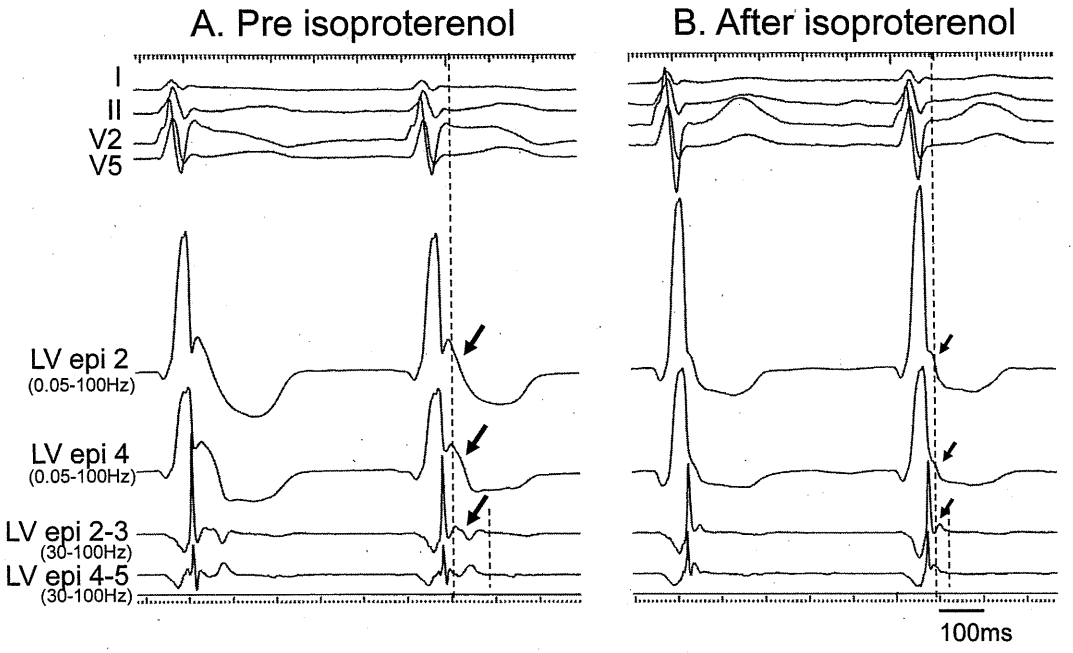

図８　心内心電図　A：Isoproterenol 投与前，B：Isoproterenol 投与後
Isoproterenol を単回静注すると，J 波の著明な減高（LV epi 2, 4），out of QRS potential の短縮（LV epi 2-3, 4-5）する所見を認めた。

分極相異常を反映している可能性が高いと報告している[7]。また Abe らは，特発性心室細動の症例において体表面心電図の J 波と加算平均心電図の LP について日内変動とその関連性を検討すると，J 波の増高とともに LP が増大し，さらに J 波の増高が副交感神経の亢進とも関連していることを報告している[8]。この結果は J 波が LP と密接に関連し，そして J 波が再分極相異常を反映していることを示唆していると考えられる。

我々の経験した症例での左室心外膜側の J 波，および J 波と同時相で記録される out of QRS potential も pilsicainide にて増高・延長し，心房ペーシング，isoproterenol にて減高・短縮する性質を示すことから，脱分極異常よりもむしろ再分極異常を示唆する所見と考えられた。また加算平均心電図における LP も quinidine 投与にて減少し，Aizawa らの報告と同様に再分極相異常を反映していると考えられた。そして左室心外膜側の J 波および out of QRS potential は記録方法が異なるが同一の成分を記録している可能性が示唆された。

Shinohara らは，J 波を認める特発性心室細動患者の 1 例において，薬物およびペーシングによる体表面心電図 J 波の変化を詳細に検討し報告している[9]。この報告でもやはり心房ペーシングと isoproterenol で J 波が減高しているが，disopyramide でも減高し，procainamide では不変であったとしている。我々は Na チャネル遮断薬として pilsicainide を使用したが，体表面心電図 J 波は ST 上昇の影響により不明瞭となるも心外膜側 J 波は明らかに増高していた。純粋な Na チャネル遮断薬である pilsicainide との相違も一因と考えられるが，左室心外膜側にて直接記録することにより J 波の変化が明瞭に記録されたためと予想された。

以前より Brugada 症候群では再分極相異常と脱分極相異常，両者の存在が数多く報告されて

きた。脱分極相異常を示唆する所見の1つとして加算平均心電図にて記録されるLPの存在があるが[10]，今回の検討では再分極相異常が加算平均心電図でのLPとして記録される可能性を示唆している。ただしこの1例のみの検討であり，今後さらなる患者群での検討が必要と考えられる。また再分極相異常の存在とともに脱分極相異常の合併も心室細動自然発作の出現には重要とされ，Brugada症候群には両者が併存し密接に関連している可能性も予想される[11]。

今回我々が行った心外膜側電位の記録は左室側壁のみで，他の部位での検討は行えていない。体表面心電図のJ波が下壁および側壁誘導にて広範に記録されたことから，左室側壁心外膜側の記録のみで十分にJ波が記録可能であったと考えられた。また左室心外膜側にて直接電位を記録することが可能であったため，J波の変化がより明瞭に記録されたと考えられた。そして対側の心内膜側ではJ波の成分は記録されず，J波を形成する要因がより心外膜側に存在することが示唆された。J波の出現している領域や活動電位持続時間のばらつきを検討するなどの目的から，左室の側壁のみならず前壁や後壁での記録も同時に行うことができれば，さらにJ wave syndromeの詳細が明らかにされる可能性も高い。

BoineauらはJ波が，乳頭筋群を含む左室心内膜側構造物とPurkinje線維走行の複雑性により生じる脱分極完了のばらつきを原因として形成されると報告している[12, 13]。ただし自律神経や薬剤に対する変化やVF発生の機序に関しては十分に検討されていない。今回の我々の検討は左室側壁領域の一部のみであり，また左室心内膜側の構造に関しては検討できておらず，今後Boineauの説に関しても研究を進めていきたい。

結　語

Brugada症候群患者において，再分極異常の性質を示す左室心外膜側J波がout of QRS potentialとしても記録され，J wave syndromeの成因を考えるうえで興味深い症例と考えられ報告した。

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討論

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中里祐二（順天堂大学浦安病院循環器内科）

土谷（EP Expert Doctors-Team Tsuchiya）：非常に面白い発表だと思います。Out of QRS potential の周波数は 0.01 Hz から 100 Hz でしたか。
演者（田中）：0.05 Hz から 100 Hz です。
土谷：0.05 Hz から 100 Hz ですか。そのハイパスを切り上げていくと，ウエーブが消える時相がわかると思うのです。それによって周波数がわかると思いますが，だいたい，out of QRS potential の周波数は何 Hz ぐらいですか。というのが，depolarization であれば，心筋の伝導の周波数は 10 Hz とか 20 Hz ぐらいです。そのことによって，実際 depolarization の frequency なのか，repolarization phase と関係した frequency なのか，周波数を見ることで見当がつくのではないかなと思ってお伺いしています。
演者：バイポーラで記録した out of QRS potential は，30 Hz から 100 Hz で記録しています。
土谷：いやいや先生，バイポーラでは駄目で，ユニポーラで検討する必要があると思います。
演者：Out of QRS potential はバイポーラとユニポーラで記録し検討しています。ユニポーラでいわゆる J 波が著明に記録されました。
土谷：J 波というのは，out of QRS potential とは違うものですか。LV epi 2 とあるその後ろの potential ですが，そこの部分は……。
演者：これがいわゆる J 波が見えたというユニポーラでの記録で，その短縮とか延長を詳細に検討したのは，とくにバイポーラで測定しました。
土谷：バイポーラは 2 つの電位差を見ているので，相当な情報がオミットされてしまいます。ですから，ユニポーラでその周波数を見るのが重要だと思って質問させていただきました。
演者：普段我々が見ている加算平均心電図も一応バイポーラの記録を周波数を変えてみているので，out of QRS を論じるときには，バイポーラで見たのですが。
土谷：それはそうですが。ですから，いま話しているのは伝導速度の問題です。I ポイントで伝導速度を見るときには，バイポーラはすでにこの時点でユニポーラの微分と一緒です。プラス，バイポーラの 30 Hz のハイパスを入れると，もう 1 回微分になるのです。ですから，ユニポーラの 2 回微分したものが，バイポーラの 30 Hz のハイパスを入れた後と同じ現象になってしまうので，結局加速度を見ているということです。ですから，I ポイントで伝導性を見るためにはユニポーラの frequency を検討することが重要だと，私は言いたかったわけです。そこでユニ

ポーラの frequency を見て，通常の場合は心筋の伝導速度は 10 Hz，20 Hz のレベルですから，そのユニポーラの frequency の周波数を見ることによって伝導遅延なのか，それとも repolarization の異常なのかが，ある程度推定がつくのではないかと考えて質問させていただきました。

藤木（静岡赤十字病院）：先生の最後の結論で，pilsicainide で J 波が増強するとおっしゃいましたね。

演者：はい。

藤木：Pilsicainide で J 波が増強しているのは，epicardial 電極の J 波。体表面心電図では消えてしまいますね。

演者：体表面心電図の J 波は ST 上昇でマスクされている印象です。

藤木：それはすごく大事なことではないかと思います。というのは，epicardial の電位はみんな普段見ていないので，心室細動例で実際に J 波があっても，12 誘導心電図で J 波として認識できないタイプがあるかもしれない。そういう意味です。

演者：その可能性はあると思います。

藤木：そういう場合は，pilsicainide を投与する具合によって，あるポイントでは体表面でも J 波が高くなるとか，あるポイントを過ぎると消えてしまう。そういう心表面での J 波の出来事を，体表面心電図で何とか detect する方法は考えておられますか。

演者：今のところは，そこまでは検討できていませんが，心内の電位で J 波が局在している症例は，可能性はあると思います。

山分（横浜南共済病院）：今の質問と少し関連しているのですが，pilsicainide を使って体表面での J 波が消失しているにもかかわらず，epicardial の J 波が増強しているのは，transmural voltage gradient の増強とは関係なく，QRS が延長したのは伝導遅延のみが関係していると考えてよろしいですか。

演者：J 波が体表面で見えなくなっているだけで，側壁では悪くなっていると考えています。確かに pilsicainide を静注すると伝導遅延で悪くなる可能性ももちろんあると思いますが，今回の検討では，ペーシングしたときの反応等から，再分極相の変化もあることがわかったということです。

山分：この方は electrical storm を起こしたということですが，先生のご経験では，Brugada 症候群で右側胸部以外の誘導で J 波が出ている症例は，VF が出やすいとか予後が悪いとかいう傾向はあるのでしょうか。

演者：鎌倉先生らのグループがそういう発表をされていまして，当施設でも何例か側壁の心外膜側の電位をとっており，J 波が記録できる症例もいます。

共同演者（永瀬）：ほとんど今の説明でいいと思います。土谷先生の話に関しては，確かに言われるとおりなので，帰ってフィルターについては検討したいと思います。後は，左室心外膜での記録は，直接心臓の近くで記録するので，より局所的な情報が明瞭になると思われます。体表面心電図は非常に遠くから記録するので，局所の変化がある程度マスクされて薄まってしまい，全体的には ST 上昇に見えてはいるのですが，局所的な電位は，こちらのほうが正しいものを見ているのではないかと思います。局所の J 波が大きくなったことを，体表面で検討するのは，難しいケースがあるだろうと考えています。

座長（鎌倉）：コメントですが，私は実は Brugada の方の心磁図を記録しているのですが，Brugada の一部の方は，最終興奮部位が右室流出路ではなく左室流出路にきます。おそらくそのような症例では，LV の心外膜側で伝導遅延として記録できるのではないかと思います。ところでこの症例は，pilsicainide 負荷したときに，高位肋間の第 2 肋間や，第 3 肋間で ST 波形が coved 型になっていませんでしたか。

演者：2 肋間上も coved 型になっています。

座長（鎌倉）：体表面上では I，aVl 誘導は，2 肋間，1 肋間上の V1–V3 誘導に位置的に近いのですが，I，aVl 誘導の ST 波や J 波が pilsi-

cainideで増強する場合は高位肋間でのV1–V3誘導のST上昇が生じていることが少なくないのです。逆に，II，III，aVfでは，J波が小さくなったのではないでしょうか。このII，aVfではJ波がありますよね。そのへんはどうですか。

演者：IIとaVfも，STが上昇して見えづらくなったのかなと思っています。

座長（鎌倉）：STもJ波も小さくなったかもしれませんね。いずれにしてもII，III，aVf誘導で早期再分極があるものと，Brugada症候群においてI，aVl誘導で早期再分極を合併するものが同じかどうかは，さらに検討する必要があるかと思います。

共同演者（永瀬）：あと，Brugadaの場合，おそらくpilsicainideを静注すると右室側の伝導遅延やST上昇が増悪したり，軸が変わったりすることにより，J波がマスクされるケースがあるので，局所にJ波があったとしても，見えなくなるケースがあるのではないかと思っています。

座長（鎌倉）：どうもありがとうございました。

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EDITORIAL

ECG Marker of High-Risk in Asymptomatic Patients With Brugada Syndrome

Kengo F. Kusano, MD

Brugada syndrome (BS) is a distinct form of idiopathic ventricular fibrillation (VF) characterized by a unique ECG pattern consisting of ST elevation in the anterior precordial leads with/without right bundle branch block-like morphology.[1] It is generally accepted that patients with the type 1 ECG pattern (coved type) and with ventricular tachyarrhythmias (symptomatic BS patients) must receive an implantable cardioverter defibrillator to prevent a second VF attack.[2] However, in those without syncope, family history or documented VF (asymptomatic BS patients), the best strategy is controversial because the prevalence of Brugada-type ECG change by daily medical check-ups has been reported to be approximately 0.1–1.0% in healthy subjects, but their prognosis is good compared with that of symptomatic patients.[3] However, some asymptomatic BS patients occasionally become symptomatic and sudden cardiac death can occur with the first VF attack. Therefore, a marker that can differentiate high-risk asymptomatic patients from low-risk asymptomatic patients is needed. To date, several invasive and noninvasive parameters have been proposed for identification of patients at risk of VF, including spontaneous type 1 ST elevation,[3] characteristics of the S wave,[4] presence of late potentials,[5] coexisting atrial fibrillation,[6] augmented ST elevation after exercise,[7] fragmented QRS wave,[8] early repolarization pattern in the inferior/lateral leads,[3] third intercostal ECG[9,10] and inducibility of VF using programmed electrical stimulation, but the usefulness of these indexes remains controversial.

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The results of a study by Miyamoto et al published in the Journal[11] indicate the interesting possibility of using new ECG criteria for identifying high-risk asymptomatic BS patients. They performed computer-based ECG analysis for more than 100,000 patients and detected spontaneous type 1 ECG in 185 (0.18%) of the patients. Detailed examination was performed in 31 of these 185 patients, and 16 patients were diagnosed as high-risk BS (syncope: 87.5%, aborted

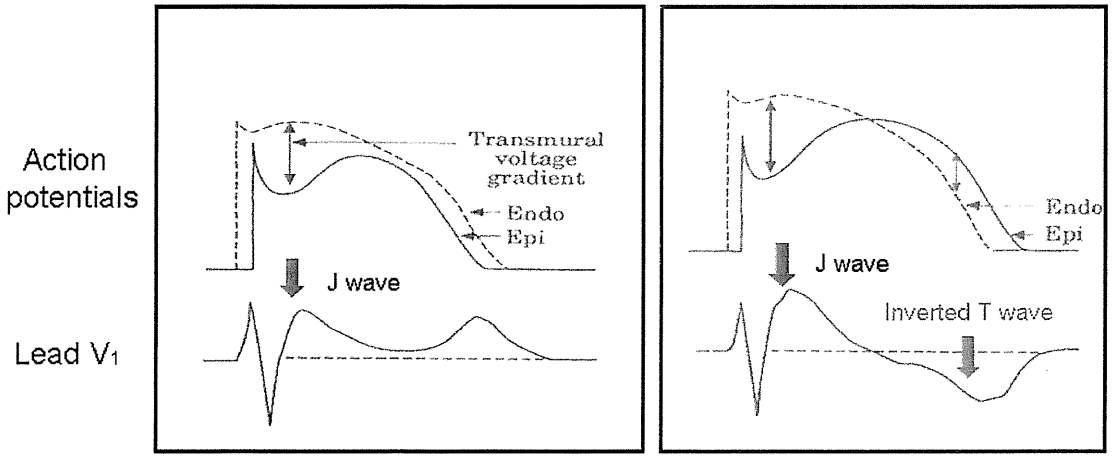

Figure. Mechanism of ST-T abnormality in Brugada syndrome. Note that a marked accentuation of the action potential notch and prolongation of repolarization in the right ventricular epicardial (Epi) but not endocardial (Endo) cells results in T-wave inversion in surface ECG lead V_1. (Modified from *Eur Heart J* 2001; **22:** 356–363.)

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sudden cardiac death/documented VF: 68.8%). They concluded that a more negative T wave in lead V_1 (<−1.05 μV), longer PQ interval (>170 ms) and family history of sudden death are associated with life-threatening events.

ECG patterns associated with BS have been classified into 3 types.[12,13] Type 1 ECG is characterized by ≥2-mm J-point elevation, coved-type ST-T segment elevation, and inverted T-wave in leads V_1 and V_2. Type 2 ECG is characterized by ≥2-mm J-point elevation, ≥1-mm ST-segment elevation, saddleback ST-T segment, and a positive or biphasic T-wave. Type 3 ECG is the same as type 2 except that the ST-segment elevation is <1 mm. Among the 3 types, only the type 1 ECG is diagnostic of BS. An experimental study[13] has revealed that the mechanism of ST-T abnormality in the right precordial leads is an outward shift of ionic currents during early repolarization (transmural voltage gradient) causing a marked accentuation of the action potential notch and prolongation of repolarization in the right ventricular epicardial but not endocardial cells (**Figure**). This discriminating electrophysiologic mechanism is thought to be associated with ST-segment elevation (J wave) and T-wave inversion in this syndrome. In a human study using the activation recovery interval (ARI) method, Nagase et al demonstrated that the inverted T wave associated with the type 1 ECG is due to a preferential epicardial ARI prolongation secondary to accentuation of the action potential notch in the right ventricular outflow tract,[14] consistent with the results of the experimental study. The data obtained in previous studies support the results of the present study. However, it has also been reported that this ECG pattern is very dynamic and often concealed during follow-up, and repeated ECG recordings should therefore be performed so as not to miss high-risk BS patients.

In BS, depolarization abnormalities, including prolongation of the P wave, PQ interval and QRS width, are sometimes observed, particularly in patients having severe forms of the gene mutation (SCN5A). Therefore, it would not be surprising that prolongation of the PQ interval is a risk marker in this syndrome, so we also need a careful attention to this marker.

In conclusion, although the number of high-risk patients was small, the follow-up period was short and further study is needed to reach a definitive conclusion, Miyamoto et al have provided important clinical evidence of a simple ECG marker for differentiating high-risk patients with BS-type ECG.

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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

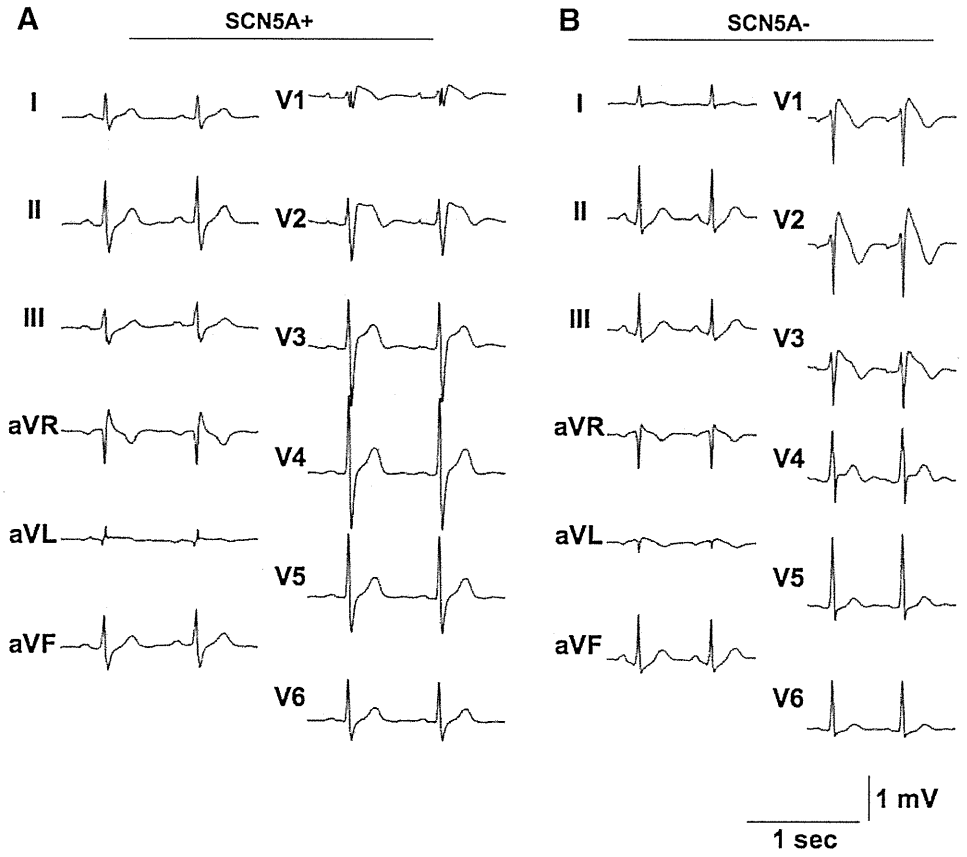

Fig. 1. Representative ECGs of patients with or without SCN5A mutation. (A) ECG of a patient with SCN5A mutation (SCN5A+), R282H (47 years old, male). (B) ECG of a patient without SCN5A mutation (SCN5A−) (42 years old, male).

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.

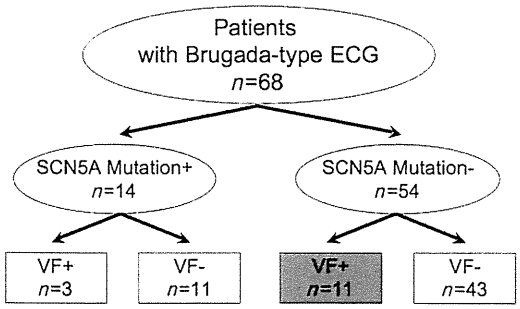

Fig. 2. Study profile.

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−

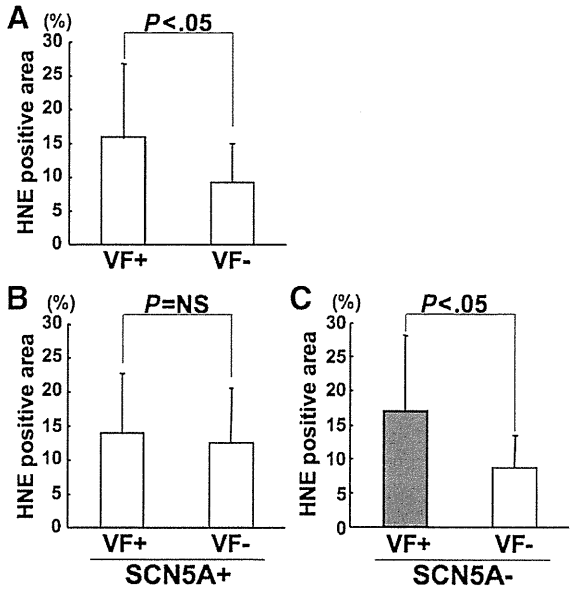

Fig. 3. HNE-modified protein-positive area. (A) HNE-modified protein-positive area in patients with spontaneous VF (VF+ group) vs. without (VF− group). (B) HNE-modified protein-positive area in patients with SCN5A mutation (SCN5A+). (C) HNE-modified protein-positive area in patients without SCN5A mutation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

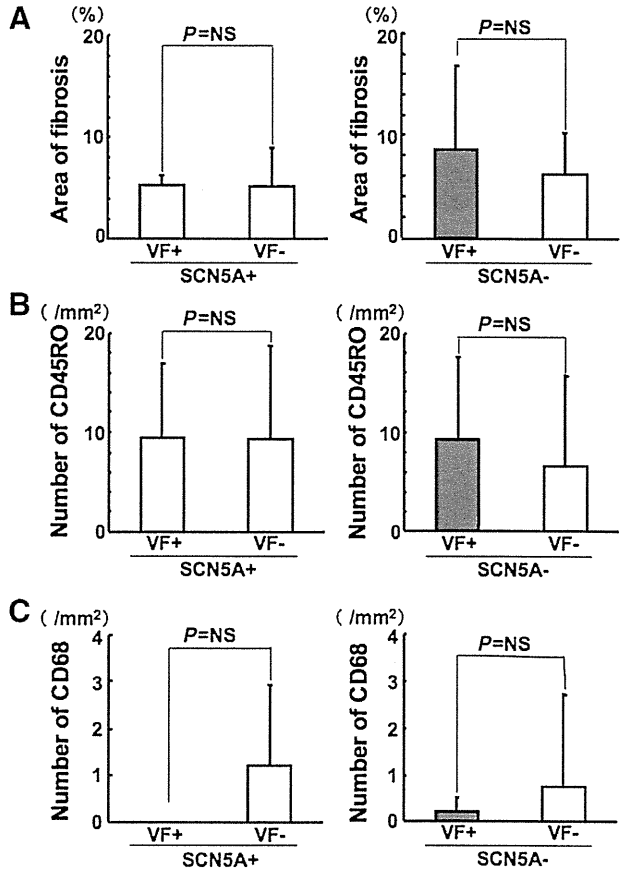

Fig. 4. Histological and immunohistochemical analyses. (A) Areas of fibrosis in SCN5A+ and SCN5A− patients in the VF+ and VF− groups. (B) Numbers of CD45RO-positive cells in SCN5A+ and SCN5A− patients in the VF+ and VF− groups. (C) Numbers of CD68-positive cells. Data are expressed as means±S.D.

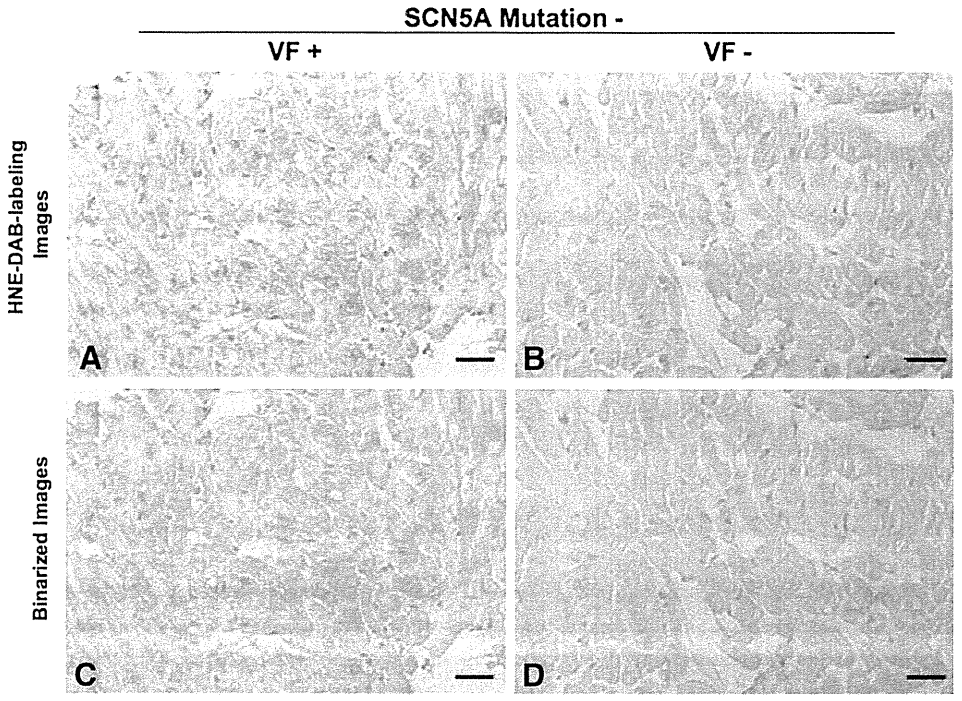

Fig. 5. Representative figures of HNE-modified protein staining. Representative immunostainings (brown) for HNE-modified protein by diaminobenzidine (DAB) (A and B) and binarized images (green) using WinROOF Image software (C and D) in the myocardium from a patient without SCN5A mutation and with spontaneous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

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BACKGROUND Patients with Brugada syndrome (BS) often have spontaneous changes in their electrocardiogram (ECG).

OBJECTIVE To evaluate the significance of ECG alterations, we investigated the relationships between the ECG and the occurrence of ventricular fibrillation (VF) in both patients and an experimental model of BS.

METHODS In study 1, we evaluated ECG alterations in BS patients with (VF+, n = 33) and without (VF−, n = 41) spontaneous VF. We defined type 0 ECG as coved-type ST elevation without a negative T wave, which represents the existence of loss-of-dome (LOD) type action potentials (APs). In study 2, we optically mapped epicardial APs and recorded transmural ECGs in 34 canine right ventricular tissues with a drug-induced BS model by a combination of pinacidil and pilsicainide.

RESULTS In study 1, changes in ST level ≥0.2 mV were more frequent in the VF+ group than in the VF− group ($P < .01$). Spontaneous ECG alterations and appearances of types 1 and 0 ECGs were more frequent in the VF+ group than in the VF− group ($P < .01$). In study 2, BS model with spike-and-dome (SAD) epicardial APs exhibited type 1 ECG. Deepening of the phase 1 notch of the APs induced heterogeneous conversion of the APs (SAD→LOD) and resulted in ECG conversion from type 1 to type 0. Significant AP heterogeneity often appeared during AP alterations and initiated phase 2 reentry. Tissues having ventricular tachycardia (VT; n = 20) had more frequent alterations in APs and ECG than in tissues without VT (n = 14; $P < .01$).

CONCLUSION ECG alterations, especially conversion between types 0 and 1, are associated with significant AP heterogeneity that can initiate VF in BS.

KEYWORDS: Sudden death; Electrocardiography; Ventricular fibrillation; Brugada syndrome

ABBREVIATIONS **AP** = action potential; **APD** = action potential duration; **BS** = Brugada syndrome; **CL** = cycle length; **ECG** = electrocardiogram; **f-QRS** = fragmented QRS; **ICD** = implantable cardioverter-defibrillator; **LOD** = loss-of-dome; **LP** = late potential; **LV** = left ventricle; **RBBB** = right bundle branch block; **RV** = right ventricle; **SAD** = spike-and-dome; **VF** = ventricular fibrillation; **VT** = ventricular tachycardia

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Introduction

Brugada syndrome (BS) is characterized by ST elevation in the right precordial leads, episodes of ventricular fibrillation (VF),[1] and sudden cardiac death in patients generally 30–50 years old.[2,3] Type 1 electrocardiogram (ECG), with ≥0.2 mV coved-type ST elevation followed by a negative T wave, as defined by the Report of the Second Consensus Conference of Brugada Syndrome, is the only ECG type diagnostic of BS.[4] Although detection of type 1 ECG is important to predict patient prognosis,[2,5] ECGs in patients with BS often vary spontaneously between normal and type 1, making it difficult to assess the risk of VF.[3,4,6] Repetitive recordings are necessary to detect and verify the presence of type 1 ECG.[6,7] ECG alterations are also associated with frequent implantable cardioverter-defibrillator (ICD) discharges.[7]

Experimental studies have shown that type 1 ECG reflects spike-and-dome (SAD) type action potentials (APs)[8–11] and that a shallow or absent negative T wave reflects loss-of-dome (LOD) type AP in the right ventricular (RV) epicardium.[9–11] Although some clinical studies reported coved ST elevation without negative T waves in the ECGs of patients with BS,[10] the significance of that particular ECG variation and its prediction of VF risk are unknown.

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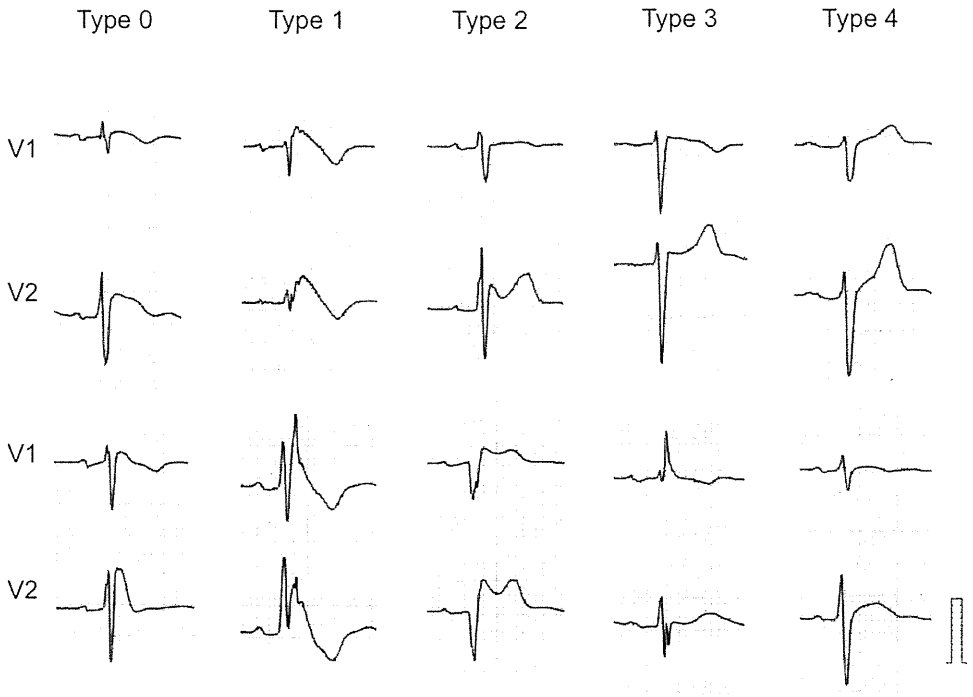

Figure 1 Classification of ECG types. Two typical ECGs are shown in each type. Type 0 is defined as an ECG with coved-type ST elevation ≥2 mm and a shallow negative T wave (≤1 mm) or having no negative T wave. Type 1–3 ECGs are defined by the Consensus Reports of Brugada Syndrome. Normal ECG is defined as normal-appearance ECG with or without early repolarization.

In the present study, we evaluated spontaneous alterations in the ECGs and in the ST level during follow-up in BS patients with and without VF and the underlying mechanism and impact of ECG alterations on the occurrence of ventricular arrhythmias in an *in vitro* experimental model of BS.

Methods

Clinical studies

The study groups comprised 70 males and four females with Brugada-type ECGs (mean age 59 ± 13 years), divided into those with (VF+ group) and without (VF− group) documented VF. To minimize the inclusion of patients with new onset of VF in the future, the VF−group consisted of elderly patients (≥60 years old at their first visit), because new-onset VF is rare in this population and we wished to minimize this additional variable.[2,3] Naturally, conclusions from our study can only be directed to the specific population we studied. All patients had type 1 ECG (54 spontaneous and 20 pilsicainide-induced[10]). Of the 20 patients with pilsicainide-induced type 1 ECG, 17 were in the VF− group and three in the VF+ group. No patients were from the same family. Echocardiography and chest X-ray were performed in all patients, and no abnormalities were found. All patients underwent an electrophysiological study after risks were explained and written informed consent obtained.

Standard 12-lead ECGs (0–150 Hz filter) and additional V1–V3 at the third intercostal space were recorded simultaneously. To evaluate the alterations in ECG type and ST level, we acquired ECGs during the initial and at each scheduled (3–6 months of follow-up) and any unscheduled visits and during any in-hospital stay. We evaluated the alterations in the ECG at rest (usually 2 hours before or after meal) and excluded the ECGs recorded with any stress (such as exercise test, drug challenge test, full stomach, and febrile illness).

We evaluated RR, PQ, and QRS intervals in lead II and QT interval, ST level at J point, and existence of fragmented QRS (fQRS)[12,13] in leads V1–V3 and V5 of the 12-lead ECG at their first visit. ECGs during follow-up were classified as type 0, 1, 2, 3, or normal. Types 2 and 3 ECGs were defined as in the Reports of the Second Consensus Conference of Brugada Syndrome.[4] We defined type 1 as in the Consensus Report, that is, having ST elevation with negative T wave, but added the criterion of T-wave depth >1 mm. We defined type 0 ECG as having coved ST elevation ≥2 mm and a shallow or absent negative T wave (depth ≤1 mm). Our previous experimental studies showed that type 0 ECG represented the existence of LOD type APs in the RV epicardium[9–11] (Figure 1). When multiple ECG types were observed, for example, when leads V1, V2, and V3 showed type 1, 0, and 2 ECGs, respectively, we classified the ECG as the type with the lowest number, type 0 in this example. We realize that the addition of new ECG categories complicates the BS classification, but the existing classification does not include the type 0 ECG.

The electrophysiological study, as we reported previously,[12] was performed in 54 patients. Induction of ventricular arrhythmia was attempted without antiarrhythmic drugs. The criterion for ventricular arrhythmia was induction of sustained polymorphic ventricular tachycardia (VT) or VF by programmed electrical stimulation from the RV apex, RV outflow tract, or left ventricle, using a maximum of two extra stimuli shortened to intervals reaching ventricular refractoriness at two cycle lengths (CLs).

Genetic analysis[12] for SCN5A mutations was performed in 43 patients in compliance with guidelines for human genome studies at the Ethics Committee of Okayama University.

In vitro studies

The animal investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). We harvested hearts, as we have done previously,[11,14] from 34 anesthetized adult male mongrel dogs and immediately perfused the hearts through the aorta with a cardioplegic solution. We isolated tissues of two sizes, ~2.5 × 2.5 × 1.0 cm^3 (epicardial × epicardial × transmural, 21 free-wall preparations) and ~0.8 × 2.5 × 1.0 cm^3 (13 transmural preparations) from the RV free wall. Each preparation contained a branch of the right coronary artery (diameter ~1 mm). We inserted into each artery separate perfusion and pressure monitoring cannulas. The tissues were mounted in a warmed chamber with their epicardial (free-wall preparation) or cut-exposed transmural (transmural preparation) recording surfaces up, perfused with Tyrode's solution at an arterial pressure of 40–50 mmHg at 36.5°C ± 0.5°C, and immersed in the perfusion efflux.

We paced the endocardial surface of the tissue at a CL of 2,000 ms. Two silver electrodes were placed in the bath, 5 mm away from the epicardium (anode) and the endocardium (cathode), to register a transmural ECG.[10,11,14]

The tissue preparations were stained with di-4-ANEPPS (Biotium, Inc., Hayward, CA, ~4 mmol/L) and immobilized with cytochalasin D (Fermentek ltd, Jerusalem, Israel, 20–30 μmol/L), which has been shown not to alter canine electrophysiology.[15] We verified the physiological conditions of the preparations as we have done before.[12] An optical mapping system with a 256-element (16 × 16) photodiode camera collected the fluorescence from a 19.5 × 19.5 mm^2 observation area on the tissue surface and converted it into 256 channels of electrical signals (APs).[10–12,14]

As we have done before,[10,11,14] we induced Brugada-type transmural ECGs at 36.5°C ± 0.5°C with pilsicainide (2.5–12.5 μmol/L, Asubuio Pharma, Tokyo, Japan) and pinacidil (1.25–12.5 mmol/L, Sigma Chemical, St. Louis, MO). The doses of drugs were increased progressively and simultaneously until the tissues developed the characteristic epicardial AP of BS.[8,10,11,14,16] We statistically analyzed APs at the recording sites along the epicardial and endocardial layers in the transmural preparations. Transmural dispersion of AP duration (APD) was calculated as the difference between the endocardial and epicardial APDs. We defined the epicardial regions having the longest and shortest APDs as EPI 1 and EPI 2, respectively. The depth of the phase 1 notch of the AP relative to the height of phase 0 depolarization was used as a surrogate indicator of the effects of transient outward potassium current.[8,11] We defined SAD type AP as APs with a deep phase 1 notch and an upstroke leading to the large phase 2 dome. LOD type AP was defined as the existence of a deep phase 1 notch with succeeding down-sloping phases 2 and 3 (without a large phase 2 dome). We defined AP heterogeneity when both types of APs existed in the epicardium simultaneously in the optical APs recordings.

We used both free-wall and transmural preparations to analyze epicardial AP parameters and used transmural tissues to analyze endocardial AP parameters and dispersion between epicardial-endocardial APs. Polymorphic VT in the experimental study was identified by three consecutive beats of rapid nonpaced activations with changing contours.[11,14] We compared the ECG and APs between the tissues with and without any ventricular arrhythmia.

Statistics

Continuous data were expressed as mean ± SD values. Comparisons among means were performed with two-way analysis of variance coupled with Scheffe's test. Comparison of two groups were made with Student's *t*-test for unpaired data (patients data) and paired data (longitudinal experiment data), as appropriate. Categorical data and percentage frequencies were analyzed by nonparametric test (Mann-Whitney *U*-test). Fisher's exact test was performed for the comparison of proportions among groups. Markers of ECG alteration were used in multiple logistic regression analyses to assess independent predictors of the VF episode. Significance was defined as $P < .05$. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the manuscript as written.

Results

ECG alterations in patents with and without VF

There were no differences in patients' background between the VF− and VF+ groups, except older age (≥60 years old) in the VF− group. Patients in the VF+ group had their first VF attack at 48 ± 11 years of age, and only four (12%) patients were ≥60 years old (61–73 years old). There was no new onset of VF or syncope in the VF− group during follow-up. Programmed electrical stimulation induced VF more frequently in the VF+ group (66% vs. 10%; Table 1). There was no difference in the effective refractory period at the RV apex (pacing CL 400 ms) between VF+ and VF− patients (225 ± 15 vs. 230 ± 18 ms, respectively; P = NS).

The number of recorded ECGs was 13.8 ± 9.6 in the VF+ group (range 2–39) and 10.0 ± 7.3 in the VF− group (range 3–33; P = NS).

Table 1 shows the ECG parameters and the incidence of each ECG type during follow-up. Spontaneous ECG alterations were common, especially in the VF+ group. ST level variations (≥0.20 mV) during follow-up were more prominent in the VF+ group than in the VF− group. The appearance of type 0, 1, and normal ECGs was frequent in the VF+ group (Figures 2, 3). In contrast, the VF− group had fewer ECG alterations, mild ST level variation, and less frequent occurrences of type 0 and 1 ECGs. The ECGs in the VF− group rarely converted to normal ECG (Figure 4).

Table 1 Clinical and ECG parameters in patients with Brugada syndrome

	Asymptomatic	VF	*P*
Patients background			
n	41	33	—
Female	3	1	ns
Age	68 ± 6	48 ± 11	<.001
Follow-up period	60.8 ± 37.6	75.7 ± 53.9	NS
Family history	8 (19)	11 (35)	NS
SCN5A mutation (n = 43) (%)	4 (15)	4 (24)	NS
PES induced VF (n = 46) (%)	3 (10)	19 (66)	.0008
ECG alterations (%):			
ECG type	18 (44)	32 (97)	<.0001
ST change ≥2 mm	6 (15)	30 (91)	<.0001
Spontaneous appearance of ECG type during follow-up (%):			
Type 0	2 (5)	23 (70)	<.0001
Type 1	24 (59)	30 (91)	.0014
Type 2	29 (71)	25 (76)	NS
Type 3	6 (15)	11 (33)	NS
Normal	5 (12)	20 (61)	<.0001
ECG Parameters			
II			
RR, ms	949 ± 188	958 ± 168	NS
PQ, ms	193 ± 42	183 ± 38	NS
QRS, ms	105 ± 13	118 ± 17	.0007
V1			
QT, ms	377 ± 43	405 ± 39	.0048
ST level, mV	0.16 ± 0.10	0.25 ± 0.16	.0026
Spikes	2.4 ± 0.8	2.9 ± 1.0	.0189
V2			
QT, ms	386 ± 35	407 ± 51	.0495
ST level, mV	0.29 ± 0.16	0.39 ± 0.25	.0303
Spikes	2.6 ± 0.9	3.3 ± 1.1	.0064
V3			
QT, ms	386 ± 30	391 ± 40	NS
ST level, mV	0.19 ± 0.11	0.25 ± 0.15	NS
Spikes	1.7 ± 0.8	2.1 ± 1.1	NS
V5			
QT, ms	386 ± 34	376 ± 24	NS
ST level, mV	0.03 ± 0.05	0.06 ± 0.06	.0368
Spikes	1.2 ± 0.5	1.3 ± 0.5	NS
fQRS			
Total spikes (V1–3)	6.6 ± 1.7	8.3 ± 2.3	.0011
Existence of fQRS (%)	24 (56)	30 (91)	.0001

Note: PES: programmed electrical stimulation.

There were longer QRS and QT intervals (V1 and V2), higher ST level (V1, V2, and V5), more spikes (V1, V2, and total spikes), and higher incidence of fQRS in the VF+ group than in the VF− group.

In 17 patients of the VF+ group, we compared ECGs recorded ≤1 week before or after the VF attack (without therapeutic drugs) and ECGs recorded ≥1 year after the last VF episode. ECG recordings ≤1 week before or after the VF attack had a high incidence of type 0 and 1 ECGs (18% and 59%, respectively) compared with the ECGs recorded ≥1 year from VF (0% and 47%, respectively).

Multiple logistic regression analysis (with the following variables: ST level, ECG types, and fQRS) indicated that the appearance of type 0 ECG (P = .0478), normal ECG (P = .0356), and variations of ST level ≥0.20 mV (P = .0061) were independently associated with VF. Induced VF by programmed stimulation was not associated with ECG alterations.

Alterations in ECG and in AP in the *in vitro* model of BS

Before BS induction, the tissues had a normal transmural APD gradient (longer in the endocardium than in the epicardium), a small phase 1 notch in the epicardial AP, and a small J wave with a positive T wave in the transmural ECG (normal in Figure 5). There was no AP heterogeneity in the epicardium and no spontaneous AP or ECG changes. The combination of pinacidil and pilsicainide induced a deep phase 1 notch and a prominent delayed phase 2 dome (SAD) in the epicardial AP and a transmural ECG with a large J-ST elevation and a negative T wave, characteristics of BS type 1 ECG. Further deepening of the phase 1 notch abolished

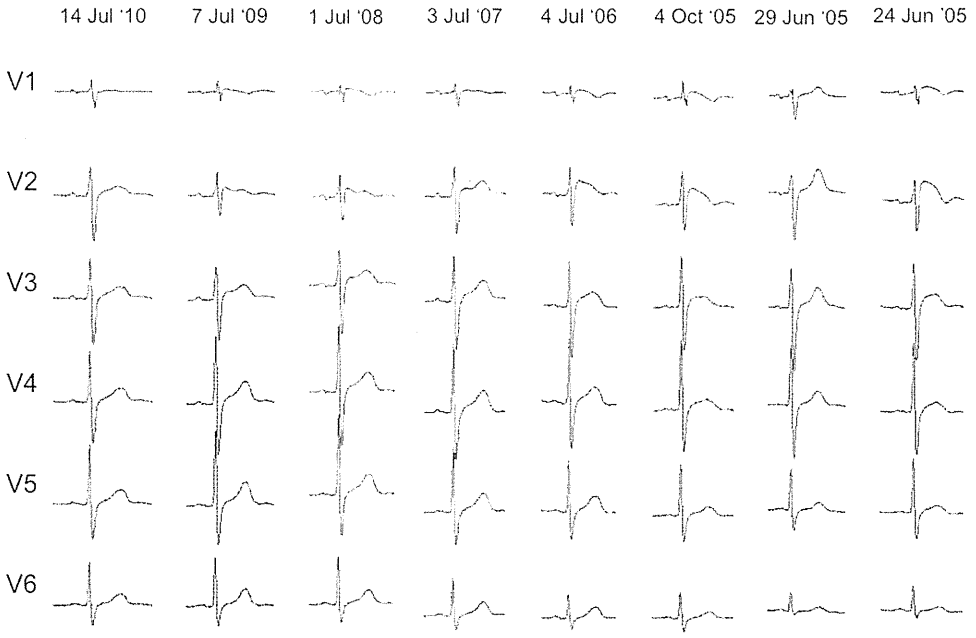

Figure 2 Spontaneous alterations in ECG types in the VF+ group. This ECG series was recorded in a 51-year-old BS male with VF. Various ECG types (types 0–3 and normal) were observed in leads V1 and V2 during follow-up.

the phase 2 dome (LOD) in the epicardial AP and changed the ECG from the type 1 to type 0 (Figures 5 and 6). The loss of the phase 2 dome occurred regionally, causing coexistence of both SAD and LOD types of APs (in the EPI 1 and EPI 2 regions, respectively) within the epicardium. Traveling of the phase 2 dome from EPI 1 to EPI 2 caused phase 2 reentry and initiated polymorphic VT in 20/34 tissues.

Compared with the tissues without VT (VT− tissues), the tissues with VT (VT+ tissues) had longer maximum APDs (in the EPI 1 region and the endocardium), larger APD dispersions (intraepicardium and between the EPI 2 region and the endocardium), and a deeper phase 1 notch in the epicardial APs (EPI 1 and EPI 2). AP heterogeneity and shifting between the LOD and SAD types AP occurred more frequently in the VT+ tissues. Transmural ECG showed longer QT interval and larger J wave in the VT+ tissues than in the VT− tissues (Table 2). Since VT was associated with AP changes and heterogeneity in the epicardium, changes in the type and ST level (≥0.10 mV) of

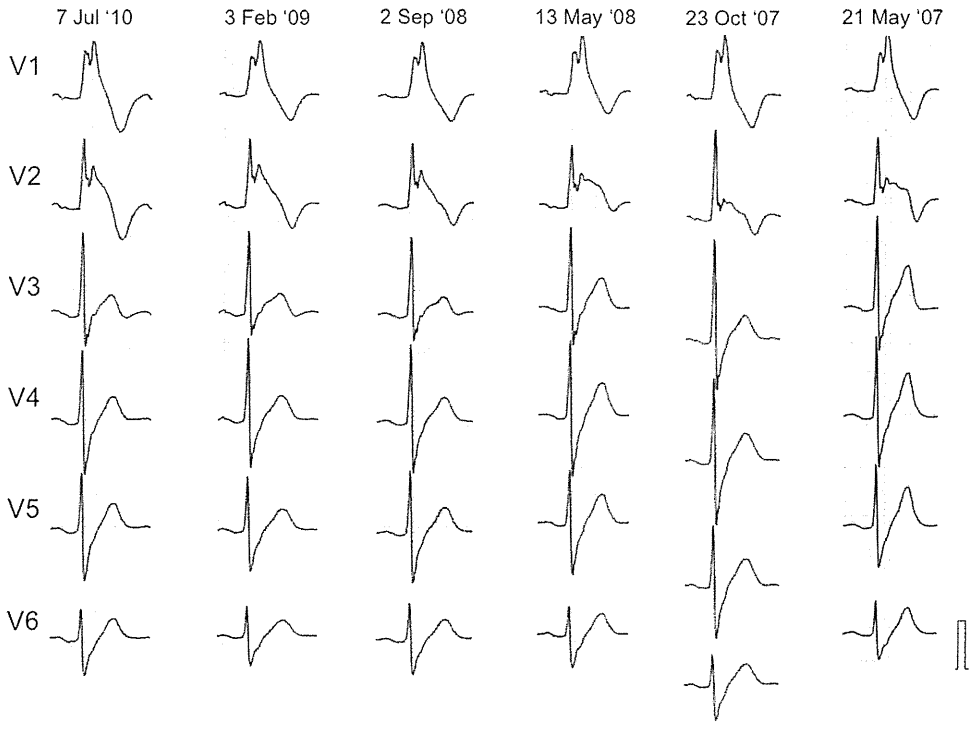

Figure 3 Spontaneous variations in ST elevation in the VF+ group. This ECG series was obtained from a 34-year-old male with VF. All ECGs represent type 1 ECG in leads V1 and V2 during follow-up. Variations (≥2 mm) in the ST level were observed.

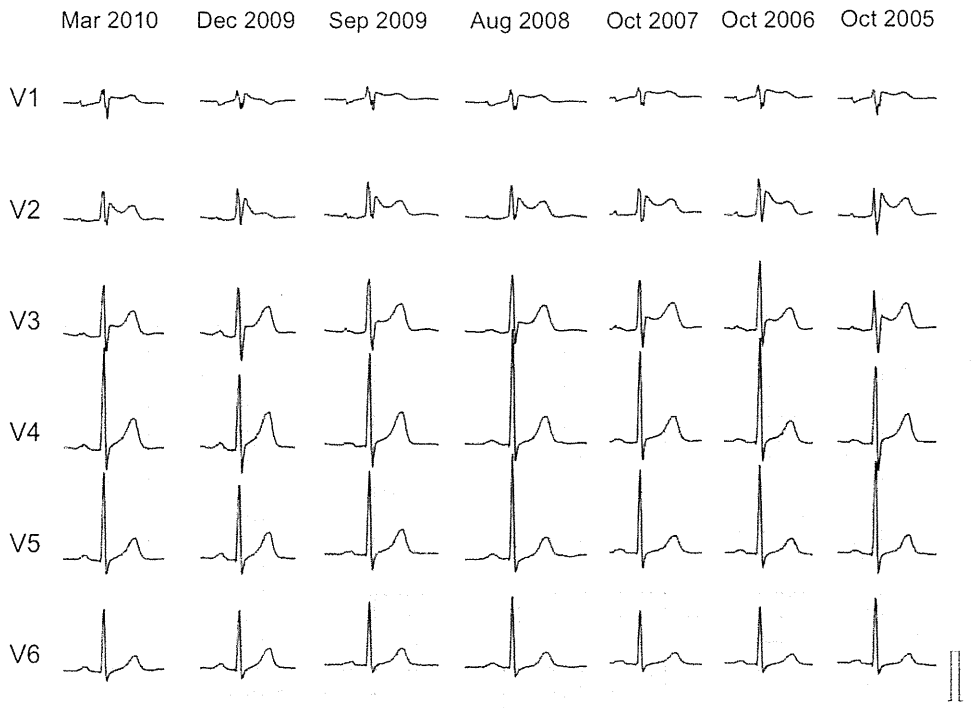

Figure 4 Minor alterations in the ECG type and ST level in the VF− group. This ECG series was obtained from an asymptomatic 61-year-old male. All ECGs showed type 2 ECG with minor ST level variation (<2 mm) in leads V1 and V2.

the ECG were usually observed at the onset of VT (Figure 6Aa–Ac and 6B). However, AP changes might not produce visible changes in ECG (e.g., Figure 6Ad) if AP changes occurred only in a small (<0.5 cm^2) region or far from the ECG electrode (anode; >1–1.5 cm).

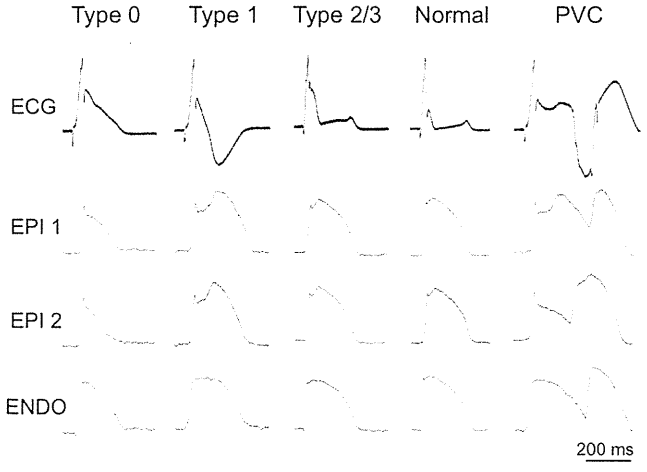

Figure 5 ECG and transmural APs in an experimental model of BS. Transmural ECG types are labeled as in the clinical ECG types. Type 0 ECG represents the LOD type AP in the epicardium, and type 1 the SAD type AP in the epicardium. Types 2, 3, and normal ECGs represent a small phase 1 notch and a small phase 2 dome in the epicardium. The heterogeneity of the epicardial APs initiated phase 2 reentry. Pacing CL = 2,000 ms. EPI 1 and EPI 2 = the longest and the shortest APDs in the epicardium, respectively. ENDO = endocardium.

Discussion

New observations

We observed a greater incidence of spontaneous type 0, 1, and normal ECGs in the VF+ group than in the VF− group, although both groups had type 1 ECGs. We also observed frequent and prominent ST level variations and T-wave changes in the VF+ group. In the canine tissue models of BS, we demonstrated regional alternating presence and absence of the phase 2 dome in APs, leading to significant RV epicardial heterogeneity, alterations in the ECG, and, finally, polymorphic VT. In both patients and in the isolated tissue model of BS, longer QT interval and ST level variation were associated with the occurrence of VT/VF. Therefore, changing ECG type and ST elevation were important risk factors of VT/VF in BS.

Changing ECGs in BS

Although type 1 ECG is a well-recognized diagnostic predictor of VF,[2,4,5] fluctuations and changes in the type of ECG over time have also been reported.[3,6,7] Many factors affect the ECG type and ST level. For example, bradycardia,[17] vagal nerve activation,[18] full stomach,[19] glucose-insulin test,[20] fever,[21] exercise test,[22] and pharmacological agents[10,23,24] can all cause short-term variations in the ambulatory ECG.[25,26] Repeat ECG recordings during follow-up hospital visits also can detect long-term alterations in the ECG type and ST level.[6,7,27] Autonomic balance, aging, and sex can all contribute to these long-term alterations. Veltmann et al[6] reported frequent spontaneous ECG fluctuations during 17.7 months of follow-up in patients with BS but did not correlate the

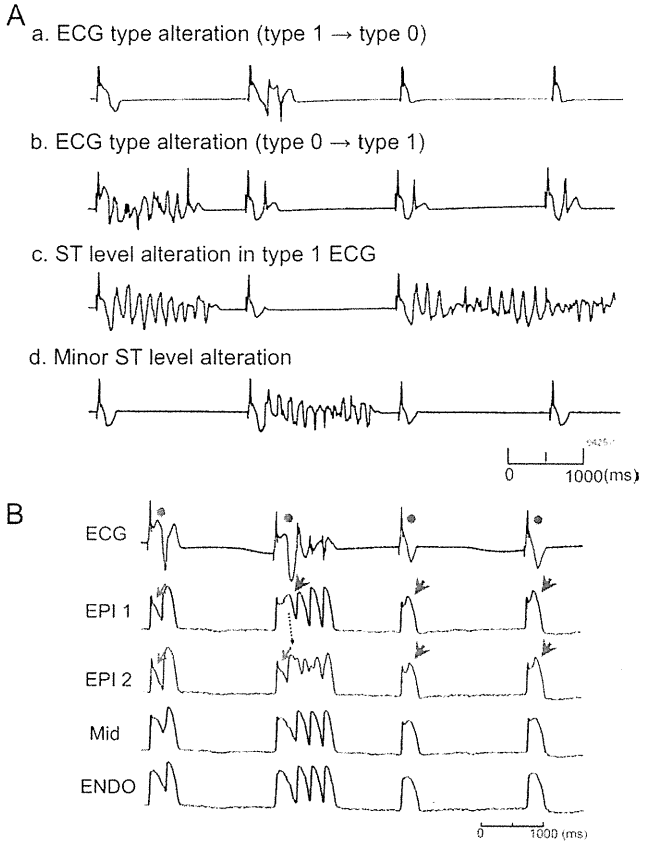

Figure 6 Changes in ECGs in an experimental model of BS. **A:** (a) Conversion from type 1 to type 0 ECG. The couplet of ventricular complexes occurred during the ECG conversion. (b) Conversion from type 0 to type 1 ECG. The first beat shows type 0 ECG, followed by initiation of polymorphic VT. Type 1 ECGs appeared after the termination of VT. (c) ST level variation in type 1 ECG. All paced beats show type 1 ECG. Polymorphic VT occurred at the beats with high ST elevation. (d) Minor ST level alteration. There were no identifiable alterations in the ECG when the area with AP alteration was far from the ECG anode (on the epicardial side). **B:** Alterations in ECG and transmural APs. The first beat shows that the epicardium has LOD-type APs (*small blue arrows*). Premature ventricular complex in the first beats occurred from outside of the mapping area. The second beat shows that AP alteration occurred in the EPI 1 but not in the EPI 2, resulting in AP heterogeneity and polymorphic VT. The third and fourth beats show that the epicardium had only SAD type APs (*large arrows*) without AP heterogeneity and thus without ventricular arrhythmias. Pacing CL = 2,000 ms. EPI 1 and EPI 2 = the longest and the shortest APDs in the epicardium, respectively. Mid = midmyocardium; ENDO = endocardium.

ECG alterations with clinical characteristics. Richter et al[7] reported the detection of type 1 ECG was associated with a high incidence of appropriate shock from ICD during 48 months follow-up and concluded that repetitive ECG recordings detected the type 1 ECG and the risk of VF.[3]

The present study demonstrated that ECG changes, which suggest unstable repolarization processes, occurred more frequently in the VF+ group than in the VF− group. The conversion to normal appearance ECG from Brugada-type ECGs also represented repolarization instability and AP heterogeneity. Thus, ECG alterations and conversions are potentially important risk factors that were not previously noted.

The *in vitro* experiments demonstrated the mechanistic links between the ECG alterations and VT in BS via unstable repolarization in the RV epicardium with simultaneous presence and frequent shifting of APs with SAD and LOD, leading to phase 2 reentry and changing ECG. Therefore, variations in the ECG morphologies (ECG types and ST level) suggest a proarrhythmic substrate. Moreover, the spontaneous type 0 ECG was an independent risk factor for VF.

Different from the previous reports,[6] this study revealed that changing ECGs are one of the predictors for occurrence of VF in BS. Control subjects in the previous study[6,27] included younger patients without VF compared with those in this study. We excluded such young cases to avoid the inclusion of the young patients who might have VF in the future. The difference in the control subjects compared with in the previous study can explain the different results in this study.

We evaluated the long-term alterations in ECGs in the clinical part of the study, although we examined short-term alteration in ECGs in the experimental part. Although it has been reported that several acute stress tests (such as full

Table 2 Transmural ECG and APs parameters in an experimental model of BS

	VT (−)	VT (+)	*P*
n	14	20	—
Drugs, μM			
Pilsicainide	10.0 ± 2.7	8.7 ± 3.3	NS
Pinacidil	8.4 ± 3.9	7.3 ± 5.4	NS
APD, ms			
EPI 1	243 ± 21	267 ± 36	.0048
EPI 2	218 ± 19	201 ± 37	NS
ENDO	231 ± 12	250 ± 11	.0124
APD dispersion, ms			
EP1-EP2	17 ± 8	66 ± 46	.0004
EP1-END	7 ± 7	13 ± 7	NS
EP2-END	14 ± 8	33 ± 16	.0274
Phase 1 notch, %			
EPI 1	19 ± 5	23 ± 6	.0340
EPI 2	22 ± 8	31 ± 6	.0008
ENDO	7 ± 4	2 ± 2	.0142
Conduction time, ms			
EPI 1	43 ± 8	46 ± 14	NS
EPI 2	41 ± 9	47 ± 14	NS
ENDO	24 ± 10	22 ± 9	NS
ECG			
QT	265 ± 27	306 ± 54	.0142
J width, ms	43 ± 14	67 ± 36	.0212
J voltage, ms	0.80 ± 0.49	0.90 ± 0.39	NS
AP variation (SAD ↔ LOD) (%)	6 (43)	21 (100)	.0001
AP Heterogeneity (%)	6 (43)	21 (100)	.0001
ECG variation (%)	3 (21)	20 (100)	<.0001
ST change (>0.1 mV)	3 (21)	20 (100)	<.0001
Type 0↔1 (%)	3 (21)	20 (100)	<.0001

Note: EPI 1 and EPI 2: the longest and shortest APDs in the epicardium, ENDO: endocardium.