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Original article

Deterioration of the circadian variation of heart rate variability in Brugada syndrome may contribute to the pathogenesis of ventricular fibrillation

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ABSTRACT

Aims: Abnormal sympathetic innervation triggers ventricular fibrillation (VF). We examined the circadian variation of autonomic nervous system and its relevance to risk stratification of VF in patients with Brugada syndrome (Brs).

Methods: We enrolled 12 male Brs patients with documented VF (Brs-S; mean age, 42 ± 4 years), 17 without documented VF (Brs-N; mean age 48 ± 4 years), and 16 age- and gender-matched controls. The clinical data, 12-lead electrocardiography (ECG), signal-averaged ECG, electrophysiological study (EPS), and heart rate variability from 24 h Holter ECG were compared between the groups.

Results: The low frequency components (LF) in Brs-S and Brs-N and high frequency components (HF) in Brs-S patients were significantly lower than in the controls (409.8 ± 128.6 ms², 329.5 ± 108 ms² vs. 945.3 ± 111.3 ms²; 135.1 ± 73.8 ms² vs. 391.8 ± 63.9 ms², respectively). The circadian variation of the LF and LF/HF decreased in the Brs patients, the standard deviation (SD) of LF/HF (<2.5) and SD of LF (<400 ms²) had sufficiently high sensitivity (96.6%) and specificity (92.9%) for the diagnosis of Brs. Most of the Brs-S patients (83.3%) were located under the line formed by the SD/mean of HF = SD/mean of LF in the scatter plots.

Conclusion: Lack of the circadian variation of autonomic function occurs in Brs, and this may contribute to the pathogenesis of VF.

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Introduction

Many reports have documented the role of abnormal sympathetic innervation as a trigger of ventricular fibrillation (VF) [1–6]. Brugada syndrome (Brs) is one of the major causes of VF and a significant circadian peak from midnight to early morning in VF has been reported in patients with Brs [7]. Presynaptic sympathetic abnormalities of the heart in patients with Brs have been documented using positron emission tomography and [¹²³I]m-iodobenzylguanidine-single-photon emission computed tomography [8,9]. A predominance of a vagal tone has also been reported in patients with Brs using the head-up tilt

test [10,11]. With regard to the heart rate variability (HRV), some studies have reported decreased HRV in patients with Brs. However, most of those reports dealt with asymptomatic patients and there are only a few reports with various results discussing differences in the HRV between symptomatic and asymptomatic Brs [10,12,13]. There are many reports discussing the risk stratification of VF in Brs [14–23]. However, these parameters were inadequate and the risk stratification of VF in Brs remains controversial. The relationship between the autonomic nervous system and VF in Brs patients remains to be fully elucidated.

We investigated the circadian variations in autonomic nervous system using the HRV from 24-hour Holter electrocardiography (24-h ECG) in patients with Brs. Furthermore, we investigated whether an abnormal autonomic nervous system was related to the pathogenesis of a higher incidence of lethal ventricular arrhythmias associated with Brs.

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Methods

Subjects

We enrolled 12 male patients with documented VF (Brs-S group; mean age, 42 ± 4 years), 17 without documented VF (Brs-N group; mean age, 48 ± 4 years), and 16 age- and gender-matched controls (mean age, 43 ± 4 years) without structural heart disease, arrhythmias, and diseases affecting the autonomic nervous system. Among the enrolled patients, seven had a family history of sudden cardiac death. Noninvasive examinations revealed normal cardiac structure and function in all patients. Brs was definitively diagnosed when a type 1 ST-segment elevation was observed in >1 right precordial lead (V_1 to V_3) in the presence or absence of a sodium channel-blocking agent and in conjunction with one of the criteria of the Brs consensus conference [11]. A baseline examination revealed a spontaneous type 1 Brugada ECG pattern in 27 patients and type 2 Brugada ECG pattern at baseline in 2 patients who underwent a pharmacological challenge test with a pilsicainide injection (1 mg/kg body weight/10 min), which subsequently converted to a type 1 ECG pattern in more than one right precordial lead.

We retrospectively compared the following parameters in the Brs-S and Brs-N groups: (1) a history of syncope and family history of sudden cardiac death; (2) occurrence rate of paroxysmal atrial fibrillation; (3) 12-lead ECG parameters and type of Brugada ECG; (4) signal-averaged ECG findings; (5) electrophysiological study (EPS) parameters and induction of VF/ventricular tachycardia (VT) with burst pacing and triple paired extrastimuli from the right ventricular apex and right ventricular outflow tract; and (6) genetic analysis for the SCN5A gene which encodes for the α -subunit of the human cardiac sodium channel (hNav1.5).

We thoroughly investigated the HRV and also compared the power and fluctuation in the time and frequency domains of the HRV in the Brs-S, Brs-N, and controls.

The study was approved by Ethical Review Committee of our institution.

Twelve-lead ECG findings

The 12-lead ECG was recorded at a speed of 25 mm/s and an amplification of 1 cm/mV. We measured the RR, PQ, QRS, and corrected QT (QTc) intervals in lead V_6 and the J-point amplitude in leads V_1 and V_2 (STJ). We classified the Brugada ECG patterns into types 1–3, according to the criteria of the Brs consensus conference [11]. Because the ST configurations in the right precordial leads are known to show a day-to-day variation, more than five ECGs were recorded on different days for each patient. When a spontaneous type 1 Brugada ECG pattern was recorded at least once from a patient with Brs, that patient was identified as having a type 1 ECG pattern.

Signal-averaged ECG findings

Late potentials were analyzed using an FP-705LP system (Fukuda Denshi, Tokyo, Japan). The ECG was recorded using Frank X, Y, and Z leads during sinus rhythm, and the signals from 300 beats were amplified, digitized, averaged, and filtered with backward and forward filters at a high band-pass frequency of 40 Hz. The filtered QRS duration (f-QRS), root mean square voltage of the terminal f-QRS segment (RMS 40), and duration of low-amplitude signals $<40 \mu\text{V}$ in the terminal f-QRS segment (LAS 40) were calculated. Late potentials were identified as being present when two of the following criteria were satisfied: f-QRS >114 ms, RMS 40 $<20 \mu\text{V}$, or LAS 40 >38 ms [24].

Heart rate variability

Each subject underwent 24-h ECG monitoring, and we analyzed the total heart beats, minimum, maximum, and mean heart rate during 24 h. We obtained heart beats without premature beats and other arrhythmias, and used them for the analysis of the HRV (SCM-6600TM, Fukuda Denshi or MARSTM, Ambulatory ECG system ver.8, GE Medical Systems, Milwaukee, WI, USA). We performed time domain and frequency domain analyses (Fast Fourier transformation) with the data. The time domain indices were as follows: the standard deviation (SD) of normal sinus RR intervals (SDNN), SD of the average normal sinus RR intervals every 5 min (SDANN), average SD of normal sinus RR intervals every 5 min (ASDNN), and root mean square of successive normal sinus RR interval difference (rMSSD). The frequency domain indices were as follows: very low frequency component (VLF): 0.003–0.04 Hz; low frequency component (LF): 0.04–0.15 Hz that reflects the modulation of the sympathetic and parasympathetic tone by baroreflex activity; high frequency component (HF): 0.15–0.4 Hz that reflects the modulation of the vagal tone; and the ratio of the LF to HF [25]. The method of normalizing is [Normalized LF] = $(\text{LF} - [\text{mean of LF}]) / [\text{SD of LF}]$. The data were normalized to have mean = 0 and variance = 1. LF* is the moving average of the LF. Here, the LF* at time t is given as the average of the LFs at time t , $t - 1$ hour and $t + 1$ hour. None of the subjects took any antiarrhythmic agent and we confirmed all of the subjects with implantable cardioverter defibrillators were in regular sinus rhythm without pacing during recording the 24-h ECG monitoring.

EPS and VT/VF induction

After informed consent was obtained from the patients and their families, an EPS was performed in all patients. Three 5 French-quadrupolar electrode catheters with 5-mm inter-electrode spacing were positioned in the high right atrium, bundle of His, and right ventricular apex. Programmed ventricular stimulation was performed in all patients with burst pacing [up to 230 beats per min (bpm)] and up to triple extrastimuli at two different drive cycle lengths (400 and 600 ms) from the right ventricular apex and right ventricular outflow tract. The shortest coupling interval of premature beats was limited to 200 ms.

Sequence analysis of SCN5A using genomic DNA

Peripheral blood samples were obtained from all patients, and the genomic DNA was extracted from leukocytes according to the standard protocol using a QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, Germany). All SCN5A exons and their splice sites were amplified by a polymerase chain reaction from 2.5 ng of genomic DNA with GO Taq DNA polymerase (Promega, Madison, WI, USA). The samples were resequenced using the 64 sense and antisense primers (approximately 300 bp each) of SCN5A with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Statistical analyses

Statistical analysis was performed using commercially available software (JMP 8.0.2 Software, SAS Institute Inc., Cary, NC, USA). Values are presented as the mean \pm standard error (SE). A Mann-Whitney U test was used to compare the continuous variables between two groups. The continuous variables among the three different groups were analyzed using Kruskal-Wallis and Steel-Dwass tests. The χ^2 test and Fisher's exact test were used to evaluate the differences in the categorical variables between the subgroups. We used a repeated measures of ANOVA with a Friedman's Test to evaluate the HF, LF, and LF/HF every hour in the three

groups. A value of $p < 0.05$ was considered statistically significant. Moreover, a cluster analysis was used to generate a dendrogram (Ward's minimum variance algorithm) grouping a set of objects in a similar wave of the circadian variation [26].

Results

The clinical characteristics and electrocardiographic and electrophysiological findings in the Brs-S and Brs-N groups are shown in Table 1. A previous history of paroxysmal atrial fibrillation was more frequent in the Brs-S group than in the Brs-N group. The proportion of patients with syncope history, patients with a family history of sudden cardiac death, ECG parameters, rate of positive late potentials, EPS parameters including the induction rate of VF, and a positive mutation rate of SCN5A were similar between the two groups. Table 2 shows a comparison of the 24-h ECG findings and results of the time and frequency domain analyses in the Brs-S, Brs-N, and controls. The total heart beats and mean heart rate during 24 h were significantly lower in the Brs-S group than in the controls ($90\,122 \pm 4908$ beats vs. $106\,328 \pm 4070$ beats, $p = 0.0281$ and 68 ± 3 bpm vs. 77 ± 2 bpm, $p = 0.0303$, respectively). The other parameters of the time domain analysis were similar among the three groups. With regard to the frequency domain analysis, the LF power was significantly lower in the Brs-S and Brs-N groups than that in the controls (409.8 ± 128.6 ms² and 329.5 ± 108 ms² vs. 945.3 ± 111.3 ms², $p = 0.0006$, respectively). In addition, the HF power was significantly lower in the Brs-S group than in the controls (135.1 ± 73.8 ms² vs. 391.8 ± 63.9 ms², $p = 0.0112$). Fig. 1(a) contains the scatter plots of the SD of LF vs. SD of LF/HF for the three groups. Most of the patients in Brs had a smaller SD of LF and LF/HF than those in the controls. We set the SD cut-off values for the LF/HF at < 2.5 and for the LF at < 400 ms². Those cut-off values had sufficiently high sensitivity (96.6%) and specificity (92.9%) for the diagnosis of Brs. Fig. 1(b) also shows the scatter plots of the SD/mean of the HF and that of the LF, where most of the patients in the Brs-S group (83.3%) were located under the line indicated by the SD/mean of HF = SD/mean of LF. We plotted the HF, LF, and LF/HF every hour (Fig. 2) and found that the LF and LF/HF circadian variation decreased more in the Brs-S and Brs-N groups than in the controls.

In addition, in the dendrogram obtained by a cluster analysis of the circadian variation of normalized LF*, we found a large group that consisted of only patients with symptomatic Brs. In the Brs-S patients, the nocturnal LF changes decreased, particularly from

Table 1

Comparison of the clinical, electrocardiographic and electrophysiologic findings between Brs-S group and Brs-N group.

	Brs-S group (N = 12)	Brs-N group (N = 17)	p value
Clinical data			
PAF	3 (25%)*	2 (12%)	0.0407
Syncope	3 (25%)	3 (18%)	0.6302
Family History of SCD	3 (25%)	4 (24%)	0.9274
SCN5A	2 (17%)	0 (0%)	0.1249
Twelve leads ECG findings			
RR (ms)	967 ± 41	895 ± 33	0.1855
PQ (ms)	185 ± 11	169 ± 9	0.2538
QRS (ms)	156 ± 26	149 ± 21	0.8281
QTc (ms)	414 ± 9	424 ± 7	0.4105
V1STJ(mV)	4.0 ± 1.4	2.9 ± 1.3	0.5600
V2STJ(mV)	4.3 ± 1.2	2.9 ± 1.1	0.4194
Spontaneous type 1 ECG	10 (83%)	13 (76%)	0.6532
Signal averaged ECG findings			
Positive LPs	8 (67%)	13 (76%)	0.5077
fQRSd (ms)	173.2 ± 9.7	164.4 ± 7.7	0.4831
RMS 40 (V)	48.2 ± 2.4	13.3 ± 1.9	0.8741
LAS 40 (ms)	48.2 ± 5.2	48.4 ± 4.1	0.9792
EPS findings			
AH (ms)	106.3 ± 6.5	99.9 ± 4.8	0.4408
HV (ms)	47.6 ± 3.4	50.7 ± 2.5	0.4649
AVN ERP (ms)	383.3 ± 30.3	359.2 ± 21.8	0.5307
SNRT (ms)	1299 ± 255	1385 ± 187	0.7894
VF induction	10 (83%)	9 (53%)	0.0931

Data were mean ± SE. Brs-S group, patients of Brugada syndrome with documented ventricular fibrillation; Brs-N group, patients of Brugada syndrome without documented ventricular fibrillation; PAF, paroxysmal atrial fibrillation; SCD, sudden cardiac death; ECG, electrocardiography; STJ, J-point amplitude; LP, late potential; EPS, electrophysiologic study; ERP, effective refractory period; SNRT, sinus node recovery time; VF, ventricular fibrillation.

* $p < 0.05$ Brs-S group vs. Brs-N group.

evening to midnight, and drastically rose at midnight compared with control patients (Fig. 3).

Discussion

The major finding of this study was that the HRV decreased in patients with Brs over 24 h, and lack of the circadian variation of the HRV may contribute to the pathogenesis of life-threatening arrhythmias in Brs. We could distinguish Brs using the cut-off value of the SD of LF and SD of LF/HF with a high sensitivity and specificity. In addition, we could distinguish the patients with Brs-S using scatter plots of the SD/mean of the HF and LF, to some extent. Furthermore, we found a large group consisting of only patients with

Table 2

Comparison of the 24 h-Holter ECG findings including results of time domain and frequency domain analysis among Brs-S group, Brs-N group and controls.

	Brs-S group (N = 12)	Brs-N group (N = 12)	Controls (N = 16)	p value
Total hearts beats	90 122 ± 4908*	93 888 ± 3948	106 328 ± 4070	0.0281
Maximum heart rate (bpm)	127 ± 6	118 ± 4	131 ± 5	0.1088
Minimum heart rate (bpm)	47 ± 2	50 ± 2	52 ± 2	0.2837
Mean heart rate (bpm)	68 ± 3	70 ± 2	77 ± 2	0.0303
Mean RR interval (ms)	891 ± 32	865 ± 27	813 ± 29	0.1829
SDNN (ms)	131.7 ± 12.7	123.4 ± 10.7	154.9 ± 11.4	0.1312
SDANN (ms)	114.5 ± 12.2	109.4 ± 10.2	140.4 ± 10.9	0.1052
ASDNN (ms)	57.5 ± 5.7	49.5 ± 4.8	59.7 ± 5.1	0.3197
rMSSD (ms ²)	28.8 ± 3.2	26.1 ± 2.7	28.2 ± 3.2	0.7903
VLF (ms ²)	1463.9 ± 405.5*	1044.9 ± 340.7*	3215.2 ± 351.1	0.0002
LF (ms ²)	409.8 ± 128.6*	329.5 ± 108*	945.3 ± 111.3	0.0006
HF (ms ²)	135.1 ± 73.8*	143.1 ± 62*	391.8 ± 63.9	0.0112
LF/HF	3.25 ± 0.53	2.62 ± 0.45	3.76 ± 0.46	0.2156

Data were mean ± SE. Brs-S group, patients of Brugada syndrome with documented ventricular fibrillation; Brs-N group, patients with Brugada syndrome without documented ventricular fibrillation; SDNN, standard deviation of normal sinus RR intervals; SDANN, standard deviation of average of normal RR intervals every 5 min; ASDNN, average of standard deviation of normal sinus RR intervals every 5 min; rMSSD, root mean square differences of successive RR intervals; VLF, very low frequency; LF, low frequency; HF, high frequency; LF/HF, ratio of LF to HF.

* $p < 0.05$ vs. controls.

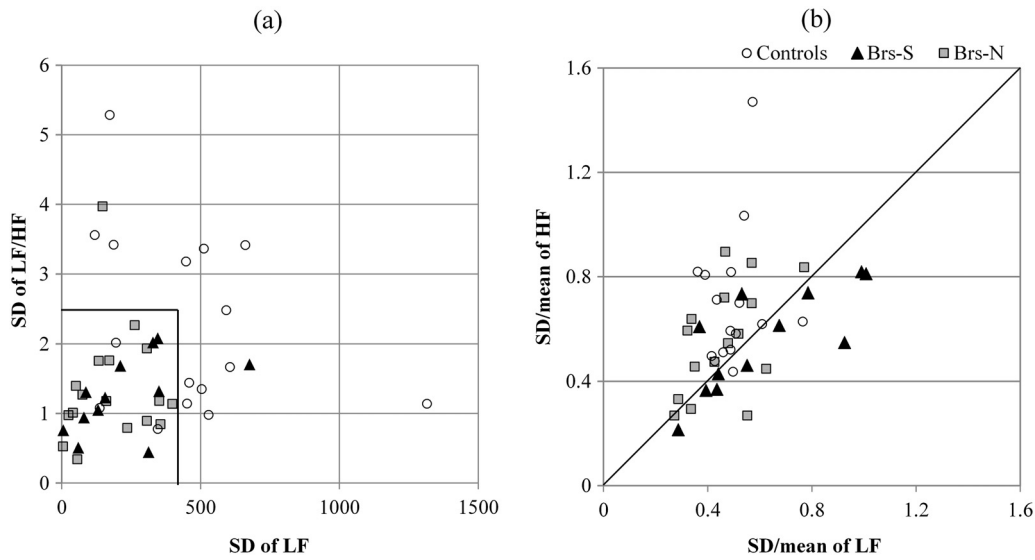


Fig. 1. (a) The scatter plots of the standard deviation (SD) of LF versus the SD of LF/HF for each subject in the three groups. Most of the patients with Brugada syndrome fall within a range of the SD of LF/HF <2.5 and the SD of LF <400 ms². (b) The scatter plots of the SD/mean of HF and the SD/mean of LF for each subject in the three groups. Most of the patients in Brs-S group (83.3%) were located under the line indicated by [SD/mean of HF]=[SD/mean of LF]. Brs-S, Brugada syndrome with documented ventricular fibrillation; Brs-N, Brugada syndrome without documented ventricular fibrillation; LF, low frequency components; HF, high frequency components.

Brs-S whose circadian variation of the normalized LF differed from that of patients with Brs-N and the controls.

Many studies have documented the role of abnormal sympathetic innervation as a trigger of VF [1–6]. Most ventricular arrhythmic events are nocturnal, particularly in patients with Brs [7], suggesting the influence of the autonomic nervous system.

Previous studies reported presynaptic sympathetic abnormalities of the heart in patients with Brs using positron emission tomography and [¹²³I]m-iodobenzylguanidine-single-photon emission computed tomography [8,9]. Adopting a different technique for testing the autonomic nervous activity, Kostopoulou et al. reported the predominance of the vagal tone in Brs using the head-up tilt

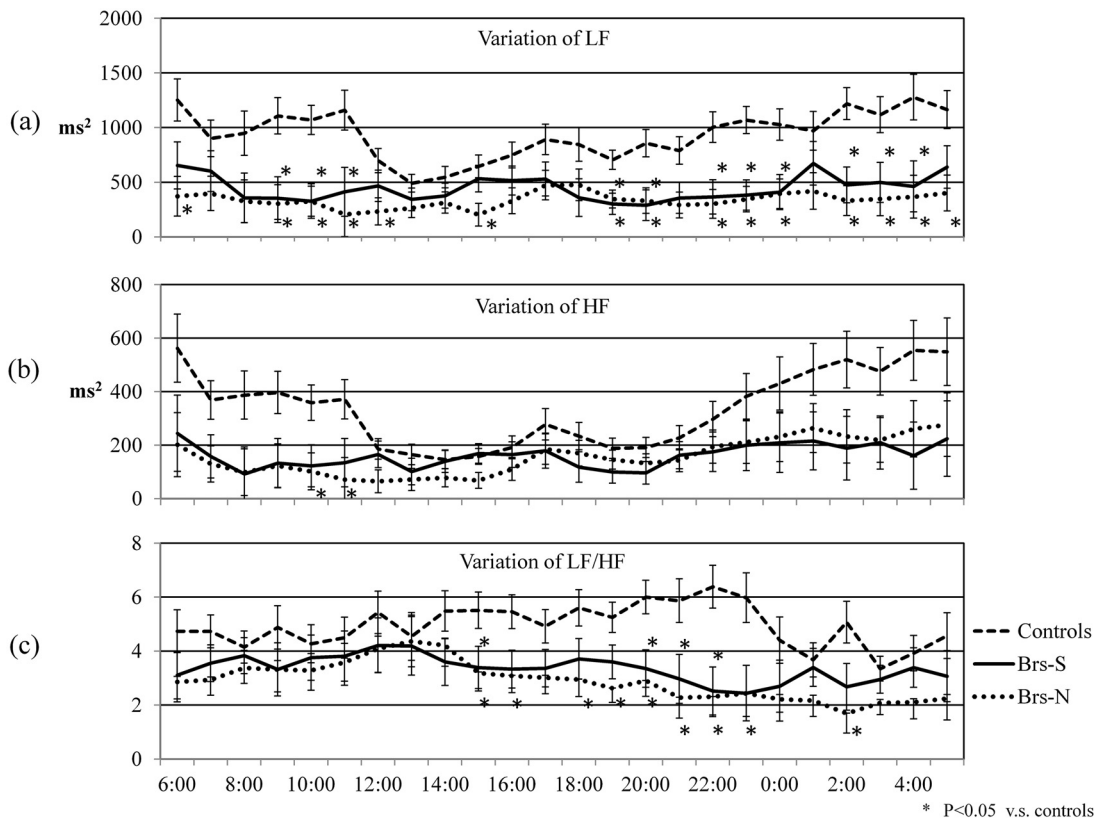


Fig. 2. The LF (a), HF (b) and LF/HF (c) every hour were plotted in the groups. The LF and LF/HF circadian variation decreased more in the Brs-S and Brs-N groups than in the controls group. Brs-S, Brugada syndrome with documented ventricular fibrillation; Brs-N, Brugada syndrome without documented ventricular fibrillation; LF, low frequency components; HF, high frequency components.

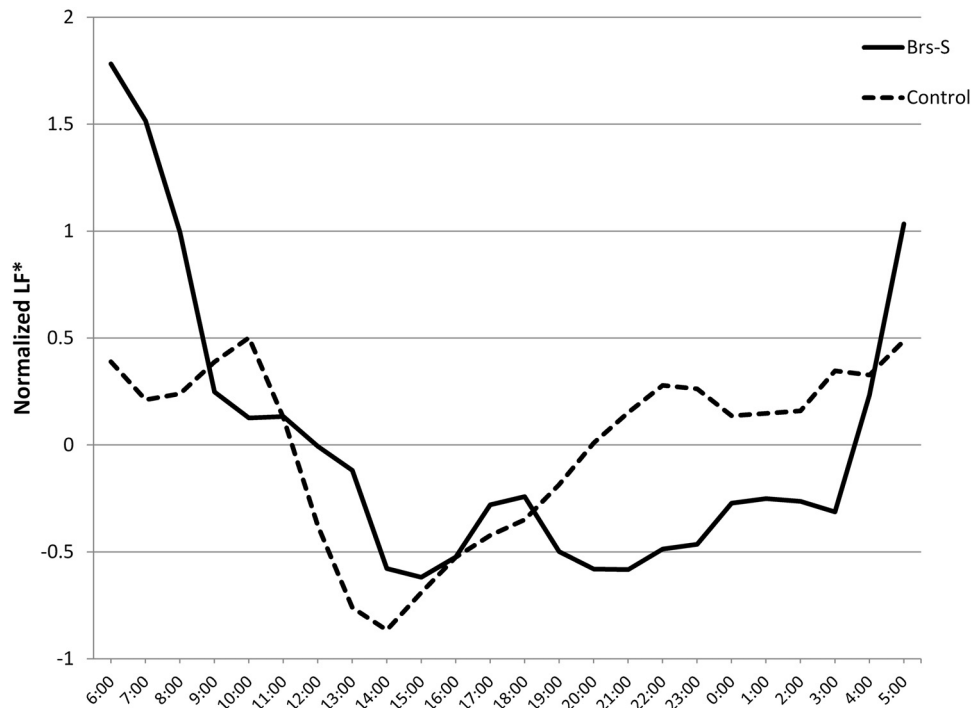


Fig. 3. The circadian variation of the normalized LF* in most Brs-S patients in the dendrogram obtained by a cluster analysis. Their normalized LF decreased from evening to midnight and drastically rose at midnight. The LF* is the moving average of the LF. Here, the LF* at time t is given as the average of the LFs at time t , $t - 1$ hour and $t + 1$ hour. Brs-S, Brugada syndrome with documented ventricular fibrillation; LF, low frequency components.

test, but a positive test result did not have any prognostic value for life-threatening arrhythmias. With regard to the HRV, the same report revealed that the HRV was similar between the patients with Brs and the controls [9]. Pierre et al. reported that patients with a Brugada-like ECG had lower HRV and QT/RR slopes than control subjects during the night [27]. Krittayaphong et al. reported that patients with asymptomatic Brs had a lower HRV or vagal tone at night than controls [10]. Most patients with Brs in previous reports were asymptomatic, and there have been no reports discussing the difference with regard to the HRV in patients with Brs with and without VF. Despite serious efforts of many researchers, controversies remain with regard to the risk stratification of VF in patients with Brs [14–23] and whether abnormal autonomic nervous system activity is related to the higher incidence of lethal ventricular arrhythmias in Brs.

In our study, there was no significant difference in the presence of a family history of sudden cardiac death, 12-lead ECG parameters, rate of positive late potentials, EPS parameters, inducibility of VF, or rate of *SCN5A* mutations between the Brs-S and Brs-N groups. In our report, a past history of atrial fibrillation was more prevalent in our subjects with Brs than in men of the same generation, and it was significantly higher in the Brs-S group than in the Brs-N group. Recently, Rodríguez-Mañero et al. reported that atrial fibrillation could be one of the first clinical manifestations of latent Brs [28]. Takagi et al. reported the clinical characteristics and risk stratification in patients with symptomatic Brs and a high incidence of a previous history of atrial fibrillation in patients with symptomatic Brs [23].

As for the investigation of the 24-h ECG, the total heart beats and mean heart rate from the 24-h ECG were significantly lower in the Brs-S and Brs-N groups than in the controls. Previous papers reported the manifestation of sinus node dysfunction in patients with Brs with *SCN5A* mutations [29]. In our study, the sinus node function in the EPS and frequency of *SCN5A* mutations were similar between our Brs-S and Brs-N groups.

An HRV analysis showed that the LF and HF power were lower in patients with Brs than in the controls. These results suggest that the vagal activity in patients with Brs consistently decreased in past reports [10,11]. Further investigation of the fluctuation in the HRV in our study revealed that the circadian variations in LF and LF/HF over 24 h were lower in patients with Brs than in the controls. The differences in the HRV in Brs patients with and without VF and similar investigation were already reported in a few papers as various results. Hermida et al. reported that 5 min averaged NN intervals (SDANN) was an independent marker of arrhythmic events in Brs patients, but frequency domain analysis of HRV were similar between symptomatic and asymptomatic Brs patients [12]. Their symptomatic patients included patients with history of syncope without VF history. Krittayaphong et al. reported that LF and HF in night time were lower in Brs patients than those in asymptomatic Brs ECG patients [10]. In the other reports, Nakazawa et al. reported that during the night (00.00–05.00 h), the HF was significantly higher and LF/HF was significant lower in Brs with VF than those without [13]. We could distinguish the patients with Brs with a high sensitivity and specificity using the SD of LF and SD of LF/HF cut-off values. In addition, we could distinguish the patients with Brs-S using the plots of the SD/mean of the HF and SD/mean LF in scatter plots to some extent. To the best of our knowledge, this was the first trial to quantitatively distinguish patients with Brs and stratify high-risk patients using circadian fluctuations in the HRV.

The circadian variation of the LF and LF/HF in Brugada syndrome patients may represent deterioration in circadian variation of sympathetic and parasympathetic nerve balance. We cannot clearly explain the mechanism why circadian variation of the LF and LF/HF was impaired in Brugada syndrome. Kies et al. presumed that autonomic innervation in Brugada syndrome describes an enhanced presynaptic norepinephrine recycling with preserved β -adrenoceptor density [6]. But, in their reports, they did not assess parasympathetic innervation. In another report, Taggart et al. reported that heart–brain interaction including autonomic

nerve activity was related to the cardiac arrhythmia [30]. The mechanism of impaired circadian variation of autonomic activity in patients with Brs remains to be solved.

In addition, in the dendrogram obtained from a cluster analysis of the circadian variation of the normalized LF, we found a large group consisting of only patients with Brs-S. The patterns of the circadian variation in all patients were heterogenic and it was extremely difficult to draw an absolute line between the patients with symptomatic and asymptomatic Brs. However, in this large group consisting of only patients with Brs-S, an identical pattern was indicated where the nocturnal changes in the LF decreased, particularly from evening to midnight and drastically rose at midnight.

In conclusion, there was a lack of the circadian variations in the HRV with Brs, and this autonomic nervous abnormality may contribute to the pathogenesis of life-threatening arrhythmias in Brs patients.

Limitations

There were several limitations in the present study. We examined only a small number of patients in a single institution; thus, the findings in our study need to be validated in a larger group of patients. The HRV data greatly varies and differs with age. All the subjects in our study were male and most were approximately 40 years old. Thus, our results may reflect only a limited cohort of patients with Brs. The relationship between the HRV and Brs has to be widely verified; however, to the best of our knowledge, this is the first report demonstrating fluctuations in the HRV and lethal arrhythmias in patients with Brs.

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