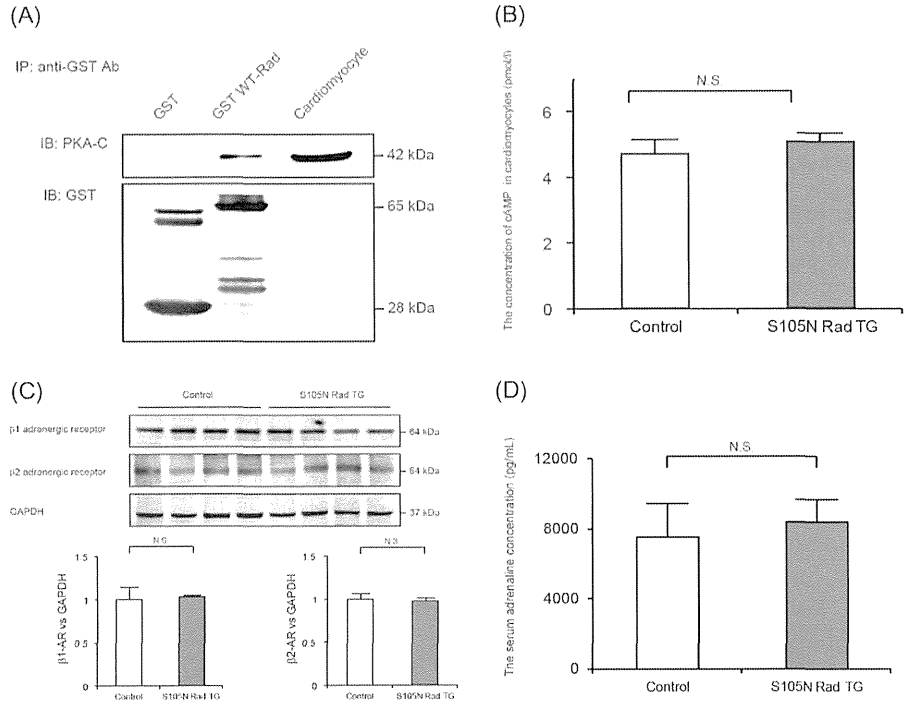

Fig. 4. PKA activity and cAMP/PKA signaling in S105N Rad heart. (A) GST-pull-down assay with immunoblotting of the antibody of PKA-C and GST, implicating the interaction between Rad and PKA-C. (B) Concentration of cAMP in S105N-Rad TG and control hearts (control ($n = 6$) vs S105N Rad TG ($n = 12$). (C) Western blots of β1-AR and β2-AR protein in S105N Rad TG and control hearts (control ($n = 8$) vs S105N Rad TG ($n = 8$). (D) The serum adrenaline concentration in S105N Rad TG mice and controls (control ($n = 5$) vs S105N Rad TG ($n = 5$)).

The other crucial signaling pathway that regulates RyR activity is a calmodulin-dependent kinase II (CaMKII) pathway. However, there were no significant changes in CaMKII phosphorylation between control and S105N Rad TG hearts (data not shown).

We finally analyzed the protein and messenger RNA (mRNA) expression levels of a range of these proteins from S105N Rad TG and control mice. Immunoblots of Ca^{2+} handling proteins showed no significant differences in the protein expressions of SERCA2a, PLB, and NCX between control and S105N Rad TG hearts, while phosphorylated PLB at Serine 16, which is a PKA phosphorylation site, was increased in S105N Rad TG samples (data not shown).

4. Discussion

This is the first report to show that Rad could regulate SR Ca^{2+} release channel activity via direct interaction with a PKA catalytic subunit. In this study, we found that ablation of Rad activity in a dominant-negative manner increased the amplitude of $[Ca^{2+}]_i$ transients as well as the frequency of Ca^{2+} sparks, resulting in abnormal cellular excitability including triggered activities (EADs and DADs) and Ca^{2+} waves. Consistent with this, the RyR2 activity was increased by dominant-negative suppression of Rad. The mechanism for this might be at least in part due to the upregulation of PKA signaling via direct interaction between Rad and a PKA catalytic subunit.

4.1. Rad regulates excitation–contraction coupling via upregulation of RyR2 activity

Reduction of Rad activity in S105N Rad TG cardiomyocytes led to the significant increase in the amplitude of peak $I_{Ca,L}$ as well as $[Ca^{2+}]_i$ transients, compared with wild-type littermate mouse control cells. Furthermore, the frequency of Ca^{2+} sparks was also significantly enhanced in S105N Rad TG cardiomyocytes. In contrast, there was no significant difference in caffeine-evoked $[Ca^{2+}]_i$ transients between control and S105N Rad TG cardiomyocytes, suggesting that Rad-mediated modulation of the $[Ca^{2+}]_i$ transient and Ca^{2+} spark were not attributable to the changes in SR Ca^{2+} content. Therefore, we investigated the activity of SR Ca^{2+} release channel RyR2, and found that p-RyR2 Ser^{2809} was significantly upregulated by the suppression of Rad activity in mouse hearts as well as at the single-cell level. Dysfunctional Ca^{2+} handling proteins such as L-type Ca^{2+} channel and RyR2 in the S105N Rad TG mice could induce intracellular Ca^{2+} overload, and also induction of ventricular arrhythmias due to the changes in triggered activities shown herein and in our previous report [9].

In contrast to Wang et al., whose data demonstrated that Rad downregulation did not change the properties of Ca^{2+} sparks and Ca^{2+} wave frequency, our data clearly showed an increased frequency of Ca^{2+} sparks and Ca^{2+} wave in S105N Rad TG cardiomyocytes, compared with control cells [11]. The possible explanation for this discrepancy is that the cardiomyocytes used in the present study were freshly isolated and thus retained many necessary signal transduction molecules that may be downregulated during cell culture, as used in the previous report. According to their data, Ca^{2+} spark frequency tended to be greater in Rad-downregulated cells compared with controls, although there were no significant differences. Wang et al. also demonstrated that β-adrenergic stimulation by ISO did not restore Rad-mediated inhibition of $I_{Ca,L}$ [11], whereas $I_{Ca,L}$ could be enhanced in Rad-downregulated cells, supporting our data that Rad may inhibit PKA phosphorylation via direct interaction with a PKA catalytic subunit. Taken together,

several distinct mechanisms for RGK-mediated L-type Ca^{2+} channel regulation have been proposed in cardiomyocytes as followed: (1) channel trafficking, (2) reduction of channel-open probability, and (3) phosphorylation by PKA. However, the predominant mechanism underlying the regulation of L-type Ca^{2+} channels may depend on experimental conditions including cell type, use of cultured cells, and disease states, and further studies are clearly warranted.

4.2. Rad directly interacts with PKA and regulates PKA signaling

RyR2 is regulated by many signaling molecules including protein kinases (eg., calmodulin dependent kinase II, PKA, channel stabilizing protein calstabin2, protein phosphatases, and sorcin). Among these molecules, PKA can phosphorylate RyR2 at Serine 2809 [16,17], and an association of Rad with β-adrenergic signaling was implicated previously [11], and interactions between Rad and the β-adrenergic pathway were suggested. Although there were no significant differences in the expression of adrenergic receptor, cAMP levels, or the concentration of catecholamines between S105N Rad and control hearts, the phosphorylation of PKA was significantly increased in S105N Rad hearts and cardiomyocytes. Finally, PKA acts downstream of β-adrenergic signaling and upstream of RyR2 Ser^{2809}. Notably, the GST-pulldown assay in this study revealed for the first time a direct interaction of Rad with PKA catalytic subunits. Thus, the hyperphosphorylation of L-type Ca^{2+} channels by PKA might contribute to the Rad-mediated increase in $I_{Ca,L}$ in addition to the channel trafficking regulation, as consistent with Rem-induced regulation of $I_{Ca,L}$ [18]. However, such a contribution would not have much of an impact because no changes were observed in the steady-state activation and inactivation of L-type Ca^{2+} channels between S105N Rad TG and control cells. Thus, this study provided no significant evidence for upregulation of the activities of other PKA substrates including phospholamban and SERCA2a. We speculate that another molecule might be required in the interaction between Rad and PKA. One candidate for this is a PKA scaffolding protein, which could ensure that PKA accesses its phosphorylation substrate correctly. The association of Rad with PKA might therefore be regulated according to the interaction sites and other interacting molecules, and further studies are needed to investigate this hypothesis.

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Disclosure

None.

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Tachycardia-dependent augmentation of "notched J waves" in a general patient population without ventricular fibrillation or cardiac arrest: Not a repolarization but a depolarization abnormality?

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BACKGROUND J waves can be observed in individuals of the general population, but electrocardiographic characteristics are poorly understood.

OBJECTIVE The purpose of this study was to examine the J-wave dynamicity in a general patient population.

METHODS The responses of J waves (>0.1 mV above the isoelectric line in 2 contiguous leads) to varying RR intervals were analyzed. Patients with aborted sudden cardiac death, documented ventricular fibrillation, or a family history of sudden cardiac death were excluded. The J-wave amplitude was measured at baseline, in beats with short RR intervals in conducted atrial premature beats (APBs) or atrial stimulation during the electrophysiology study, and in the beats next to APBs with prolonged RR intervals.

RESULTS Mainly notched J waves were identified in 94 of 701 (24.5%) general patients (13.4%), and APBs were present in 23 of 94 (24.5%) patients. The mean baseline amplitude of J waves was 0.20 ± 0.06 mV at the baseline RR interval of 853 ± 152 ms, 0.25 ± 0.11 mV at the RR interval in the conducted APB of 545 ± 133 ms (P = .0018), and 0.19 ± 0.08 mV at the RR interval of 1146 ± 314 ms (P = .3102). The clinical characteristics were not different between patients with and without tachycardia-dependent augmentation of J waves. Augmentation of J waves was confirmed by the electrophysiology study: 0.28 ± 0.12 mV vs 0.42 ± 0.11 mV at baseline and in the beats of atrial stimulation, respectively (P = .0001). However, no bradycardia-dependent augmentation (>0.05 mV) was observed. Such tachycardia-dependent augmentation can represent depolarization abnormality rather than repolarization abnormality.

CONCLUSION J waves in a general patient population were augmented at shorter RR intervals, but not at prolonged RR intervals. Mechanistically, conduction delay is most likely responsible for this.

KEYWORDS J waves; Rate dependency; Early repolarization; Conduction delay

ABBREVIATIONS **APB** = atrial premature beat; **ECG** = electrocardiogram/electrocardiography/electrocardiographic; **EPS** = electrophysiology study; **ER** = early repolarization; $\mathbf{I_{to}}$ = transient outward current; **VF** = ventricular fibrillation

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Introduction

An early repolarization (ER) pattern is defined as ST-segment elevation that is usually observed in the precordial leads and is referred to as a normal QRS-T variant.[1–5] ER patterns were originally used to differentiate ST-segment elevation due to acute myocardial infarction or acute pericarditis and have been viewed as a benign electrocardiographic (ECG) finding.[6,7]

Notches or slurs in the terminal portion of the QRS complex have been observed in association with idiopathic ventricular fibrillation (VF)[8–11] and have been labeled as J waves.[12] In reports that have established a link between J waves and VF/sudden cardiac death, the key factor is the presence of J waves irrespective of ST-segment elevation.[8–11] In the general population, such J waves can be observed in people without symptoms and without a history of aborted sudden cardiac death.[13,14] Distinguishing individuals with benign J waves from those with malignant J waves who are at risk of sudden cardiac death caused by VF is critically important.[9,12,13,15,16] However, controversy also exists surrounding the genesis of J waves: that is, J waves as a manifestation of repolarization[17] or depolarization abnormality.[18,19] The ECG and/or electrophysiological characteristics specific to each mechanism are still lacking.

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In previous studies,[8,20] we analyzed the response of J waves to a sudden change in the RR interval and confirmed that pause-dependent augmentation of the J-wave is characteristic of patients with idiopathic VF. In this study, we examined the dynamicity of J waves in a general patient population that was not at risk of developing VF or sudden cardiac death by analyzing the responses of J waves to sudden changes in RR intervals.

Methods

Study population

This study involved adult patients less than 80 years of age who had visited our hospital in 2012–2013 for general diseases or for an electrophysiology study (EPS). When J waves were identified, they were analyzed at varying RR intervals induced by the atrial premature beat (APB) (the APB group).

The study group had no history of syncope, cardiac arrest, documented ventricular tachycardia, or VF based on medical and family history. Patients with common diseases such as hypertension, diabetes mellitus, and obesity were included, but those with ischemic heart diseases, such as angina pectoris or myocardial infarction, hypertrophic or dilated cardiomyopathy, valvular heart diseases, or heart failure with more than mild severity, or chronic lung diseases were excluded from the study. There was no evidence of primary electrical disorders, such as Wolff-Parkinson-White syndrome, QT interval prolongation[21] or shortening,[22] Brugada syndrome,[23] or J-wave–associated idiopathic VF,[8–11] based on clinical history, physical examinations, and laboratory findings (including ECG and echocardiographic findings). None of the patients had bundle branch block that can mask J waves.[24,25] Patients with fragmented QRS complexes were excluded.[26]

In addition, the responses of the J-wave to varying RR intervals were analyzed by the EPS during atrial stimulation (the EPS group). All patients of the EPS group met the inclusion criteria, and none showed inducible sustained ventricular tachycardia or VF during the EPS. The EPS was performed after obtaining a written informed consent.

ECG analysis

ECGs were analyzed for RR, PR, QT intervals and J waves. Bazett's formula was used to correct the QT interval. J waves were defined as (1) a notch or slur in the terminal portion of the QRS complex and (2) an amplitude of >0.1 mV above the isoelectric line in at least 2 contiguous leads in the inferior (II, III, and aVF) or left precordial (V_4 to V_6) leads or in either of the high lateral leads (I or aVL). Late r (r′) waves following S waves were not included as J waves in this study unless notching or slurring was evident in other leads.

When J waves were present, the type (notch or slur), the localization in the 12-lead ECG, and the morphology of the ST segment were determined as described in the original reports.[15,16] When 2 types of ER patterns were present, the type was determined in the leads to reveal the maximum amplitude. The localization was classified according to the leads with J waves: inferior (II, III, and aVF), high lateral (I or aVL), and left precordial (V_4 to V_6).[9,12] ECGs were also analyzed for the presence of concomitant elevation of the ST segment.

ECGs were read after 5-fold magnification by 2 cardiologists. The J-wave amplitude was determined in the leads to reveal the maximum amplitude. When there was disagreement on J waves, the cardiologists mutually discussed the results to reach an agreement.

Data analysis

The prevalence of J waves was determined in the entire study population during the initial analysis. The clinical characteristics were then obtained from patients with J waves. Hypertension, diabetes mellitus, and dyslipidemia were diagnosed according to the published criteria or if the patients had been treated for these diseases. In the APB group, the dependency of the J-wave amplitude on RR intervals was assessed by comparing the J-wave amplitude among the baseline beat (as the mean of 3 successive beats), in the conducted APB (short RR interval), and in the beat next to the APB with prolonged RR interval.

The clinical characteristics were compared between patients with and without APBs and also between patients with and without tachycardia-dependent augmentation.

In the EPS group, the J-wave amplitude was analyzed among the baseline beats, the conducted beats of atrial stimulation, and the beats after atrial stimulation to confirm the dynamicity of J waves as observed in the APB group. Atrial stimulation was used only to diagnose arrhythmia or to evaluate atrioventricular conduction, though not systematically in the present study. When available, the J-wave amplitude was compared at different paced cycle lengths to evaluate the rate dependency.

J waves were identified as augmented if the amplitude increased by ≥ 0.05 mV, as unchanged for the change between -0.05 and <0.05 mV, and as decreased for the change <-0.05 mV.

Statistical analyses

Continuous variables are expressed as means ± SD, and categorical variables are expressed as absolute numbers and percentages. Statistical comparisons between the groups were made using the t test or analysis of variance for continuous variables and Pearson χ^2 test for categorical variables. JMP software (Statistical Discovery Software, version 5.0.1, SAS Institute Inc, Cary, NC) was used to perform statistical analysis. A 2-sided P value of $<.05$ was considered statistically significant. This study was approved by the Institutional Review Board of Tachikawa Medical Center.

Results

Prevalence of J waves in the general patient population

J waves with an amplitude >0.1 mV were present in 94 of 701 patients (13.4%) and were larger in men than in women

(15.3% vs 9.8%, respectively), although this difference was not statistically significant ($P = .0510$). The mean age was 61 ± 12 years. There were 68 male patients (72.3%). The J-wave amplitude was 0.19 ± 0.06 mV. The type of J waves was notch in 53 (56.4%) and slur in 41 (43.6%). ST-segment elevation (>0.1 mV) in either lead was found in 10 patients (11.0%). Of the 94 patients with J waves, 23 (24.5%) patients had APBs on a 12-lead ECG (the APB group). The clinical and ECG characteristics were similar between patients with and without APB (Table 1).

J-wave dynamicity in the APB group

The mean baseline amplitude of J waves was 0.20 ± 0.06 mV in 23 patients with APB. None of the patients showed ST-segment elevation >0.1 mV. J waves were notch type, with slur type in other leads. Horizontal/downward ST segments were identified in 10 patients (43.5%). J waves were located in the inferior lead in 20 patients (87.0%), in inferior and left precordial leads in 2 patients (8.7%), and in high lateral leads in 1 patient (4.3%).

The baseline RR interval was 853 ± 152 ms and shortened to 545 ± 133 ms in the conducted APBs ($P < .0001$; Figure 1). The RR interval in the next normal beat was prolonged to 1146 ± 314 ms, which was significantly different from the baseline value ($P < .0001$). The J-wave amplitude was significantly increased from 0.20 ± 0.06 mV at baseline to 0.25 ± 0.11 mV in the conducted APBs ($P = .0018$). The J-wave amplitude of the beat next to the APB did not differ from the baseline value ($P = .3102$), but was smaller than that of the APB: 0.19 ± 0.08 mV ($P = .0060$).

Comparisons between patients with and without tachycardia-dependent augmentation

In the APB group, 10 of 23 patients showed augmentation of the J wave at short RR interval and the remaining 13 (56.5%) did not. Age was higher in patients with augmentation than in those without augmentation, but was not statistically significant: 72 ± 5 years vs 57 ± 5 years ($P = .0707$). Other clinical and ECG characteristics were not different between the 2 groups (Table 2). The RR interval was shortened from 806 ± 47 to 498 ± 41 ms in the group with augmentation and from 890 ± 14 to 586 ± 137 ms in the group without augmentation. The J-wave amplitude was augmented by 0.10 ± 0.05 mV in 10 patients with augmentation and by 0.00 ± 0.01 mV in 13 patients without augmentation ($P < .0001$). Other variables were similar between the 2 groups.

Reproduction of J-wave dynamicity during the EPS

Among 171 patients who underwent EPS in the last 2 years, patients with structural heart diseases or those with fragmented QRS complexes were excluded, and 9 patients (5.8%) showed J waves that were analyzed during atrial stimulation. One patient showed respiratory changes in the amplitude of J waves, and the dynamicity of J waves was examined in 8 patients (4.7%) by the EPS during atrial stimulation (the EPS group; Table 3). The mean age was 51 ± 15 years, and 5 were men (62.5%). These 8 patients were studied for paroxysmal atrial fibrillation (n = 2), atrial flutter (n = 1), atrial tachycardia (n = 1), atrioventricular block (n = 1), and nonsustaining ventricular tachycardia (n = 3). Either constant pacing (n = 5), atrial premature stimulation (n = 1), or both constant pacing and atrial premature stimulation (n = 2) were applied.

None of the patients showed ST-segment elevation >0.1 mV in association with J waves. Notched J waves were found in 8, with slurring in 4 patients (50.0%). The location was the inferior lead in 7 (87.5%) and inferior and left precordial leads in 1 (12.5%). Horizontal/downward ST segments were identified in 3 patients (37.5%).

The baseline RR interval was 838 ± 238 ms, and the mean J-wave amplitude was 0.28 ± 0.12 mV. The RR interval was shortened to 436 ± 83 ms ($P = .018$) in the conducted beats of atrial stimulation, and the J-wave amplitude was increased to 0.42 ± 0.11 mV ($P = .0001$; Table 3 and Figure 2). The J wave was augmented (≥0.05 mV) in all patients by 0.12 ± 0.04 mV. When the RR interval was prolonged to 1019 ± 237 ms after atrial stimulation ($P = .0239$), the J-wave amplitude did not differ from the baseline value (0.28 ± 0.12 mV; $P = .6508$), but a decrease or increase in the J-wave amplitude were observed

Table 1 Characteristics of the patients with APBs

Characteristic	All patients with J waves (n = 94)	Patients with APBs (n = 23)	Patients with no APB (n = 71)	P
Age (y)	61 ± 12	63 ± 20	59 ± 11	.1799
Sex: male	68 (72.3)	16 (69.6)	52 (73.2)	.7127
J-wave amplitude (mV)	0.19 ± 0.06	0.20 ± 0.06	0.19 ± 0.07	.6246
RR interval (ms)	889 ± 176	853 ± 152	900 ± 183	.3017
PR interval (ms)	165 ± 29	168 ± 23	166 ± 26	.6201
QRS width (ms)	95 ± 16	95 ± 9	97 ± 10	.4714
QTc interval ($ms^{1/2}$)	401 ± 61	408 ± 41	409 ± 23	.9769
Hypertension	39 (41.5)	11 (47.8)	28 (41.5)	.4796
Diabetes mellitus	17 (18.1)	4 (17.4)	13 (18.3)	.9205
Hyperlipidemia	9 (9.6)	2 (8.7)	7 (9.9)	.8678
BMI (≥25 cm/kg^2)	6 (6.4)	1 (4.4)	5 (7.0)	.6327

Values are presented as mean ± SD or as n (%).
APB = atrial premature beat; BMI = body mass index; QTc = corrected QT.

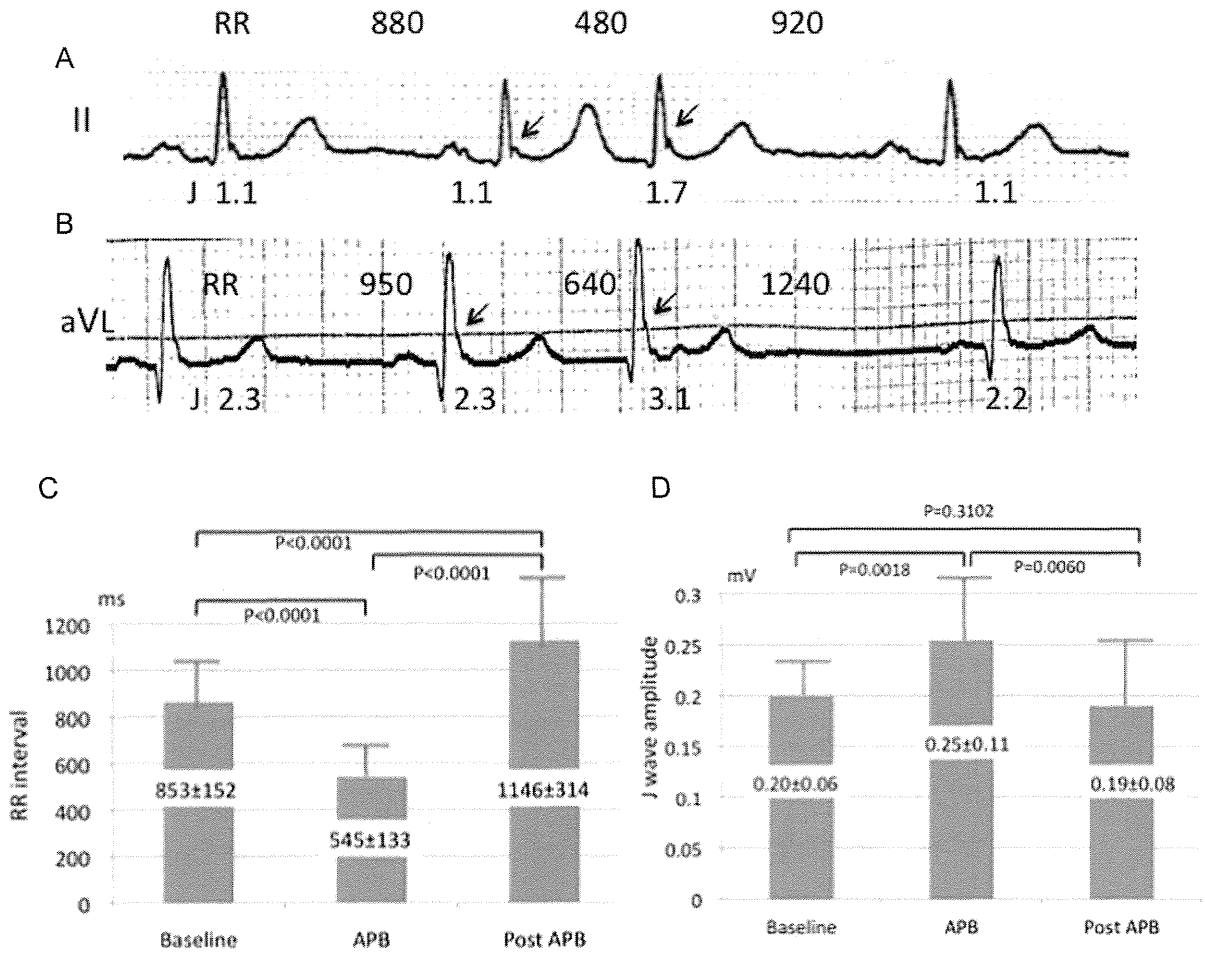

Figure 1 Changes in the J-wave amplitude with varying RR intervals in the conducted APB. **A:** RR intervals and the corresponding J-wave amplitudes for the notch-type ER. The J-wave amplitude (in mm) was augmented at shorter RR interval in the conducted APB. **B:** Dependency of the J-wave amplitude (slur-type ER) on varying RR intervals. **C and D:** RR intervals and the corresponding J-wave amplitudes. The J-wave amplitude was augmented at short RR interval in the conducted APB, but not altered in the beat with RR interval prolongation in the beat next to the APB. APB = atrial premature beat; ER = early repolarization.

in 3 and 2 patients, respectively (Table 3). J waves after stimulation were smaller than those of the stimulated beat at the atrium ($P < .0001$).

During constant pacing of the atrium, the J-wave amplitude was constant in 1–2 conducted beats. At 2–3 differently paced cycle lengths, rapid pacing of the atrium showed a

Table 2 Comparisons of ECG parameters between patients with and without augmentation of the J-wave amplitude

Characteristic	Patients with augmentation (n = 10)	Patients without augmentation (n = 13)	*P*
Age (y)	72 ± 5	57 ± 5	.0707
Sex: male	8 (72.7)	10 (72.2)	.8589
J-wave amplitude (mV)	0.20 ± 0.02	0.20 ± 0.02	.7240
RR interval (ms)	806 ± 47	890 ± 14	.1919
PR interval (ms)	172 ± 8	167 ± 7	.5979
QRS width (ms)	94 ± 3	95 ± 2	.7199
QTc interval ($ms^{1/2}$)	411 ± 13	407 ± 12	.8213
CI of APB/atrial pacing	498 ± 41	586 ± 137	.1265
Change in J-wave amplitude (mV)	0.10 ± 0.05	0.00 ± 0.01	<.0001
Notched J waves*	4 (40.0)	4 (30.8)	.6455
Horizontal/downward ST segments	4 (40.0)	6 (46.2)	.7677

Values are presented as mean ± SD or as n (%). RR intervals were significantly shortened by APBs or by atrial stimulation ($P < .0001$) followed by a significant prolongation ($P < .0001$).

APB = atrial premature beat; CI = coupling interval of atrial premature beat or atrial stimulation; ECG = electrocardiographic; QTc = corrected QT.

*With slur type in other leads.

Table 3 Clinical and ECG characteristics of the EPS group

Case	Age(y)	Sex	Diagnosis	Location	RR interval: baseline/AS/post-AS (ms)	J-wave amplitude: baseline/AS/post-AS (mV)
1	34	M	PAF	Inf	1240/420/1240	0.15/0.25/0.12
2	57	M	AFL	Inf	740/470/940	0.30/0.37/0.32
3	63	M	AT	Inf + LP	600/400/1080	0.18/0.32/0.15
4	35	F	NSVT	Inf	760/440/1150	0.45/0.55/0.40
5	55	M	PAF	Inf	920/500/950	0.31/0.46/0.31
6	61	F	NSVT	Inf	700/400/780	0.38/0.55/0.38
7	71	F	AVB	Inf	730/600/880	0.37/0.50/0.40
8	34	M	NSVT	Inf	1100/500/1300	0.12/0.35/0.12

AFL = atrial flutter; AS = atrial stimulation; AT = atrial tachycardia; AVB = atrioventricular block; ECG = electrocardiographic; EPS = electrophysiology study; F = female; Inf = inferior; LP = left precordial; M = male; NSVT = nonsustaining ventricular tachycardia; PAF = paroxysmal atrial fibrillation.

rate-dependent augmentation of the J wave in the 3 patients studied (Figure 3).

Discussion

J waves, mainly notch type, were observed in a general patient population. All the patients denied episodes of syncope or cardiac arrest and sudden cardiac death in family members. J waves were augmented in the conducted beat of the APB with short RR interval and remained unchanged in the next sinus beat with prolonged RR interval. The same J-wave dynamicity was reproduced by varying RR intervals during the EPS. Augmentation of the J-wave amplitude at shorter RR interval and no augmentation at prolonged RR interval were quite different from those observed in idiopathic VF. Such dynamicity of J waves, tachycardia-dependent augmentation, could be best explained by conduction delay: that is, depolarization abnormality.

ER and J waves

The ER pattern is a common ECG variant, originally characterized by J-point elevation manifested as terminal QRS slurring or notching associated with concave upward ST-segment elevation and prominent T waves in at least 2 contiguous leads. The ER pattern can be observed in people

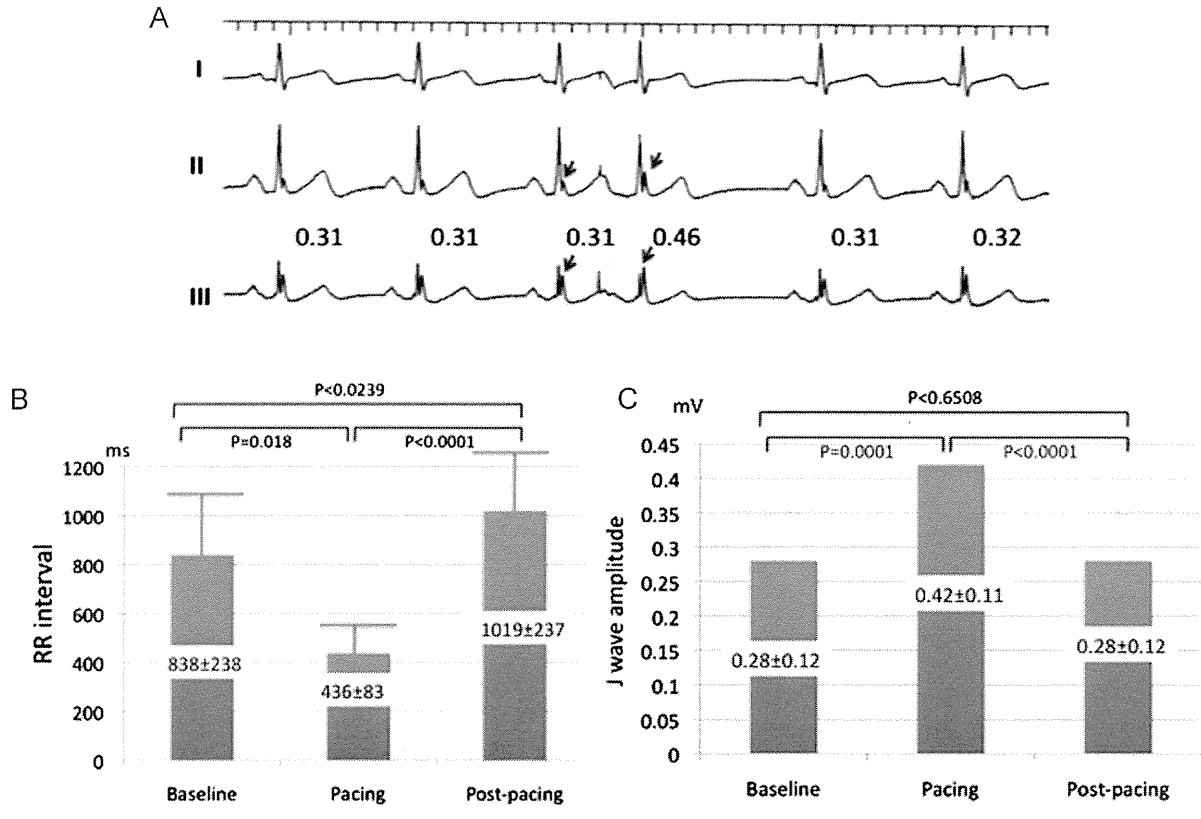

Figure 2 Changes in the J-wave amplitude during atrial stimulation in the EPS. **A:** Measurements of the J-wave amplitude during atrial premature stimulation. The J-wave amplitude (in mm) was augmented in the conducted beat of atrial premature stimulation. **B:** RR intervals were significantly altered during atrial stimulation. **C:** J-wave amplitude was augmented at short RR interval, but not altered at prolonged RR interval. EPS = electrophysiology study.

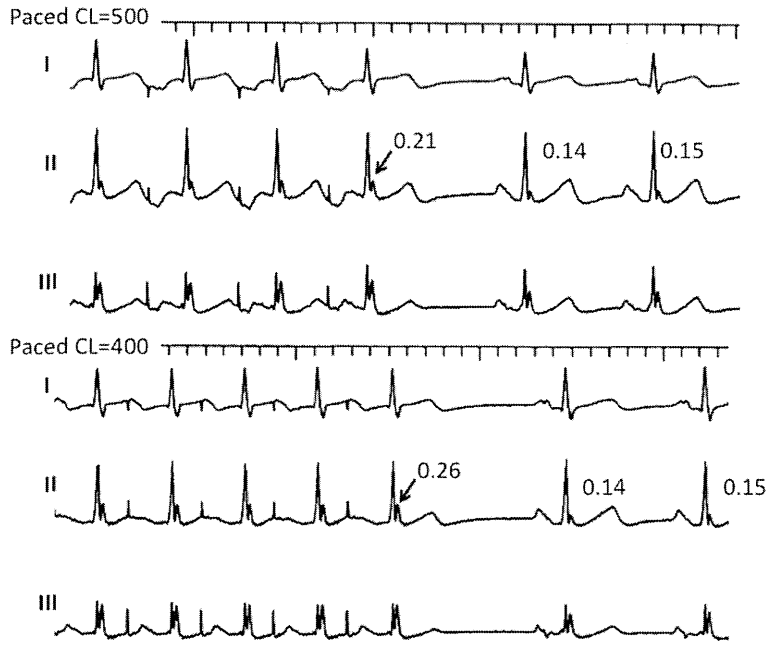

Figure 3 Rate-dependent augmentation of the J-wave amplitude by constant pacing (upper panel: at 500 ms; lower panel: at 400 ms of the paced cycle length). The J-wave amplitude (in mV) was not altered in the postpacing beat with the prolonged RR interval. CL = cycle length.

in population-based studies.[1–5] In contrast, J waves, notch or slur types, have been intimately related to idiopathic VF in recent published studies.[8–11] On the basis of the spatial distribution of J waves, a classification scheme using type 1 to type 4 terminology has been proposed for J waves.[12] This classification may need further confirmation, providing pathophysiological substrates across the 4 types.[27,28] In addition, the ST-segment morphology that follows the J wave was shown to be a higher risk of sudden cardiac death.[15,16]

Mechanistically, the J wave is considered an ECG manifestation of the transmural voltage gradient that is created by phase 1 notch in epicardial myocytes where transient outward currents (I_{to}) are dominantly expressed, resulting in phase 1 notch of the action potential.[12,17] J waves may be alternatively explained by depolarization abnormality,[18,19] though some characteristics of J waves in patients with idiopathic VF and in patients with Brugada syndrome can be best explained by ER.[17] We need more tools to differentiate J waves that result from multiple mechanisms.

Response of J waves to bradycardia/pause

Since 1992, we have analyzed bradycardia-dependent augmentation of J waves in patients with idiopathic VF,[8] and it was proved that bradycardia-dependent augmentation occurs in the beats after premature ventricular beats when the RR interval was prolonged.[9,11] Recently, it was confirmed that the J wave is augmented in the beats after APBs, premature ventricular beats, or during atrioventricular block when the RR interval was suddenly prolonged.[20] This bradycardia-dependent augmentation of J waves seems to be a striking characteristic that is specific to idiopathic VF, and mechanistically, such dynamicity would be best explained by pause-dependent augmentation of I_{to}: ER.[12,17] In the present study, significant bradycardia-dependent augmentation of J waves was not observed in the general patient population.

Response of J waves to tachycardia

J waves were attenuated by atrial pacing when applied to control electrical storms of VF in idiopathic VF.[29] In a patient with idiopathic VF, Nagase et al[30] found that the J-wave amplitude is attenuated by atrial pacing at a higher rate. These findings would be a reverse aspect of bradycardia-dependent (pause-dependent) augmentation of J waves.

In the present study, J waves, mainly notch type with or without slur in other leads, were augmented when the RR interval was shortened by conducted beats of the APB or atrial stimulation, showing tachycardia-dependent augmentation (Figures 1–3).

This finding is consistent with our previous study in which J waves of the patients with post–myocardial infarction were augmented in the conducted beats of the APB with shorter RR intervals.[31] Such J waves were not augmented in the beat next to the APB with prolonged RR intervals, which indicates absence of bradycardia-dependent augmentation.

In the present study, we treated notched J waves observed in individuals who were considered to be unrelated to VF or sudden cardiac death, supporting the observation that J waves can be benign. Tachycardia-dependent augmentation of J waves can be due to abnormal depolarization, that is, conduction delay, while J waves in patients with idiopathic VF were found to be attenuated by atrial pacing at higher rates.[28,29]

Clinical implications

Mechanistically, J waves can be due to ER, conduction delay, or an altered activation pattern over the ventricle. The J waves showing bradycardia-dependent augmentation must be I_{to}-mediated type, and I_{to} increases at a lower rate.[12,17] The attenuation of J waves by atrial pacing at a higher rate would also explain that J waves are I_{to}-mediated because I_{to} decreases at a higher rate.[17] Such J-wave dynamicity was exclusively observed in patients with idiopathic VF so far.[20,28,29]

By contrast, augmentation of J waves at a higher rate must be explained by conduction delay or phase 3 block. This can be observed in J waves of individuals in the population-based study or in the general patient population in which idiopathic VF or sudden cardiac death is unlikely. Augmentation at a lower rate was not evident in these individuals as is the case in the present study or as was the case with the control subjects in an earlier study.[20]

Therefore, the J wave must represent an ECG phenotype of diverse mechanisms, and an analysis of responses of J waves to varying RR intervals can delineate the characteristics of J waves resulting from different mechanisms and may be used for the risk stratification of J waves.

Study limitations

The limitation of the study is a small number of patients. However, the responses to tachycardia, to conducted APBs or to atrial stimulation during the EPS, and to bradycardia after APB or atrial stimulation were quite different from those observed in patients with idiopathic VF. In spite of the significantly different dynamicity reproduced by varying RR intervals, half of the patients, that is, those of a general population in the present study and those with idiopathic VF in the earlier study,[20] showed no rate-dependent change in the J-wave amplitude and the underlying mechanism remains to be elucidated. Furthermore, no difference in the ECG characteristics was observed between patients with and without augmentation of the J-wave amplitude at a higher or lower rate. Since patients with only slur-type ER were limited in this study, we need further study with a large number of subjects to elucidate the genesis of J waves.

Conclusion

J waves in the general patient population, mainly notch type and unrelated to idiopathic VF, were augmented in amplitude at shorter RR intervals, but not at prolonged RR intervals. Conduction delay must be the underlying mechanism of such tachycardia-induced augmentation of J waves. Analyzing J waves in the ECG with APB or during atrial stimulation in the EPS can be useful for delineating the mechanisms underlying J waves.

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CLINICAL PERSPECTIVES

J waves have been established as a marker of sudden cardiac death, but they might be observed in individuals of the general population with benign long-term outcomes. However, J waves can be an electrocardiographic (ECG) phenotype of multiple mechanisms, but the ECG and/or electrophysiological characteristics specific to each mechanism are still lacking. In this study, the responses of notched J waves to varying RR intervals were studied in ECG recordings of the patients of a general patient population. None had experienced aborted sudden cardiac death or had documented ventricular fibrillation (VF) or a family history of sudden cardiac death, suggesting a benign nature of J waves. The conducted atrial premature beats with short RR intervals showed augmentation of the J-wave amplitude—a tachycardia dependency—but no augmentation was observed in the following beats with prolonged RR intervals. The same phenomenon was confirmed in the conducted beats of atrial premature stimulation or rapid pacing of the atrium by the electrophysiology study. Mechanistically, such tachycardia-dependent augmentation can be explained by depolarization abnormality. Such tachycardia-dependent augmentation can represent depolarization abnormality rather than repolarization abnormality. The analysis of the responses of J waves to varying RR intervals would be useful for delineating the mechanisms underlying J waves and discriminate benign J waves from malignant ones.

Time-Domain T-Wave Alternans is Strongly Associated with a History of Ventricular Fibrillation in Patients with Brugada Syndrome

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Time-Domain T-Wave Alternans and Brugada Syndrome. *Aims:* **T-wave alternans (TWA) is an indicator of vulnerability to ventricular arrhythmias and is useful for predicting sudden cardiac death (SCD) in patients with various structural heart diseases. We evaluated whether high levels of time-domain TWA on ambulatory ECG (AECG) are associated with a history of ventricular fibrillation (VF) in Brugada syndrome (BrS) patients.**

Methods and Results: **We examined the associations among VF history, family history of SCD, spontaneous type 1 electrocardiogram (ECG), late potentials, VF induction by programmed electrical stimulation, and TWA in 45 BrS patients (44 males; mean age, 45 ± 15 years). TWA analyzed from 24-h AECG recordings using the modified moving average method was positive in 13 of 43 patients (30%). Patients with a history of VF had a significantly higher incidence of a positive TWA test (82% vs. 13%; P < 0.001) and spontaneous type 1 ECG (92% vs. 38%; P = 0.007) than those without VF history. Multivariate analysis indicated that positive TWA (OR 7.217; 95% CI 2.503–35.504; P = 0.002) and spontaneous type 1 ECG (OR 5.530; 95% CI 1.651–34.337; P = 0.020) were closely associated with VF history. Spontaneous type 1 ECG had high sensitivity (92%) but low specificity (63%). Positive TWA was a reliable marker with high sensitivity and specificity (82% and 88%, respectively).**

Conclusion: **Elevated time-domain TWA on AECG confirms arrhythmia risk in symptomatic BrS patients without the need for provocative stimuli.** *(J Cardiovasc Electrophysiol, Vol. 25, pp. 1021-1027, September 2014)*

ambulatory monitoring, Brugada syndrome, implantable cardioverter-defibrillator, sudden death, T-wave alternans, ventricular fibrillation

Introduction

Stratification of risk for ventricular fibrillation (VF) in patients with Brugada syndrome (BrS) remains challenging and controversial. VF history and syncope in BrS patients with a spontaneous type 1 electrocardiogram (ECG) at baseline are considered to be strong indicators of risk for cardiac arrhythmic events during follow-up.[1-4] A variety of possible BrS risk markers has emerged in previous retrospective and small study populations.

In the largest registry of BrS patients (FINGER registry) reported by Probst *et al.*, symptoms and a spontaneous type 1 ECG were predictors of arrhythmic events but inducibility of ventricular tachyarrhythmias (VT) and a family history of sudden cardiac death (SCD) were not.[5] Priori *et al.* also reported in the PRELUDE registry that VT/VF inducibility is unable to identify high-risk patients, whereas the presence of a spontaneous type 1 ECG, history of syncope, ventricular effective refractory period of <200 milliseconds, and QRS fragmentation are useful for identifying candidates for a prophylactic implantable cardioverter-defibrillator (ICD).[6] In those 2 large BrS registries, the role of induction of VF during electrophysiologic study (EPS) was downgraded.

The recent HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes reported the current status of risk stratification in BrS.[7] Importantly, most BrS patients are asymptomatic, and ICD treatment for primary prevention of VF is invasive and has recently been reported to be potentially hazardous.[8] Achieving a balance between harm and efficacy is therefore subtle and requires further investigation.

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Depolarization and repolarization abnormalities have been implicated in the pathogenesis of polymorphic ventricular arrhythmias in BrS.[9] T-wave alternans (TWA) is an ECG phenomenon of a beat-to-beat alternation in the shape, amplitude, or timing of the ST segment. Microvolt TWA has been identified as a predictor of malignant arrhythmias, SCD, and cardiovascular and total mortality in patients with ischemic cardiac events or heart failure,[10-12] but its positive predictive value varies depending on the population of patients studied.[12,13] In BrS patients, spectral analysis of microvolt TWA is of uncertain value.[14,15]

Recently, time-domain TWA using a 24-h ambulatory ECG (AECG) has been found capable of predicting SCD.[16,17] Because VF tends to occur during daily activity or sleep in BrS patients, we retrospectively investigated the association of VF history with microvolt TWA analyzed by the time-domain modified moving average (MMA) method from AECG recordings without provocative stimuli in patients with BrS.

Methods

Study Population

The study population was comprised of 45 consecutive Japanese BrS patients (44 males; mean age 45 ± 15 years), who were admitted to Hiroshima University Hospital between 2001 and 2011. The study was approved by the ethics review committee of our institution. Absence of structural heart disease was confirmed in these patients by noninvasive methods (physical examination, ECGs, exercise stress tests, echocardiography, and cardiac magnetic resonance imaging/computed tomography) and invasive studies (coronary angiography with acetylcholine and left ventricular cineangiography). Pilsicainide challenge was conducted in all patients to confirm the diagnoses of BrS. BrS was definitively diagnosed when type 1 ST elevation was observed in >1 right precordial lead (V_1–V_3) in the presence or absence of a sodium channel blocker in conjunction with 1 of the following criteria of the BrS consensus conference: documented VF, polymorphic VT, family history of SCD at <45 years of age, coved-type ECG in family members, inducibility of VT with EPS, syncope, or nocturnal agonal respirations.[18]

Baseline examinations revealed a spontaneous type 1 Brugada ECG pattern in 24 of 45 patients, who were enrolled in our study. A pharmacological challenge test via pilsicainide injection (1 mg/kg body weight/10 minute) was performed in the 21 remaining patients with a type 2 Brugada ECG pattern in the baseline examination to confirm BrS diagnosis. These patients were also enrolled in our study. The ECG pattern in all 21 patients converted to a type 1 (coved type) ECG pattern with ST-segment elevation of >0.2 mV in >1 right precordial lead. We retrospectively compared the clinical and electrophysiological parameters, including 12-lead ECG, inducibility of VF, late potentials (LPs), *SCN5A* mutations, and time-domain TWA in these 45 patients and investigated associations with their histories of VF.

Clinical, 12-Lead and Signal-Averaged Electrocardiographic and Electrophysiological Study Data

The following clinical data were collected: age, sex, history of VF, history of syncope, and family history of SCD (<45 years of age), or BrS diagnosis. A 12-lead ECG was utilized in all 45 BrS patients. A signal-averaged ECG was recorded and analyzed in 40 patients using the EP-705LP system (Fukuda Denshi, Tokyo, Japan). Three parameters were assessed using a computer algorithm: (1) total filtered QRS duration (f-QRS), (2) root-mean-square voltage of the terminal 40 milliseconds of the f-QRS complexes (RMS 40), and (3) duration of low-amplitude signals of <40 μV of the f-QRS complexes (LAS 40). LPs were identified when 2 of the following criteria were satisfied: f-QRS ≥114 milliseconds, RMS 40 <20 μV, or LAS 40 ≥38 milliseconds.[19]

After informed consent was obtained from the patients or for children from their families, EPS was performed in 36 BrS patients (7 with documented VF, 7 with syncope alone, and 22 asymptomatic patients). Three 5F quadripolar electrode catheters with 5-mm interelectrode spacing were positioned in the upper right atrium, His bundle region, and right ventricular apex. Programmed ventricular stimulation was performed in all patients with burst pacing (up to 230 bpm) and up to triple extra stimuli at 2 different drive cycle lengths (400 and 600 milliseconds) from the RVA and right ventricular outflow tract. The shortest coupling interval of the premature beats was limited to 200 milliseconds.

Measurement of Time-Domain TWA

ECG was recorded in all subjects using a 24-h AECG device. No antiarrhythmic drugs were administered to any patients during the AECG recordings. Time-domain TWA was assessed via the MMA method using the MARS PC system (GE Healthcare Inc., Milwaukee, WI, USA). This method has been described in detail.[20] In brief, the MMA algorithm separates odd and even beats into separate bins and creates median templates for both the odd and even complexes every 15 seconds. These templates are superimposed and the entire JT segment is analyzed for alternans. The difference between the odd and even median complexes at any point is defined as the TWA value. These templates of superimposed complexes are easily examined visually to verify the presence and magnitude of TWA. The MMA method has an adjustable update factor that permits control of the influence of new incoming complexes on the median templates; the update factor used for the acquisition of data was 1/8. TWA was analyzed in leads V_5 (CM5) and V_2 (NASA) from routine 24-h AECG recordings during daily activity. The maximal TWA voltage at a heart rate of <120 bpm was derived automatically in these 2 leads. Manual editing was performed to exclude beats affected by noise or artifact. The MARS PC system displayed the maximal magnitude of TWA on the screen, with superimposed complexes and the TWA trend over 24-h in both leads V_2 and V_5. The 15-second period when heart rate dropped rapidly was excluded in estimating peak TWA. We visually inspected all TWA events >40 μV and recorded TWA during each person's peak event. TWA was measured by 2 cardiologists unfamiliar with the patients' information. Lin's concordance result of the reproducibility of TWA was 0.79. None of the patients exhibited fever or was taking antiarrhythmic drugs including quinidine or β-blockers during the AECG recordings.

Sequence Analysis of SCN5A Using Genomic DNA

Peripheral vein blood samples were obtained from all patients, and the genomic DNA was extracted from leukocytes according to a 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 64 sense and antisense primers (approximately 300 bp each) of *SCN5A* with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Follow-Up

ICD implantation was performed in 37 BrS patients (13 with documented VF, 7 with syncope alone, and 17 asymptomatic patients). All subjects were routinely followed at the outpatient clinic of our hospital. Cardiac events were defined as SCD, aborted cardiac arrest, VF, or sustained VT documented by ICD or ECG recordings.

Statistical Analysis

Numeric values are expressed as means ± standard deviation. The chi-square test or Student's *t*-test analysis of variance was performed when appropriate to determine statistical differences. P values of <0.05 were considered statistically significant. Receiver–operator characteristic (ROC) curves were developed to estimate the optimal cutoff value of TWA. To assess the independent indicators for the occurrence of VF, a multivariable logistic regression analysis was performed. Odds ratios (OR) and confidence intervals (CI) were calculated by logistic regression test. The JMP ver. 10.0 J, statistical package (SAS Institute, Cary, NC, USA) was used for all statistical tests.

Results

Clinical Profile of the Patients

The characteristics of the 45 BrS patients are summarized in Table 1. A history of VF was documented in 13 patients. Seven patients (16%) had a history of syncopal episodes and 12 had family members with SCD <45 years of age or BrS diagnosis. In 19 of 36 patients (53%), malignant ventricular arrhythmias were inducible by ventricular electrical stimulation. LPs were present in 30 of 40 patients (75%). The mean peak value of TWA in the 43 patients was 48.5 ± 14.7 mV in lead V_2 and 47.7 ± 15.6 mV in lead V_5 (Table 1).

TABLE 1
Characteristics of the BrS Patients

Variables	
Gender	1 female, 44 males
Age (years)	45.1 ± 15.3
History of VF (n)	13 (29%)
History of syncope (n)	7 (16%)
Family history of SCD at age <45 years or BrS (n)	12 (29%)
Spontaneous type 1 ECG (n)	24 (53%)
Induction of VF by EPS (n)	19/36 (53%)
Positive LPs (n)	30/40 (75%)
TWA in lead V_2 (μV)	48.5 ± 14.7
TWA in lead V_5 (μV)	47.7 ± 15.6
SCN5A mutation (n)	1 (2%)

Results are presented as mean ± SD.
BrS = Brugada syndrome; ECG = electrocardiogram; EPS = electrophysiological study; LPs = late potentials; SCD = sudden cardiac death; TWA = T-wave alternans; VF = ventricular fibrillation.

No TWA results could be determined in 2 of 45 patients because of extrasystoles or atrial fibrillation and they were therefore excluded from the analysis. Figure 1 shows a representative case of TWA. The ECG rhythm strip in leads V_2 and V_5 (lower panel) and a high-resolution template of the QRS-aligned complexes (upper left panel) at the time of peak TWA (upper right panel; 24-h trend graph) are shown in a BrS patient with a history of VF. In this case, the peak value of TWA in lead V_2, 80 μV, indicating a high level of risk, was obtained at 22:30.[12] The peak value of TWA was achieved during the nighttime (20:00–9:00) in more than half of the BrS patients (28/43 patients, 65%).

Time-Domain TWA in Symptomatic and Asymptomatic BrS Patients

The performance of TWA for discriminating VF history in BrS patients was determined by ROC analyses (Fig. 2). TWA levels and incidence of positive tests were significantly greater in patients with a history of VF than in those without prior VF. The area under the ROC curve for TWA in lead V_2 was 0.821 and in lead V_5 was 0.645, indicating that TWA in lead V_2 had a higher degree of discrimination than that in lead V_5. The sensitivity and specificity of TWA were maximized at 60 μV in lead V_2 and 57 μV in lead V_5.

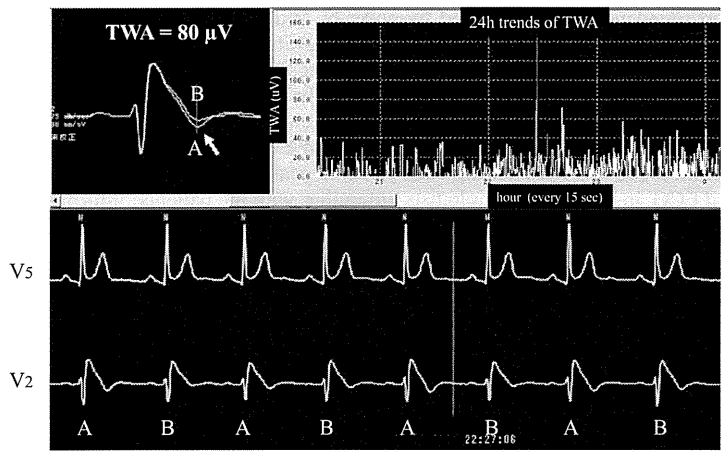

Figure 1. *Representative case of time-domain T-wave alternans (TWA) in lead V_2. The electrocardiogram rhythm strip in leads V_2 and V_5 (lower panel) and a high-resolution template of QRS-aligned complexes (upper left panel) at the time of peak TWA (upper right panel; 24-hour trend graph) in a Brugada syndrome patient with a history of ventricular fibrillation is presented. The template illustrates T-wave alternans (TWA) as a separation within the ST segment and T wave in beats A and B. TWA magnitude = 80 μV. For a high quality, full color version of this figure, please see Journal of Cardiovascular Electrophysiology's website: www.wileyonlinelibrary.com/journal/jce*

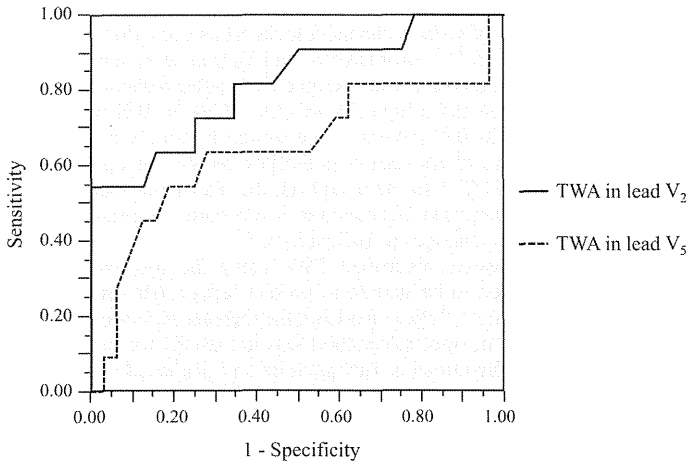

Figure 2. *Receiver–operator characteristics (ROC) analyses for discriminating the optimal cutoff value of the peak time-domain T-wave alternans (TWA). The area under the ROC curve for TWA in lead V_2 was 0.821 ($P = 0.007$) and in lead V_5 was 0.645 ($P = 0.303$), indicating that TWA in lead V_2 had better discrimination than in lead V_5. The sensitivity and specificity were maximized at 60 μV in lead V_2 and 57 μV in lead V_5, respectively.*

TABLE 2
Indicators of VF History in BrS Patients and Symptomatic/Asymptomatic BrS Using Univariate and Multivariate Analysis

	VF History (+) n = 13	VF History (−) n = 32	Univariate Analysis P Value	Multivariate Analysis OR	95%CI	P Value
Positive TWA in lead V_2 or V_5 (n)	9/11 (82%)	4 (13%)	<0.001	7.217	2.503 – 35.504	0.002
Positive TWA in lead V_2 (n)	6/11 (55%)	1 (3%)	0.002			
Positive TWA in lead V_5 (n)	5/11 (45%)	4 (13%)	0.029			
Age (<45 years), n	9 (69%)	14 (44%)	0.128			
Family history of SCD or BrS (n)	5 (42%)	7 (23%)	0.241			
History of syncope	2 (15%)	5 (16%)	0.984			
Spontaneous type1 ECG (n)	12 (92%)	12 (38%)	0.007	5.530	1.651 – 34.337	0.020
Positive LPs (n)	7/11 (64%)	23/29 (79%)	0.313			
Induction of VF by EPS (n)	3/7 (43%)	16/29 (55%)	0.560			
SCN5A mutation	0/12 (0 %)	1/30 (3%)	0.519			
	Symptomatic (+) n = 18	**Asymptomatic (−) n = 27**	**Univariate analysis P value**	**Multivariate analysis OR**	**95% CI**	**P value**
Positive TWA in lead V_2 or V_5 (n)	9/16 (56%)	4 (15%)	0.004	2.504	1.199–5.672	0.018
Positive TWA in lead V_2 (n)	6/16 (38%)	1 (4%)	0.004			
Positive TWA in lead V_5 (n)	5/11 (31%)	4 (15%)	0.200			
Age (<45 years), n	8 (44%)	14 (52%)	0.626			
Family history of SCD or BrS (n)	6 (33%)	0 (22%)	0.409			
Spontaneous type1 ECG, n	14 (78%)	10 (37%)	0.007	2.058	1.005–4.560	0.056
Positive LPs (n)	11/16 (69%)	19/24 (79%)	0.456			
Induction of VF by EPS (n)	7/12 (58%)	12/24 (50%)	0.637			
SCN5A mutation	0/17 (0%)	1/25 (4%)	0.409			

BrS = Brugada syndrome; ECG = electrocardiogram; EPS = electrophysiological study; LPs = late potentials; SCD = sudden cardiac death; TWA = T-wave alternans; VF = ventricular fibrillation.

These values were employed as the cutoff levels in this study. Table 2 shows indicators of VF history and symptomatic or asymptomatic BrS using univariate and multivariate analyses. Patients with a history of VF had a significantly higher incidence of positive TWA tests in lead V_2 or V_5 (82% vs. 13%; $P < 0.001$) and a spontaneous type 1 ECG (92% vs. 38%; $P = 0.007$) than those without VF history in univariate analysis. In multivariate analysis, positive TWA in lead V_2 or V_5 (OR 7.217; 95% CI 2.503–35.504; $P = 0.002$) and a spontaneous type 1 ECG (OR 5.530; 95% CI 1.651–34.337; $P = 0.020$) were closely associated with VF history. Positive TWA in leads V_2 or V_5 was associated with VF history with a sensitivity of 82% and specificity of 88%. A spontaneous type 1 ECG had a high sensitivity (92%) but low specificity (63%). In addition, positive TWA in lead V_2 or V_5 was the only independent indicator of symptomatic or asymptomatic BrS (OR 2.504; 95% CI 1.199–5.672; $P = 0.018$) in multivariate analysis. Of the 43 patients whose AECGs could be analyzed for TWA, 4 (9.3%) had high levels of TWA but no VF history or syncope. All of them showed positive TWA in V_5 but negative TWA in V_2. One of these patients also exhibited vulnerability based on family history of BrS. In another of these patients, VF induced by RV extrastimuli was difficult to terminate by cardioversion.

Follow-Up Study

We also examined the capacity of TWA to predict VF or syncope in a prospective manner. During the follow-up period (mean, 45.2 ± 37.9 months), cardiac events occurred in 5 of the 45 BrS patients (11%). Three of these 5 patients had a history of VF and experienced appropriate defibrillation

shocks at 1, 3, or 30 months after the start of the study. The other 2 patients had a history of syncope before the ICD implantation and experienced appropriate defibrillation shocks at 8 or 36 months after the start of the study. In the 43 patients in whom TWA could be analyzed, the incidence of subsequent ICD discharges was significantly higher in the patients with a positive TWA test than in the patients with a negative TWA test (4 of 13 patients [31%] vs. 1 of 30 patients [3%]; $P = 0.01$). The single patient with ICD discharge on follow-up but a negative TWA test was a 37-year-old male with a history of syncope and a family history of SCD. He exhibited spontaneous type 1 Brugada ECG in V_1 and type 2 Brugada ECG in V_2. He received an ICD because of syncope, family history of SCD and VF induction during EPS. During EPS, VF was induced by right ventricular stimulation but sinus node and atrioventricular node function were normal. LP test results were positive. Spontaneous VF occurred and appropriate shock was discharged 3 times at 8–10 months after ICD implantation.

Discussion

The most important finding of this study was that time-domain TWA and a spontaneous type 1 ECG were found to be associated with previous VF events in BrS patients. Positive TWA was more reliable, given its high degree of sensitivity and specificity.

Risk stratification of BrS remains controversial regardless of the variable factors reported to be useful for predicting worse outcomes in BrS patients.[7] Most studies agree that surviving VF and having a history of syncopal episodes in patients with a spontaneous type 1 ECG indicates a high risk for cardiac arrhythmic events.[1,2,5] In this study, a spontaneous type 1 ECG finding was an indicator of VF in the BrS patients, consistent with past reports. However, the ST configuration in the right precordial leads is known to show day-to-day variations, and discrimination of the type of Brugada ECG is partially dependent on each investigator.

Nademanee *et al.*[21] demonstrated clearly that a depolarization abnormality is involved in the pathogenesis of VF in BrS. They showed that fragmentation in the RVOT epicardium exists in BrS patients with a history of VF. By eliminating these fractionated electrograms by catheter ablation, VF events as well as ST elevation in the right precordial leads did not recur in 8 out of 9 patients during follow-up. Previous studies identified parameters of depolarization abnormalities that were useful for risk stratification in BrS patients.[22,23] In this study, positive LPs were evident in most BrS patients, but no difference in the rate of positive LPs was observed between BrS patients with or without a VF history.

Transmural dispersion of repolarization within the ventricular myocardium has also been suggested as an underlying mechanism of arrhythmogenesis in BrS.[24] TWA indicates repolarization instability and predicts the occurrence of serious ventricular arrhythmias leading to SCD. Regarding the source of TWA, an unstable intraepicardial and transmural dispersion of action potentials may induce fatal arrhythmias. In most patient groups, prediction of arrhythmia by macroscopic TWA is limited. Microvolt TWA is reported to be a useful predictor of fatal arrhythmias or SCD in patients with structural heart diseases including myocardial infarctions[11-14] and is a reliable parameter for arrhythmia risk stratification.[25]

The appearance of macroscopic TWA after the administration of sodium channel blockers has been reported in cases with BrS.[25-29] Morita *et al.* and Tada *et al.* reported an association between macroscopic TWA after pilsicainide administration and a high risk of clinical VF in BrS patients.[25,30] TWA in BrS patients is generated by an alternating phase 2 dome of the action potentials within the epicardium of the RVOT.[31] In other reports, the alternating occurrence of blocked phase 2 conduction was reported to produce similar T-wave changes in BrS patients.[32]

However, microvolt TWA using the spectral method is reported to be unable to predict lethal arrhythmias in BrS patients.[14,15] We considered the reasons why microvolt TWA using the spectral method was not useful for predicting fatal arrhythmias in BrS patients and proposed the following possibilities. Spectral analysis of TWA requires a specialized protocol, which mandates the elevation and stabilization of heart rate (100–110 beats/minute) for several minutes by exercise or pacing. In BrS patients, pacing at this heart rate has been reported to decrease TWA level.[29] Time-domain TWA can be analyzed from routine 24-hour AECG recordings without the need for a provocative stimulus. BrS patients are characterized by the presence of a circadian variation in their ECGs, depending on the sympathetic and parasympathetic balance, and are more likely to develop fatal arrhythmic events in a situation with parasympathetic predominance. Therefore, time-domain TWA has an advantage over spectral analysis in BrS patients as TWA occurs during bradycardia or sleep, periods of parasympathetic nerve predominance. This theory is consistent with our study results, as the peak TWA levels were achieved during the night in most of the BrS patients. These findings concur with the observation that development of VF is more common at night than daytime in BrS patients.

In 4 patients, high levels of TWA indicated latent arrhythmia risk, as neither VF nor syncope had been documented. All of them showed positive TWA in V_5 but negative TWA in V_2. One patient with negative TWA test in both V_5 and V_2 experienced a VF event and appropriate shock. The possibility exists that the ICD contributed to provoking the arrhythmia. Many ICD shocks in BrS patients are not appropriate.[8] Moreover, provocation of VF by ICDs has been reported, suggesting that patients should be retested following ICD implantation.

Considerable evidence suggests that TWA is a regionally specific phenomenon.[33] For example, in patients with right coronary artery lesions, TWA is most prominent in the right precordial leads, whereas patients with lesions in the left coronary circulation exhibit elevated TWA in the left precordial leads.[34] In most BrS patients, the ECG abnormalities are manifest principally in the right precordial leads, reflecting derangements in ionic currents including a gain of function of I_{to} attributable to genetic enhancement of this current within the right ventricle.[34] These factors are likely to account for the elevated and more predictive changes in TWA in lead V_2 than in V_5.

Several studies have reported that a conduction delay is needed to explain the susceptibility to VF in BrS patients.[22,23] Combined assessment of TWA and LPs was reported to generate a high positivepredictive value for arrhythmic events after acute myocardial infarction.[35] In another report, concurrent monitoring of depolarization and repolarization heterogeneity in conjunction with TWA could potentially provide

an early warning of impending nonsustained VT.[36] TWA has been reported as a reliable predictor of SCD in patients with organic diseases, due to conduction block, prolonged effective refractory period, or abnormal Ca cycling. Discordant alternans arises dynamically from action potential duration and conduction velocity restitution properties and markedly increases dispersion of refractoriness.[37] Thus, TWA indicates both repolarization and depolarization abnormalities.

Currently, the following noninvasive therapeutic interventions are recommended for all patients diagnosed with BrS: avoidance of drugs that may induce or aggravate ST elevation in the right precordial leads, avoidance of an excessive alcohol intake, and immediate treatment of a fever with antipyretic drugs. Quinidine has been shown to suppress spontaneous ventricular arrhythmias in clinical settings.[7,38,39] However, the only proven effective therapeutic strategy for the prevention of SCD in BrS patients is ICD implantation. Most BrS patients are asymptomatic and an ICD implantation as a primary preventative measure is invasive and recently reported to be potentially hazardous.[8] Thus, a comprehensive assessment of the situation and risk stratification of VF in BrS patients is very important. Recently, BrS patients older than 70 years likely were reported to have a lower risk but more severe conduction block than younger patients. They also reported that the diagnosis of BrS in the elderly was important because of diagnosis in the family members of elderly patients.[40]

Study Limitations

The utility of TWA on AECG to predict VF or syncope in BrS patients requires validation in a larger prospective study. It may be that a lower cut point would be more suitable to optimize negative predictivity. The number of BrS patients analyzed for TWA and the duration of follow-up were limited in this study. The present findings and prognosis of the patients analyzed for time-domain TWA must be validated in a larger prospective study with a longer follow-up period.

Conclusions

To the best of our knowledge, this is the first report of an association of high levels of microvolt TWA with VF history in BrS patients. We found time-domain analysis to be appropriate for analyzing this association. These data suggest that analysis of microvolt TWA on AECG without provocative stimuli can contribute to risk stratification in BrS patients and may allow screening of high-risk patients, including those with a family history of SCD or BrS diagnosis; spontaneous type I ECGs; survivors of VT/VF, documented sustained VT, or syncope likely due to ventricular arrhythmia, for risk of VF early in their medical history prior to the lethal arrhythmia. It would also be valuable to determine whether following ICD implantation, TWA may be capable of guiding medical therapy or other interventions to reduce the incidence of arrhythmia and attendant effects of ICD discharge in high-risk BrS patients.

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