reported. However, cases with such mutations are not frequent and the prevalence of those mutations is not clear [8]. Recently, Frustaci et al. [9] reported that lymphocytic myocarditis was observed in patients with Brugada-type electrocardiogram (ECG) who did not have SCN5A gene mutations, but the association with histological findings and occurrence of ventricular fibrillation (VF) has not been fully elucidated. Thus, the mechanism by which VF develops in cases of Brugada-type ECG without SCN5A mutation has remained unclear.

Recently, oxidative stress has been implicated in the pathophysiology of cardiac arrhythmia. Hydrogen peroxide (H_2O_2) decreases SCN5A transcription and current [10]. E2-isoketal, a highly reactive product of lipid peroxidation, potentiates inactivation of cardiac Na^+ channels [11]. Reactive oxygen species (ROS) contribute to cardiac sympathovagal imbalance in cardiomyocytes [12]. We also investigated oxidative stress levels, assessed by expression levels of 4-hydroxy-2-nonenal (HNE)-modified protein, a reliable marker of lipid peroxidation [13,14], in the myocardia of patients with Brugada-type ECG and investigated the association between VF events and oxidative stress levels in the myocardia of patients with Brugada-type ECG with and without mutation in the SCN5A gene.

2. Methods

2.1. Subjects

In the period from June 1998 to June 2008, we performed electrophysiological study and endomyocardial biopsy in 68 consecutive patients with Brugada-type electrocardiogram (ECG) (66 males and two females; mean age, 49.0 years). Brugada-type ECG was defined as coved ST-segment elevation (>0.2 mV) followed by a negative T-wave in more than one right precordial lead (V1 to V3) or third intercostal leads (V1 to V2) in the presence or absence of a sodium channel blocker (Fig. 1). Routine examinations, including cardiac echocardiography, coronary angiography, and right and left ventriculography, showed no evidence of structural heart disease in any of the patients. We examined the clinical characteristics of patients, including age, sex, spontaneous VF occurrence, history of syncope, family history of sudden death, and SCN5A mutation.

2.2. Cardiac catheterization, endomyocardial biopsy, and electrophysiological study

After providing written informed consent, all patients underwent cardiac catheterization, coronary angiography, right and left ventricular angiography, and endomyocardial

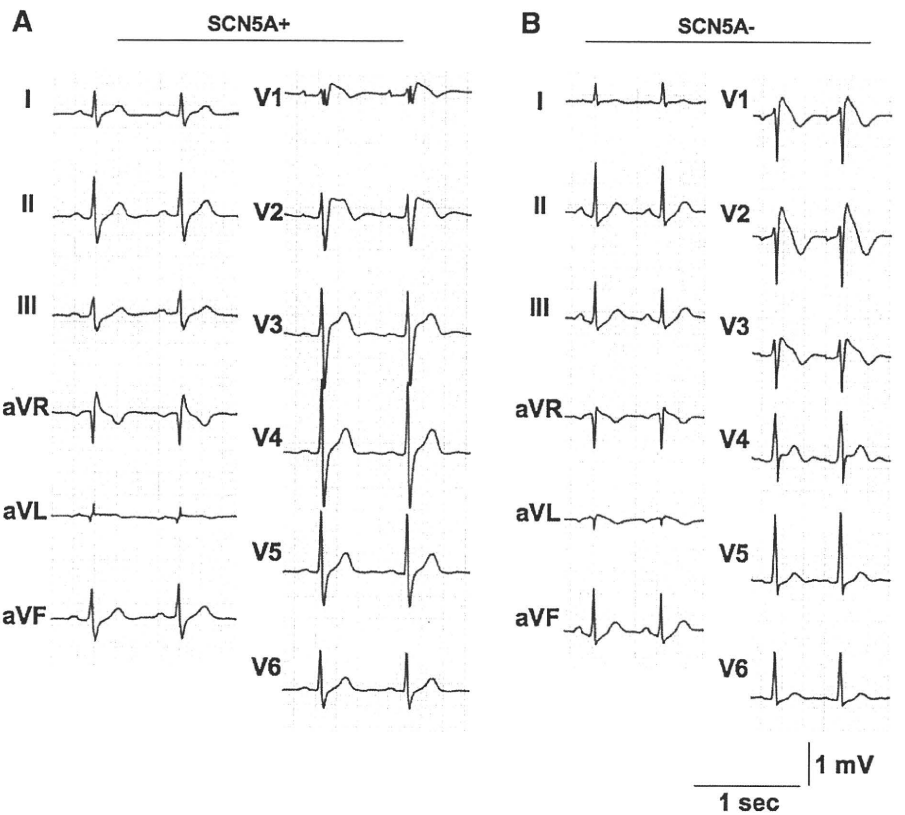

Fig. 1. Representative ECGs of patients with or without SCN5A mutation. (A) ECG of a patient with SCN5A mutation (SCN5A+), R282H (47 years old, male). (B) ECG of a patient without SCN5A mutation (SCN5A−) (42 years old, male).

biopsy. Endomyocardial biopsy samples (three or four per patient for histology) were obtained from the right ventricular (RV) side of the septum of all patients by the internal jugular approach.

The electrophysiological study was performed in all patients as reported previously [15,16]. The risks of the electrophysiological study were explained to each patient, and written informed consent was obtained from all patients. Induction of ventricular arrhythmia was initially attempted without the use of any antiarrhythmic drugs. The criterion for the induction of ventricular arrhythmia was induction of VF by programmed electrical stimulation from the RV apex, RV outflow tract, or left ventricle with a maximum of two extrastimuli at two cycle lengths.

2.3. Histology and immunohistochemistry

Endomyocardial biopsy samples were fixed in 10% formalin and embedded in paraffin. For histology, 5-μm-thick sections were cut and stained with hematoxylin and eosin and Masson's trichrome stain and examined by light microscopy.

Immunoenzymatic staining was performed using a DAKO LSAB System (Dako) according to the manufacturer's instructions, as previously described [13,14,17]. Briefly, the heart sections embedded in paraffin were preincubated with 1.5% hydrogen peroxide and normal BSA to block nonspecific reactions. CD45RO (1:100) and CD68 (1:50) antibodies (both from Dako) for the characterization of inflammatory infiltrate, and mouse monoclonal anti-HNE-modified protein antibody (1:50 dilution, NOF Medical Department) for assessment of oxidative stress were added. After incubation at 4°C overnight, the sections were incubated with biotinylated anti-mouse immunoglobulin for 20 min and subsequently with horseradish peroxidase-labeled streptavidin solution for 20 min. The slides were rinsed in cold Tris-buffered saline after each step of incubation. Peroxidase activity was visualized with diaminobenzidine (DAB) tetrahydrochloride solution.

2.4. Semiquantitative analysis of stained samples

Digital images of stained sections were taken with a Fujix Digital Camera HC-300/OL mounted on an Olympus BH-2 microscope. Color images from five randomly selected separate high-power fields (×200) in three or four sections per patient were obtained. Staining was analyzed using WinROOF Image software (Mitani Corp.) and assessed by using the following equation: stained area (%)=100×stained area (cm^2)/total sample size (cm^2).

CD45RO- and CD68-positive cells were counted by the following equation: inflammatory cell infiltration=number of CD45RO- or CD68-positive cells (n)/total sample size (cm^2).

2.5. 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. Genomic DNA was extracted from peripheral blood leukocytes by using a DNA extraction kit (Gentra, Minneapolis, MN, USA) and was stored at −30°C until use.

Twenty-seven exons of the SCN5A gene were amplified with previously reported intronic primers [18]. SCN5A gene exon 1 is a noncoding region, and we did not analyze this region in this study. Exons 6, 17–1 sense, 21, and 25 were not able to be sufficiently amplified by the primers, and we therefore designed the following intronic primers as previously described [19,20]. The primers used in this study are as follows: 5′-GTT ATC CCA GGT AAG ATG CCC-3′ (sense) and 5′-TGG TGA CAG GCA CAT TCG AAG-3′ (anti-sense) for exon 6; 5′-AAG CCT CGG AGC TGT TTG TCA CA-3′ (sense) for exon 17–1; 5′-TGC CTG GTG CAG GGT GGA AT-3′ (sense) and 5′-ACT CAG ACT TAC GTC CTC CTT C-3′ (anti-sense) for exon 21; 5′-TCT TTC CCA CAG AAT GGA CAC C-3′ (sense) and 5′-AAG GTG AGA TGG GAC CTG GAG-3′ (anti-sense) for exon 25. PCR was performed in a 20-μl reaction volume containing 50 ng of genomic DNA, 20 pmol of each primer, 0.8 mM dNTPs, 1× reaction buffer, 1.5 mM $MgCl_2$, and 0.7 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA) or TAKARA Taq (Takara Bio, Inc., Otsu, Shiga, 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 v. 1.1 cycle sequencing kit (Applied Biosystems) and analyzed on an ABI PRISM3130 XL sequencer (Applied Biosystems). The mutations were confirmed four times by independent PCR amplification and sequencing.

2.6. Statistical analysis

Data are all expressed as means±S.D. Intergroup comparison was done by Fisher's Exact Probability Test, and difference in mean values was tested by Student's t test,

Table 1
Patients' characteristics

Number	68
Age, years	49.0±11.6
Male/female	66/2
Family history of SCD (%)	19 (16.1)
Syncope (%)	12 (27.9)
ICD Implantation (%)	22 (32.8)
SCN5A Mutation (%)	14 (20.6)
Documented VF (%)	14 (20.6)
SCN5A mutation+ (%)	3 (4.4)
SCN5A mutation− (%)	11 (16.2)

Data are mean±S.D.
SCD: Sudden cardiac death; ICD: implanted cardioverter defibrillator; VF: ventricular fibrillation.

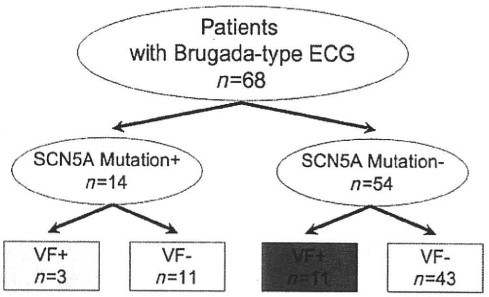

Fig. 2. Study profile.

at a critical level of 5% or lower. All data were analyzed using SPSS software (version 11.0.1).

3. Results

3.1. Patients' characteristics

Clinical characteristics of all patients with Brugada-type ECG are shown in Table 1. SCN5A mutation was detected in 14 patients. VF was documented in three patients with SCN5A mutation and in 11 patients without SCN5A mutation (Table 1 and Fig. 2).

Eleven patients (two patients with SCN5A mutation and nine patients without mutation) had histories of spontaneous VF that was converted to sinus rhythm by an external defibrillator before admission. In the other three patients (one patient with SCN5A mutation and two patients without the mutation), spontaneous VF occurred de novo after discharge from our hospital and was terminated by implantable cardioverter defibrillator therapy. There was no death in any of the patients.

3.2. Histology and immunohistochemistry

HNE-modified protein-positive area in patients with documented VF (VF+ group) was larger than that in patients without documented VF (VF− group) (VF+ group: 16.3 ±10.5% vs. VF− group: 9.3±5.7%, $P<.05$) (Fig. 3A). There were no significant differences in area of fibrosis and number of CD45RO- and CD68-positive cells between the VF+ and VF− groups.

We also checked those parameters in patients with and without SCN5A mutation. HNE-modified protein-positive areas were not significantly different in the SCN5A+ and SCN5A− patients (SCN5A+ group: 13.3±7.6% vs. SCN5A − group: 10.1±7.3%, P=NS). In SCN5A+ patients, HNE-modified protein-positive area was not significantly different between the VF+ and VF− group (VF+ group: 14.0±8.8% vs. VF− group: 12.7±7.5%, P=NS) (Fig. 3B). However, in patients without SCN5A (SCN5A−), the area was significantly larger in the VF+ group than that in the VF−

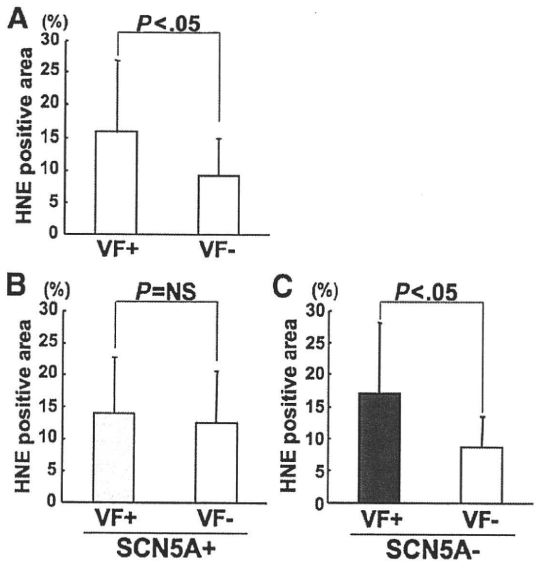

Fig. 3. HNE-modified protein-positive area. (A) HNE-modified protein-positive area in patients with spontaneous VF (VF+ group) vs. without (VF− group). (B) HNE-modified protein-positive area in patients with SCN5A mutation (SCN5A+). (C) HNE-modified protein-positive area in patients without SCN5A mutation (SCN5A−). Data are expressed as means±S.D.

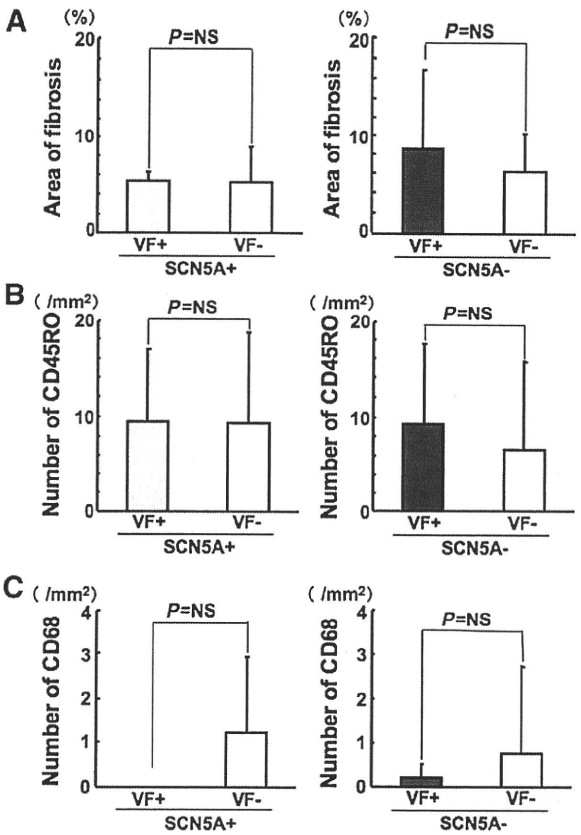

Fig. 4. Histological and immunohistochemical analyses. (A) Areas of fibrosis in SCN5A+ and SCN5A− patients in the VF+ and VF− groups. (B) Numbers of CD45RO-positive cells in SCN5A+ and SCN5A− patients in the VF+ and VF− groups. (C) Numbers of CD68-positive cells. Data are expressed as means±S.D.

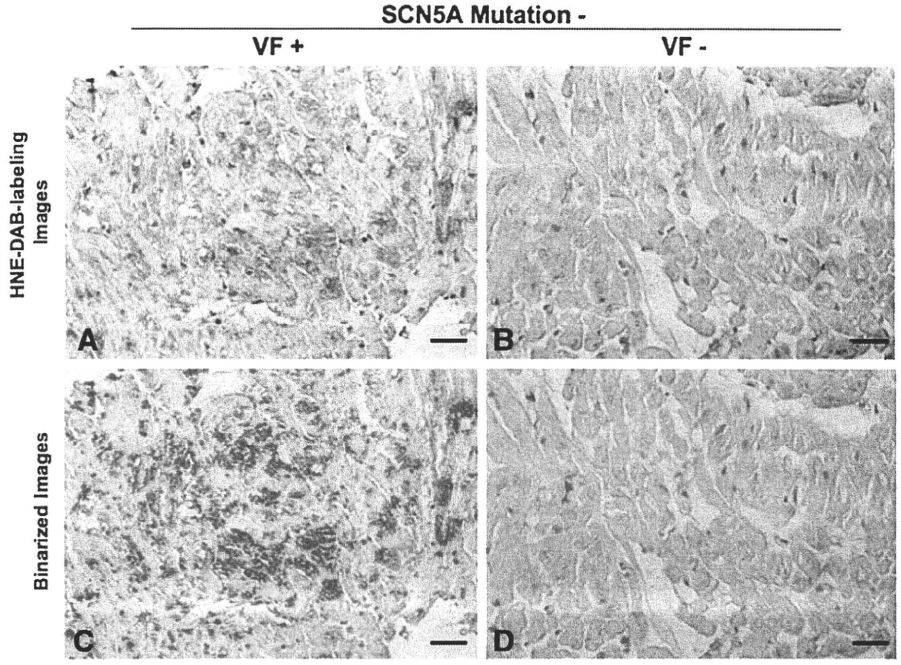

Fig. 5. Representative figures of HNE-modified protein staining. Representative immunostainings (brown) for HNE-modified protein by diaminobenzidine (DAB) (A and B) and binarized images (green) using WinROOF Image software (C and D) in the myocardium from a patient without SCN5A mutation and with spontaneous VF (A and C) and from a patient without SCN5A mutation and without spontaneous VF (B and D).

group (VF+ group: 17.0±11.2% vs. VF− group: 8.4±4.9%, $P<.05$) (Fig. 3C).

Area of fibrosis was not different between the VF+ and VF− groups in both SCN5A+ and SCN5A− patients (Fig. 4A). The number of CD45RO-positive cells was not significantly different between the VF+ and VF− groups in both SCN5A+ and SCN5A− patients (Fig. 4B). Infiltration of CD68-positive cell was rarely seen in patients in both the VF+ and VF− groups with or without SCN5A mutation (Fig. 4C).

Fig. 5 shows representative immunostainings (A and B) for HNE-modified protein in the myocardium from a patient without SCN5A mutation and with spontaneous VF (A and C) and from a patient without SCN5A mutation and without spontaneous VF (B and D). Positive immunostainings (brown) for HNE-modified protein are distinct in the cytosol of cardiac myocytes from a patient with spontaneous VF (Fig. 5A).

4. Discussion

We investigated oxidative stress levels in the myocardia of patients with Brugada-type ECG and also examined the relationship between oxidative stress levels and VF episodes. The major new finding of this work is that oxidative stress is elevated in the myocardium of patients with Brugada-type ECG who have VF episodes and do not have SCN5A gene mutations. Oxidative stress may play an important role in the occurrence of VF in patients with Brugada-type ECG without SCN5A gene mutations.

Oxidative stress induces loss of function of I_{Na}. Shang et al. [10] reported that H_2O_2 decreases SCN5A mRNA transcription and I_{Na} current. Fukuda et al. [11] reported that E2-isoketal, a highly reactive product of lipid peroxidation, potentiates inactivation of cardiac Na^+ channels. Our data showed that oxidative stress was elevated in the myocardium of BS patients with VF episodes who do not have SCN5A gene mutations. These findings indicated that loss of function of I_{Na} caused by oxidative stress is associated with the occurrence of VF in patients with Brugada-type ECG.

Oxidative stress is not related to the occurrence of VF in patients with Brugada-type ECG who have SCN5A mutation in our study. Frustaci et al. [9] reported that carriers of SCN5A mutations demonstrate myocardial cell degeneration and death. Therefore, mechanisms of VF occurrence in Brugada-type ECG patients with SCN5A mutation may be different from those in patients without SCN5A mutation. Further studies are needed to clarify the mechanisms. Since HNE-modified protein-positive areas were not significantly different in the SCN5A+ and SCN5A− patients in our study, it was thought that loss of function of the sodium channel due to SCN5A mutation did not cause oxidative stress.

ROS cause damage to lipid cell membranes in the process of lipid peroxidation. In this process, several aldehydes, including HNE, are generated as final products. HNE is recognized as the most reliable marker of lipid peroxidation [13,14]. Furthermore, exposure to a large amount of HNE (400 μmol/l) increases rat cardiac Na^+ current and causes cytotoxic effects in cardiac myocytes [21,22]. However, a small amount of HNE does not have any detectable gating

effects on I_{Na}, including I_{Na} decay, voltage-dependent activation, or the voltage dependence of channel availability [11]. Cardiac function is normal in patients with BS. Therefore, HNE in cardiac myocytes in patients with BS is thought to be at a low level and to have no cytotoxic effects and/or effect on I_{Na} current.

In conclusion, oxidative stress is elevated in the myocardium of patients with Brugada-type ECG who have VF episodes and do not have SCN5A gene mutations compared to that in the myocardium of patients without VF episodes. Loss of function of I_{Na} caused by oxidative stress may be a mechanism for VF in patients with Brugada-type ECG who do not have SCN5A mutation. Further studies are needed to clarify this point.

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Atrial electrophysiological and structural remodeling in high-risk patients with Brugada syndrome: Assessment with electrophysiology and echocardiography

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BACKGROUND: Atrial fibrillation (AF) often occurs in Brugada syndrome (BrS), and BrS patients with spontaneous AF often experience ventricular fibrillation (VF) attacks. Atrial vulnerability providing a substrate for AF is known to be enhanced in BrS, but there are no data on atrial structural attributes.

OBJECTIVE: The objective of this study was to assess atrial electrophysiological and structural characteristics in BrS and their relationships with gene mutations.

METHODS: We studied 57 patients with BrS. Intra-atrial conduction time (CT) was defined as the interval from the stimulus at the high right atrium to atrial deflection at the distal portion of the coronary sinus. Left atrial volume index (LAVI) was measured by the modified Simpson method at left ventricular end-systole using echocardiography. *SCN5A* mutations were analyzed in all patients.

RESULTS: In patients with documented VF, spontaneous AF frequently occurred and prolonged CT and increased LAVI were observed compared with those in patients without VF (all $P < .05$; LAVI: 22 ± 5 vs. 32 ± 7 ml/m^2). Even among patients without AF, CT and LAVI were still increased in patients with VF (all $P < .05$; LAVI: 22 ± 5 vs. 29 ± 5 ml/m^2). The presence of *SCN5A* mutation was associated with prolonged CT ($P < .05$) and increased LAVI ($P < .01$), but not with arrhythmic episodes.

CONCLUSION: Both atrial vulnerability and structural remodeling are enhanced in high-risk patients with BrS, even in those without AF. These morphological characteristics suggest that BrS is a form of genetic myocardial disease.

KEYWORDS Sudden cardiac death; Atrial fibrillation; Echocardiography; Genes; Remodeling

ABBREVIATIONS **AF** = atrial fibrillation; **BrS** = Brugada syndrome; **CD** = conduction delay; **CT** = conduction time; **ECG** = electrocardiogram; **ERP** = effective refractory period; **LA** = left atrium/atrial; **LAVI** = left atrial volume index; **LV** = left ventricular; **RAA** = right atrial appendage; **RAF** = repetitive atrial firing; **RVOT** = right ventricular outflow tract; **VF** = ventricular fibrillation

(Heart Rhythm 2010;7:218–224)

Introduction

Brugada syndrome (BrS) is characterized by a unique electrographic (ECG) pattern consisting of ST-segment elevation in right precordial leads and increased risk of sudden cardiac death due to ventricular fibrillation (VF) without demonstrable structural heart disease.[1–4]

We have reported that atrial arrhythmia, especially atrial fibrillation (AF), is often observed as well as ventricular arrhythmia in patients with BrS because of increased atrial electrical vulnerability.[5] We have also shown that the occurrence of spontaneous AF is more closely linked to documented VF than is mutation of the gene encoding the cardiac sodium channel, *SCN5A*.[6] However, little is known about atrial structural remodeling in BrS and its relation to electrophysiological and genetic attributes. Some recent studies have shown structural and histological abnormalities only in ventricles in patients with BrS.[7–11] In this study, we investigated atrial electrophysiological and structural characteristics in patients with BrS by electrophysiological study and echocardiography and their relationships with gene mutations.

Methods

Study population

The subjects of this study were 56 male patients and 1 female patient (mean age 48 ± 12 years) diagnosed with BrS in our hospital. We enrolled the patients in whom we recorded adequate echocardiographic images for analysis. All patients had a spontaneous type I ECG pattern based on

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Table 1 Baseline and clinical characteristics of the study population

Number of patients, n	57
Age, yrs	48 ± 12
Gender, male	56 (98%)
Body surface area, m^2	1.71 ± 0.13
Syncopal episode, %	30 (53%)
Documented VF, %	22 (39%)
Spontaneous AF, %	11 (19%)
Family history of sudden death, %	22 (39%)
Spontaneous type I ECG, %	45 (79%)
SCN5A mutation, %	11 (19%)
ICD implantation, %	36 (63%)

Values are n (%) or mean ± SD.

AF = atrial fibrillation; ECG = electrocardiogram; ICD = implantable cardioverter defibrillator; *SCN5A* = pore-forming region of the human cardiac sudden death channel; VF = ventricular fibrillation.

the Brugada Syndrome Consensus Report (1) without drug provocation (n = 45) or after provocation by administration of pilsicainide (a pure sodium channel blocker) as described previously[5] (n = 12). Twenty-two patients had a family history of sudden death at an age of <50 years. Thirty patients experienced syncope at admission, and 22 patients suffered from VF at admission or within 24 h after syncopal episodes. Eleven patients experienced spontaneous AF. None of the patients had a history of any other organic cardiovascular diseases or hypertension (Table 1).

Occurrence of spontaneous AF was evaluated by 24-h Holter monitoring, continuous monitoring during hospitalization (7 days), ECG at the time of monthly clinical follow-up, or recording of an implantable cardioverter-defibrillator if implanted.

Table 2 Clinical, genetic, electrophysiological, and echocardiographic parameters in patients with and without documented VF episode

Variable	VF (−)	VF (+)	*P* value
Clinical/ECG/genetic parameters			
Number of patients, n (male/female)	35 (34/1)	22 (22/0)	NS
Age, yrs	47 ± 12	51 ± 12	NS
Syncopal episode, n (%)	9 (26%)	21 (95%)	<.01
Spontaneous AF, n (%)	3 (9%)	8 (36%)	<.05
Family history of sudden death, n (%)	14 (40%)	8 (36%)	NS
SCN5A mutation, n (%)	5 (14%)	6 (27%)	NS
Spontaneous type I ECG, n (%)	27 (77%)	18 (82%)	NS
P-wave duration, ms	99 ± 15	110 ± 16	<.05
PQ interval, ms	177 ± 25	166 ± 17	NS
ICD implantation, n (%)	16 (46%)	20 (91%)	<.01
Electrophysiological parameters			
VF induction, n (%)	20 (57%)	16 (73%)	NS
RAF, n (%)	10 (29%)	13 (59%)	<.05
AF induction, n (%)	10 (29%)	10 (45%)	NS
ERP, ms	263.1 ± 53.1	274.1 ± 44.9	NS
CT at S1, ms	115.1 ± 16.6	129.6 ± 13.3	<.01
CT at S2, ms	133.7 ± 17.7	161.6 ± 18.0	<.01
CD, ms	19.3 ± 17.4	30.4 ± 13.0	<.05
Conduction velocity at S1, mm/ms	0.31 ± 0.05	0.29 ± 0.04	NS
Conduction velocity at S2, mm/ms	0.26 ± 0.03	0.23 ± 0.03	<.01
A1, ms	55.6 ± 14.4	63.1 ± 20.4	NS
A2, ms	71.4 ± 19.5	89.6 ± 24.3	<.01
A2/A1	1.30 ± 0.23	1.46 ± 0.25	<.05
Sinus node recovery time, ms	1,283 ± 275	1,352 ± 262	NS
Echocardiographic parameters			
LV end-diastolic volume, ml	150 ± 24	149 ± 24	NS
LV ejection fraction, %	62 ± 5	61 ± 6	NS
LV mass index, g/m^2	90 ± 17	89 ± 15	NS
Relative wall thickness	0.39 ± 0.05	0.39 ± 0.06	NS
Anteroposterior LA diameter, mm	32 ± 4	35 ± 4	<.05
Transverse LA diameter, mm	35 ± 3	37 ± 4	<.05
LAVI, ml/m^2	22 ± 5	32 ± 7	<.01
Right ventricular area, cm^2	16 ± 3	16 ± 2	NS
RVOT diameter, mm	29 ± 5	29 ± 4	NS
Peak E velocity, cm/s	62 ± 13	57 ± 7	NS
Peak A velocity, cm/s	49 ± 14	54 ± 14	NS
E/A	1.4 ± 0.5	1.2 ± 0.3	NS
E-wave deceleration time, ms	227 ± 29	233 ± 44	NS
Ea, cm/s	9.9 ± 3.1	9.4 ± 2.9	NS
Aa, cm/s	8.4 ± 1.6	7.9 ± 1.7	NS
E/Ea	7.1 ± 4.1	6.8 ± 3.1	NS

Values are n (%) or mean ± SD.

Aa = late diastolic mitral annular velocity; A1 = local atrial potential at S1; A2 = local atrial potential at S2; CD = conduction delay; CT = intra-atrial conduction time; Ea = early diastolic mitral annular velocity; ERP = effective refractory period; LA = left atrial; LAVI = left atrial volume index; LV = left ventricular; RAF = repetitive atrial firing; RVOT = right ventricular outflow tract; VF (−) = patients without documented VF; VF (+) = patients with documented VF; other abbreviations as in Table 1.

Table 3 Clinical, genetic, electrophysiological, and echocardiographic parameters in patients with and without documented VF episode among patients without spontaneous AF

Variable	VF (−) and AF (−)	VF (+) and AF (−)	*P* value
Clinical/ECG/genetic parameters			
Number of patients, n (male/female)	32 (31/1)	14 (14/0)	NS
Age, yrs	47 ± 13	48 ± 11	NS
Syncopal episode, n (%)	9 (28%)	13 (93%)	<.01
Family history of sudden death, n (%)	12 (38%)	6 (43%)	NS
SCN5A mutation, n (%)	4 (13%)	3 (21%)	NS
Spontaneous type I ECG, n (%)	25 (78%)	12 (86%)	NS
P-wave duration, ms	98 ± 16	107 ± 16	NS
PQ interval, ms	177 ± 26	167 ± 20	NS
ICD implantation, n (%)	15 (47%)	13 (93%)	<.01
Electrophysiological parameters			
VF induction, n (%)	19 (59%)	12 (86%)	NS
RAF, n (%)	8 (26%)	6 (46%)	NS
AF induction, n (%)	9 (28%)	7 (50%)	NS
ERP, ms	263.9 ± 53.9	278.5 ± 46.7	NS
CT at S1, ms	114.1 ± 15.1	125.7 ± 12.0	<.05
CT at S2, ms	133.2 ± 17.0	155.8 ± 15.6	<.01
CD, ms	18.5 ± 16.9	30.1 ± 11.0	<.05
Conduction velocity at S1, mm/ms	0.31 ± 0.04	0.29 ± 0.04	NS
Conduction velocity at S2, mm/ms	0.26 ± 0.03	0.24 ± 0.03	<.01
A1, ms	56.3 ± 14.1	64.2 ± 20.0	NS
A2, ms	72.1 ± 19.3	89.1 ± 24.5	<.05
A2/A1	1.29 ± 0.23	1.43 ± 0.25	NS
Sinus node recovery time, ms	1,277 ± 280	1,253 ± 135	NS
Echocardiographic parameters			
LV end-diastolic volume, ml	149 ± 23	147 ± 18	NS
LV ejection fraction, %	62 ± 5	62 ± 5	NS
LV mass index, g/m^2	89 ± 18	87 ± 15	NS
Relative wall thickness	0.39 ± 0.05	0.40 ± 0.06	NS
Anteroposterior LA diameter, mm	32 ± 4	35 ± 4	NS
Transverse LA diameter, mm	35 ± 3	36 ± 3	NS
LAVI, ml/m^2	22 ± 5	29 ± 5	<.05
Right ventricular area, cm^2	15 ± 3	16 ± 2	NS
RVOT diameter, mm	28 ± 5	28 ± 4	NS
Peak E velocity, cm/s	63 ± 12	56 ± 8	NS
Peak A velocity, cm/s	49 ± 15	52 ± 15	NS
E/A	1.4 ± 0.5	1.2 ± 0.3	NS
E-wave deceleration time, ms	226 ± 28	226 ± 45	NS
Ea, cm/s	10.0 ± 3.2	10.1 ± 2.5	NS
Aa, cm/s	7.9 ± 1.8	8.8 ± 1.7	NS
E/Ea	7.2 ± 4.1	5.7 ± 1.4	NS

Values are n (%) or mean ± SD.

VF (−) and AF (−) = patients without documented VF and spontaneous AF; VF (+) and AF (−) = patients with documented VF and without spontaneous AF; other abbreviations as in Tables 1 and 2.

P-wave duration and PQ-interval were measured from lead II of the surface ECG. The gene analysis of the *SCN5A* study was performed in compliance with guidelines for human genome studies of the Ethics Committee of Okayama University. Informed consent was obtained from all patients. Analysis of *SCN5A* mutation was performed as previously reported.[6,12] All of the tests that were performed were approved by the medical ethical review committees of Okayama University Hospital.

Atrial electrophysiological study

An electrophysiological study was performed in all patients as described previously.[5] A premature stimulus (S2) was delivered after 8 beats of drive pacing (S1) from the high right atrium at a basic cycle length of 600 ms. The S1–S2 interval was decreased in 10-ms steps until the effective refractory period (ERP) of the right atrial appendage (RAA) was reached.

Atrial vulnerability was evaluated and defined by the following parameters:[5,6] 1) ERP of the RAA defined as the longest coupling interval (S1–S2) at which the stimulus failed to propagate a response; 2) intra-atrial conduction time (CT) defined as the interval from the stimulus at the high right atrium to the atrial deflection at the distal portion of the coronary sinus; 3) conduction delay (CD) defined as the difference between the CT at S2 and that at S1; 4) duration of a local atrial electrogram (A) recorded at the atrial pacing site; 5) repetitive atrial firing (RAF) defined as the occurrence of 2 or more premature atrial complexes; and 6) induced AF defined as AF that was induced by extrastimulus and persisted for >30 seconds.

Measurements of echocardiographic parameters

Echocardiographic studies were performed with Vivid 7 (GE, Milwaukee, Wisconsin). LV volume and ejection frac-

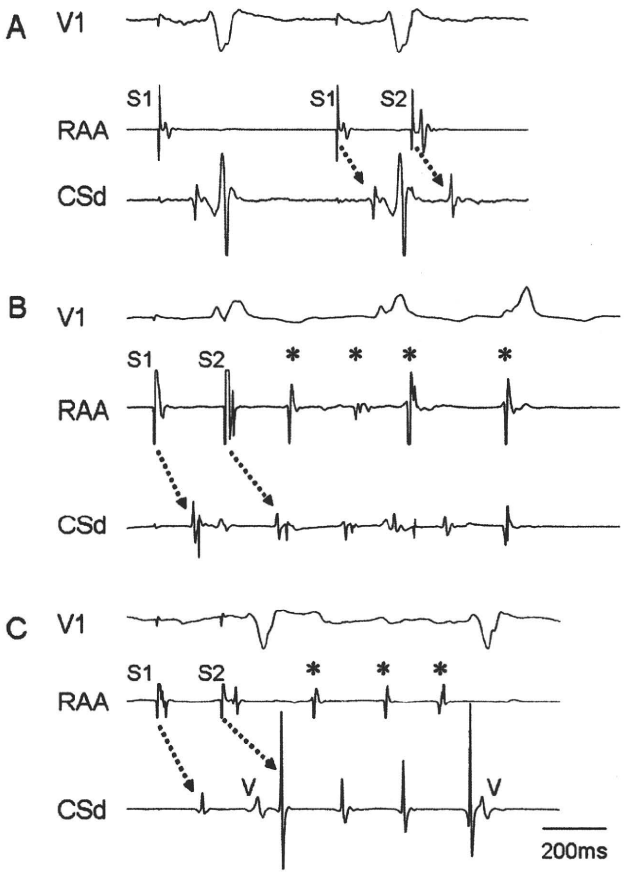

Figure 1 Occurrence of atrial vulnerability in patients with Brugada syndrome. **A:** A patient without VF and AF. This patient did not have atrial vulnerability. CT at S1 was 118 ms, CT at S2 was 126 ms, and CD was 8 ms. **B:** A patient with VF and without AF. CT at S1 was 120 ms, CT at S2 was 160 ms, and CD was 40 ms. CT at S2 and CD were prolonged compared with those in the patient without VF and AF, and RAF was also observed (*). **C:** A patient with VF and AF. CT at S1 was 138 ms, CT at S2 was 180 ms, and CD was 42 ms. CTs and CD were prolonged compared with those in the patient with VF and without AF, and RAF was induced (*). Dotted arrows indicate atrial conductions from the pacing site to the CSd at S1 and S2. AF = atrial fibrillation; CD = conduction delay; CSd = distal portion of the coronary sinus; CT = intra-atrial conduction time; RAA = right atrial appendage; RAF = repetitive atrial firing; S1 = constant pacing; S2 = extrastimuli; V = ventricular potential at the CSd; VF = ventricular fibrillation.

tion were evaluated according to the recommendations of the American Society of Echocardiography.[13] LV mass was indexed to body surface area (LV mass index). Relative wall thickness was calculated as twice the posterior wall thickness divided by the end-diastolic LV dimension. Right ventricular area was measured with a planimeter from the apical 4-chamber view, and right ventricular outflow tract (RVOT) diameter was measured in the subpulmonary region.[13]

The anteroposterior LA diameter was measured from the parasternal long-axis view at ventricular end-systole. The LA volume was measured with a planimeter from the apical 4- and 2-chamber views at ventricular end-systole by tracing the endocardial border (the biplanar modified Simpson method) excluding the pulmonary veins and the LA appendage.[13] The LA volume was indexed to body surface area (LAVI).

The transverse LA diameter was measured from the apical 4-chamber view, and theoretical conduction velocities at S1 and S2 were defined as transverse LA diameter divided by CT at S1 or CT at S2, respectively.

The peak mitral inflow early diastolic and atrial filling (E and A) velocities and the E-wave deceleration time were obtained. Peak early (Ea) and late (Aa) diastolic mitral annular velocities were measured at septal and lateral mitral annular sites by pulsed tissue Doppler imaging, and then the average values were used for analysis. E/Ea was calculated as a surrogate for LV filling pressure.[14]

Statistical analysis

Data are expressed as mean values ± SD. Student *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. Correlations were determined with the Pearson product moment correlation analysis. A value of $P < .05$ was considered to be statistically significant. All analyses were performed with SPSS II for Windows (SPSS, Inc., Chicago, Illinois).

The patients were divided into the following groups for comparisons: first, all of the patients were divided into a group with VF (n = 22) and a group without VF (n = 35); next, for excluding the influence of AF on the atrium, patients without spontaneous AF were subdivided into 2 groups according to existence of VF: patients with VF (n = 14) and patients without VF (n = 32); finally, all of the patients were divided into a group with *SCN5A* mutation (n = 11) and a group without *SCN5A* mutation (n = 46).

Results

Electrophysiological and anatomical atrial abnormalities in patients with and without VF

Patients with VF frequently experienced spontaneous AF compared with patients without VF. Incidences of *SCN5A* mutation in the 2 groups were not different (Table 2). The P-wave duration was longer in patients with VF than that in patients without VF (Table 2). The incidence of induced AF by programmed electrical stimulation and ERP were not different between the 2 groups. The CTs and CD were prolonged, and widening of the local atrial potential (A2/A1 ratio) was enhanced in patients with VF compared with those in patients without VF. Conduction velocity at S1 was identical, whereas conduction velocity at S2 was slower in patients with VF than that in patients without VF. The incidence of RAF was higher in patients with VF (Table 2).

In echocardiographic parameters, LA diameters and LAVI were significantly increased in patients with VF, although other parameters, including right ventricular area and RVOT diameter, were identical in the 2 groups (Table 2).

The LA diameters were correlated with CT at S2 (anteroposterior LA diameter: r = .440, $P = .001$; transverse LA diameter: r = .430, $P = .002$) only. In contrast, LAVI was correlated with CTs and CD (CT at S1: r = .634, $P < .001$; CT at S2: r = .715, $P < .001$; CD: r = .314, $P = .016$).

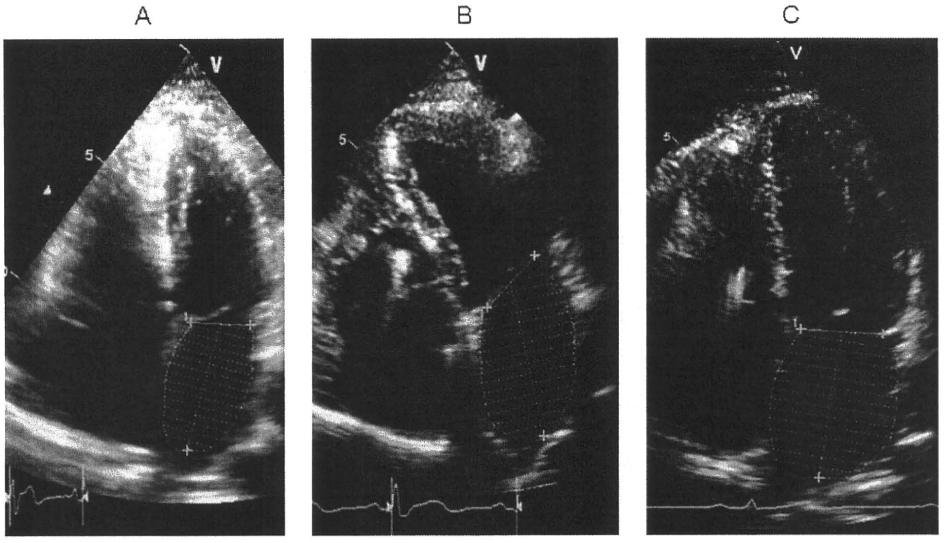

Figure 2 Echocardiographic measurements of left atrial volume in patients with Brugada syndrome. **A:** A patient without VF and AF. LAVI was 20 ml/m^2. **B:** A patient with VF and without AF. LAVI was 29 ml/m^2. **C:** A patient with VF and AF. LAVI was 33 ml/m^2. These were recorded from the same patients as those for which results are shown in Figure 1. **LAVI = left atrial volume index; other abbreviations as in** Figure 1.

Electrophysiological and anatomical atrial abnormalities in patients with and without VF who did not have spontaneous AF

There was no difference between the incidences of *SCN5A* mutation in the 2 groups. CTs and CD were significantly prolonged, and conduction velocity at S2 was decreased in patients with VF and without AF compared with those in patients without VF and AF (Table 3).

There were no significant differences in LA diameters between the 2 groups, but LAVI was significantly increased in patients with VF and without AF compared with that in patients without VF and AF (Table 3). Figure 1 and 2 demonstrate the representative cases of electrophysiological study and echocardiography, respectively.

Electrophysiological and anatomical atrial abnormalities in patients with and without *SCN5A* mutation

None of the clinical parameters were different between patients with and those without *SCN5A* mutation (Table 4). Incidence of VF induction, incidence of AF induction, incidence of RAF, and sinus node recovery time were not different between the 2 groups. ERP at the RAA, CTs, and local A2 were significantly prolonged in BrS patients with *SCN5A* mutation (Table 4). Although LA diameters showed no significant differences between the 2 groups, LAVI was increased in BrS patients with *SCN5A* mutation (Table 4).

Discussion

New observations

In the present study, high-risk patients who experienced VF had both increased atrial electrical vulnerability and advanced atrial structural remodeling. Occurrence of spontaneous AF was observed in high-risk patients with BrS as expected. The atrial electrical vulnerability and the structural remodeling were also enhanced in high-risk patients who did not have spontaneous AF. Mutation of *SCN5A* was associated with atrial electrical vulnerability and structural remodeling but not with the history of documented VF.

BrS patients with spontaneous AF

Previous studies have shown that spontaneous AF is the most common arrhythmia in patients with BrS, the incidence of AF in BrS ranging from 10% to 53%.[1,5,6,15] In the present study, spontaneous AF was observed in 19% of the subjects and in 36% of the patients who experienced VF episodes. This is consistent with previous reports that spontaneous AF is closely linked to documented VF.[6,15]

We have previously reported that atrial vulnerability in patients with BrS was enhanced compared with that in normal subjects (5) and that it was further enhanced in high-risk patients.[6]

The present study showed that atrial vulnerability was also enhanced in patients with documented VF who did not have spontaneous AF. These findings suggest that electrophysiological vulnerability exists in the entire heart, including the atria and ventricles, in patients with BrS and that the electrophysiological vulnerability increases with progression of the disease.

Atrial structural remodeling in patients with BrS

Although BrS is defined as a sudden cardiac death syndrome in the absence of cardiac structural abnormalities,[1] some investigators have shown by using various imaging techniques and histological study that cardiac structural abnormalities exist in the right ventricle in patients with BrS.[7–11]

In the present study, we demonstrated that atrial structural remodeling also occurs in patients with BrS even in the absence of spontaneous AF. In a recent magnetic resonance imaging study, no significant difference in LA size was found between patients with BrS and healthy control subjects (9). However, unlike in our study, the majority of enrolled patients were asymptomatic (25 of 30, 83%) and LA size was evaluated by LA area, not LA volume, in the aforementioned study.

Several prior studies have shown that LA enlargement is associated with LV diastolic dysfunction and elevated LV

Table 4 Clinical, genetic, electrophysiological, and echocardiographic parameters in patients with and without *SCN5A* mutation

Variable	*SCN5A* mutation (−)	*SCN5A* mutation (+)	*P* value
Clinical/ECG/genetic parameters			
Number of patients, n (male/female)	46 (45/1)	11 (11/0)	NS
Age, yrs	48 ± 12	51 ± 15	NS
Syncopal episode, n (%)	22 (48%)	8 (73%)	NS
Documented VF, n (%)	16 (35%)	6 (55%)	NS
Spontaneous AF, n (%)	7 (15%)	4 (36%)	NS
Family history of sudden death, n (%)	18 (39%)	4 (36%)	NS
Spontaneous type I ECG, n (%)	38 (83%)	7 (64%)	NS
P-wave duration, ms	102 ± 16	109 ± 18	NS
PQ interval, ms	171 ± 23	182 ± 21	NS
ICD implantation, n (%)	29 (63%)	7 (64%)	NS
Electrophysiological parameters			
VF induction, n (%)	30 (65%)	6 (55%)	NS
RAF, n (%)	18 (39%)	5 (45%)	NS
AF induction, n (%)	15 (33%)	5 (45%)	NS
ERP, ms	259.6 ± 42.0	300.0 ± 78.7	<.05
CT at S1, ms	118.7 ± 15.5	131.6 ± 22.0	<.05
CT at S2, ms	141.5 ± 20.6	161.5 ± 26.0	<.05
CD, ms	22.6 ± 16.4	30.0 ± 17.6	NS
Conduction velocity at S1, mm/ms	0.30 ± 0.05	0.29 ± 0.04	NS
Conduction velocity at S2, mm/ms	0.25 ± 0.04	0.24 ± 0.03	NS
A1, ms	57.1 ± 16.9	66.0 ± 17.6	NS
A2, ms	75.3 ± 20.0	95.3 ± 31.5	<.05
A2/A1	1.35 ± 0.23	1.44 ± 0.33	NS
Sinus node recovery time, ms	1,285 ± 241	1,479 ± 404	NS
Echocardiographic parameters			
LV end-diastolic volume, ml	147 ± 22	165 ± 29	NS
LV ejection fraction, %	62 ± 5	63 ± 5	NS
LV mass index, g/m^2	88 ± 16	97 ± 14	NS
Relative wall thickness	0.39 ± 0.05	0.37 ± 0.07	NS
Anteroposterior LA diameter, mm	33 ± 4	36 ± 4	NS
Transverse LA diameter, mm	35 ± 3	38 ± 5	NS
LAVI, ml/m^2	25 ± 6	32 ± 10	<.05
Right ventricular area, cm^2	16 ± 2	16 ± 2	NS
RVOT diameter, mm	27 ± 3	29 ± 5	NS
Peak E velocity, cm/s	60 ± 12	60 ± 10	NS
Peak A velocity, cm/s	50 ± 14	56 ± 18	NS
E/A	1.3 ± 0.5	1.2 ± 0.4	NS
E-wave deceleration time, ms	229 ± 36	231 ± 37	NS
Ea, cm/s	9.8 ± 3.0	9.1 ± 3.3	NS
Aa, cm/s	8.2 ± 1.8	7.8 ± 1.6	NS
E/Ea	6.9 ± 3.7	7.6 ± 3.7	NS

Values are n (%) or mean ± SD.
SCN5A mutation (−) = patients without *SCN5A* mutation; *SCN5A* mutation (+) = patients with *SCN5A* mutation; other abbreviations as in Tables 1 and 2.

filling pressure.[16,17] However, in the present study, parameters of LV diastolic function and hypertrophy and the E/Ea ratio were identical among groups, and none of the patients had hypertension or any organic heart disease, indicating that LA enlargement was not a result of LV diastolic dysfunction and subsequent elevated LV filling pressure. LA enlargement in BrS would therefore be due to primary myocardial damage. The possibility of primary myocardial damage is also supported by the observation of reduced conduction velocity after an extrastimulus. Thus, patients with BrS have both structural and electrophysiological remodeling of the atrium. Progression of the atrial remodeling suggests that BrS is a form of genetic myocardial disease.

SCN5A mutation and BrS

Mutations of *SCN5A* have been found in inherited arrhythmias, such as BrS,[18] progressive cardiac conduction disease,[19] and familial lone AF.[20] *SCN5A* mutation causes not only electrophysiological disorders but also organic heart diseases, such as familial dilated cardiomyopathy.[21] In the prior histological study, significant myocyte apoptosis in both the right and the left ventricular myocardium were observed in BrS patients with *SCN5A* mutation, suggesting that abnormal function of the sodium channels may lead to cellular damage and death.[10] These observations indicate that ion channel dysfunction can cause myocardial damage and fibrosis, which will then progress to arrhythmogenicity in addition to electrophysiological abnormality. We found that patients with *SCN5A* mutation had an enlarged LA as well as increased atrial vulnerability compared with those in patients without *SCN5A* mutation. These findings indicate that sodium channel disorders induced both electrophysiological and anatomical remodeling in LA in patients with BrS.

Study limitations

First, because the number of subjects in this study was relatively small, further research is needed to obtain a definitive conclusion regarding the association of LA size and

the risk of VF. Second, we could not clearly state that self-terminating spontaneous AF was never present in patients without AF, even though all of the patients underwent repeated 12-lead ECG, 24-h Holter monitoring, and strict medical interviews. Third, because most of the BrS patients with VF also had spontaneous AF, the number of patients without VF and with AF was insufficient for analysis in this study. However, we speculate that these patients would have increased atrial vulnerability and LAVI compared with those patients without VF and AF. Fourth, we measured theoretical conduction velocity (transverse LA diameter/conduction time) because we could not evaluate the exact atrial propagation of excitation. Finally, we did not evaluate atrial histology by biopsy. Detection of abnormal atrial histology would confirm the structural remodeling.

Recently, LA volume has been reported to be preferable as an accurate measurement of asymmetric remodeling of the LA chamber.[13,16] This may be the reason why LAVI, not LA diameters, showed significant differences in all comparisons and correlated well with several parameters of atrial vulnerability. In our prior study,[6] we did not find any differences in LA size in all comparisons and did not discuss the atrial structural remodeling in BrS because we had used LA linear diameter from conventional M-mode as an LA size. In contrast, we found enlarged LA in high-risk BrS patients in the present study because we measured LA volume and enrolled only patients with adequate echocardiographic images for analysis from our prior study or completely new patients with adequate images.

Quantifying right ventricular size accurately in a single 2-dimensional echocardiographic view is difficult because of its unique crescent-shaped structure.[13] This is most likely the reason why we did not find a significant difference in any echocardiographic parameters on right ventricular size among all groups.

Conclusion

This study showed that not only atrial electrophysiological remodeling but also atrial structural remodeling is augmented in patients with BrS. Moreover, atrial electrical and structural abnormalities seem to be more severe in high-risk BrS patients, and they are present even in patients without spontaneous AF. Measurement of LA volume by echocardiography will be useful for noninvasive risk stratification in patients with BrS.

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Efficacy of Low-Dose Bepridil for Prevention of Ventricular Fibrillation in Patients With Brugada Syndrome With and Without *SCN5A* Mutation

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Abstract: It has been reported that bepridil prevents ventricular fibrillation (VF) in patients with Brugada syndrome, but the comparative efficacy with and without mutation in the *SCN5A* gene has not been elucidated. The purpose of this study was to assess the efficacy of low-dose bepridil (100 mg/day) for VF prevention in patients with Brugada syndrome with and without *SCN5A* mutation. Among 130 patients with Brugada-type electrocardiogram (ECG), low-dose bepridil was administered to seven patients because of repetitive VF episodes, including three with and four without *SCN5A* mutation. Preventive effect for VF recurrence and changes of the ECG and the signal-averaged ECG were evaluated. Frequencies of VF episodes were reduced after treatment with low-dose bepridil in all three patients with the *SCN5A* mutation (before: 0.33 versus after: 0.02 episodes/month, $P < 0.01$), but not in all four patients without the *SCN5A* mutation (before: 0.43 versus after: 2.94 episodes/month, P = nonsignificant). Levels of ST-segment elevation at J points and duration of low-amplitude signals less than 40 μV in the terminal filtered QRS complex (LAS_{40}) in signal-averaged ECG were improved exclusively in patients with the *SCN5A* mutation. Treatment with bepridil prevented recurrence of VF along with improvement of ST elevation and LAS_{40} in patients with Brugada syndrome with the *SCN5A* mutation.

Key Words: Brugada syndrome, bepridil, ventricular fibrillation, *SCN5A* mutation, signal-averaged electrocardiogram, late potential

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INTRODUCTION

An implantable cardioverter–defibrillator (ICD) is the most effective life-saving device to prevent sudden cardiac death for patients with Brugada syndrome (BrS). However, frequent discharge from the ICD for repetitive ventricular fibrillation (VF) needs pharmacologic intervention to prevent VF occurrence in patients with BrS. It has been reported that quinidine, which inhibits I_{to} (transient outward current), is an effective drug to inhibit VF induction during electrophysiological study and prevent VF recurrence.[1–3] However, cessation of quinidine administration is often needed because of its intolerance such as anticholinergic effects. As another pharmacologic therapy, it has also been reported that bepridil, which is a multichannel blocker including I_{to} current,[4] is a potential drug to suppress VF in patients with BrS.[5,6] However, there have been only a few reports on the effects of bepridil on prevention of VF in patients with BrS.[5,6]

The ST elevation in patients with mutation in the cardiac sodium channel gene, *SCN5A,* is caused by both I_{to} current and decreased *SCN5A* current. On the other hand, the ST elevation in patients without the *SCN5A* mutation may be induced mainly by increased I_{to} current[7,8] The effects of I_{to} blockers may be different between patients with BrS with and without *SCN5A* mutation. Therefore, we hypothesized that there are differences in the efficacy of bepridil, a multichannel blocker including I_{to}, between patients with and without the *SCN5A* mutation.

To test this hypothesis, we assessed the efficacy of low-dose bepridil (100 mg/day) for prevention of VF and changes of the electrocardiogram (ECG) and the signal-averaged ECG (SAECG) in patients with BrS with and without mutation in the *SCN5A* gene.

METHODS

Subjects

The subjects were 130 consecutive patients with Brugada-type ECG between March 2000 and July 2007 (127 males and three females; mean age, 51.8 ± 12.9 years; age range, 27–85 years) in Okayama University Hospital. All patients showed a Type 1 ECG before or after pilsicainide. The Type 1 or 2 ECG was defined previously,[9] and if the standard