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H. 知的財産権の出願・登録状況

(予定を含む。)

1. 特許取得

   なし
2. 実用新案登録

   なし
3. その他

   第15回日本心電図学会最優秀賞を受賞

対象論文：

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Circulation: Arrhythm Electrophysiol

2009; 2: 511-523

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分担研究報告書

遺伝性不整脈疾患におけるTCAP遺伝子異常の解析

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研究要旨：心筋唯一のNaチャネルであるSCN5A遺伝子の異常により、多彩な遺伝性不整脈疾患を引き起こすことが知られている。これら心臓Naチャネル病の機序の一つとしてチャネル制御蛋白の関与が考えられる。

今回、我々は心臓Naチャネル結合蛋白であるTCAP(Z帯構成蛋白)に注目し、遺伝性不整脈疾患への関与を調べるため、不整脈患者ゲノムライブラリーを用いたTCAP遺伝子スクリーニングを行ったところ、1つの遺伝子異常、3つの多型を検出した。ホールセル・パッチクランプ法を用いた電気生理学的機能解析では、R153H変異はNa電流を減少させ、TCAP遺伝子異常は遺伝性不整脈疾患の発症に関与する可能性があると考えられた。

A．研究目的

心筋のZ帯構成蛋白の一つであるTelethonin(TCAP)は、心筋細胞の構造維持・機能に重要な働きをすることが知られている。最近、偽性腸閉塞症例において検出されたTCAP遺伝子異常が、心臓Naチャネルと結合しNa電流を変化させることが報告されたが(Mazzone JBC, 2008)、遺伝性不整脈疾患におけるTCAP遺伝子の関与については不明である。

我々は、現在まで約1000家系、2000症例に及ぶ不整脈疾患ゲノムライブラリーを構築しており、本ゲノムライブラリーを用いた遺伝子解析、また、検出された遺伝子変異の心臓Na電流に対する影響を解析した。

B．研究方法

多施設より集積した日本人遺伝性不整脈疾患ゲノムライブラリーのうち発端者547例（QT延長症候群213例、Brugada症候群284例、洞不全症候群19例、不整脈源性右室心筋症18例、拡張型心筋症7例、特発性心室細動6例)においてTCAP遺伝子スクリーニングを施行した。コントロールとして259例の正常健常人のゲノムを用いた。また、検出されたTCAP遺伝子変異に関しては、変異を導入したプラスミドを作製し、ヒト胎児腎(HEK)培養細胞に心臓Naチャネルαサブユニット、β1サブユニットと共発現させ、ホールセル・パッチクランプ法を用いた電気生理学的機能解析を行った。

(倫理面への配慮)

本研究は、京都大学医の倫理委員会にて承認済みである。

C．研究結果

547例におけるTCAP遺伝子解析の結果遺伝子異常1つ、遺伝子多型（rare variant）3つを検出した。E49Kは不整脈源性右室心筋症患者にて検出し、A97TはQT延長症候群において、E132QはQT延長症候群の2例において検出した。R153Hは、QT延長症候群、Brugada症候群の2症例にて検出した。3つのrare variant（E49K、E132Q、R153H）は、正常コントロール518アレル中それぞれ1アレル（0.19%）にて検出された。

検出された変異チャネルをHEK細胞に心臓Naチャネルと共発現させ電流計測したところTCAP-WTを加えることにより、Naチャネルは増加した。(Mock -83.3±9.3 pA/pF, WT -173.7±34.1pA/pF, -20mVにて計測)

また、Naチャネルの活性化・不活性化を解析したところ、R153Hは、不活性化曲線を-15.2mV過分極側へシフトさせ、結果としてloss-of-functionを示唆する所見であった。活性化曲線は特に変化を認めなかった。

D．考　察

TCAP遺伝子と疾患の関係に関して、Mazzoneらは、偽性腸閉塞症例において、TCAP-R76C変異

を検出し、機能解析の結果、Ｎａ電流を増加させると報告した(Mazzone et al. JBC. 2008)。心臓Naチャネル病は様々な遺伝子異常を引き起こすが、全ての患者で心臓Naチャネル変異が検出されるわけではなく、このような結合蛋白がチャネル電流に影響し遺伝性不整脈疾患の原因となり得ることが考えられた。さらなる家系解析、他の異常の機能解析が必要であると考える。

また、TCAP-R153H変異は肥大型心筋症症例において報告されている(Hayashi et al. JACC 2004)。我々がR153Hを検出した２症例はそれぞれQT延長症候群、Brugada症候群であり、心肥大は認めなかったが、解析の結果Naチャネル電流の不活性化曲線の過分極偏位を認めた。本変異と疾患との関連に関しては、さらなる家系解析やモデル動物、疾患特異的iPS細胞を用いた解析にて重要な知見が得られるのではと期待される。

E．結　論

遺伝性不整脈疾患ゲノムライブラリーにおけるTCAP遺伝子解析本の結果、遺伝子異常１つ、遺伝子多型(rare variant) ３つを検出した。うちTCAP-R153Hは、不活性化曲線を-15.2mV過分極側へシフトさせ結果としてloss-of-functionを示唆する所見であった。本研究により、TCAP遺伝子異常が遺伝性不整脈疾患に関与している可能性が示唆された。

F．健康危険情報

なし。

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H．知的財産権の出願・登録状況（予定を含む）

1．特許取得

なし

2．実用新案登録

なし

3．その他

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

EP IMAGE

Remission of Abnormal Conduction and Repolarization in the Right Ventricle After Chemotherapy in Patients With Anterior Mediastinal Tumor

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A 22-year-old man with no significant past medical history presented with dry cough that lasted for a couple of months. The patient denied accompanying shortness of breath, palpitation, edema, high fever, or syncope. He had no family history of sudden death. On examination, he was afebrile with a blood pressure of 106/63 mm Hg, pulse rate of 88 beats/min, and normal oxygen saturation. His heart sound was normal without a pericardial rub. ECG (Fig. 1A) displayed a terminal r wave (arrow a) and ST-segment elevation (arrow b) followed by negative deflection of T wave (arrow c) in lead V_1. Chest computed tomography (Fig. 1A) revealed the existence of demarcated tumor in the anterior mediastinal space that attached to the pericardium in front of the right atrium and ventricle. The tumor encompassed the right ventricular outflow tract (arrow) but did not show invasion into the intrapericardial space. The tumor was histologically diagnosed with the large B cell lymphoma from a specimen obtained by needle biopsy. He started to undergo chemotherapy including cyclophosphamide, vincristine, doxorubicin, rituximab, and prednisolone. Two months after the chemotheraphy, chest computed tomography confirmed that the lymphoma size was reduced, which was almost invisible (Fig. 1B). At that time, ECG showed the disappearance of a late r′ wave and ST-segment elevation in lead V_1 (Fig. 1B). These findings indicate that coinciding with the shrinkage of anterior mediastinal tumor, conduction disturbance, and abnormal repolarization in the right ventricle were resolved. No life-threatening arrhythmic event occurred during the follow-up.

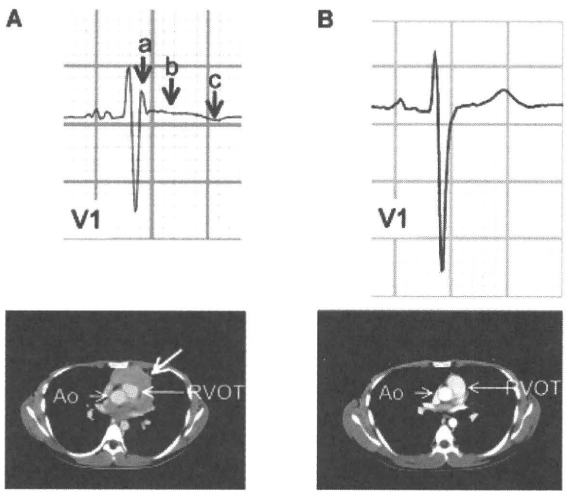

Figure 1. *A and B: ECG recording in lead V_1 and contrast-enhanced computed tomography scan before and after chemotherapy, respectively. Ao = Aorta; RVOT = right ventricular outflow tract.*

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No disclosures.

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Risk Determinants in Individuals With a Spontaneous Type 1 Brugada ECG

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Background: Spontaneous coved ST-segment elevation ≥2 mm followed by a negative T-wave in the right precordial leads (type 1 Brugada ECG) is diagnostic of Brugada syndrome (BS), but there is a false-positive rate.

Methods and Results: Computer-processed analysis of a 12-lead ECG database containing 49,286 females and 52,779 males was performed to select patients with a spontaneous type 1 Brugada ECG for an examination of the association of this ECG characteristic with long-term prognosis. There were 185 patients with a spontaneous type 1 Brugada ECG and of these, 16 (15 males; mean age, 46.7±14.0 years) were diagnosed with BS and 15 patients (all males; mean age, 50.1±13.4 years) were undiagnosed. The PQ interval was significantly longer in the diagnosed patients than in the undiagnosed patients (187.4±28.3 ms vs. 161.2±21.5 ms; P=0.0073). The T-wave in lead V_1 was more negative in the diagnosed patients than in the undiagnosed patients (−170.2±174.6 μV vs. −43.2±122.3 μV, P=0.027). Multivariate analysis revealed that a PQ interval ≥170 ms and T-wave amplitude <−105 μV in lead V_1 were independent risk stratifiers of life-threatening events. Survival analysis (mean follow-up, 78.6±81.8 months) showed that the PQ interval and a negative T-wave in lead V_1 were significantly associated with poor prognosis.

Conclusions: Analysis of a standard 12-lead ECG can stratify the prognosis of patients with a spontaneous type 1 Brugada ECG.

Key Words: Brugada syndrome; Electrocardiography; Prognosis; Risk determinant; Sudden death

Brugada syndrome (BS) is characterized by a distinct ST-segment elevation in the right precordial leads and causes sudden cardiac death.[1] This syndrome has a relatively high prevalence in East Asian countries. Patients with a coved-type ST-segment elevation in leads V_{1-3} are more susceptible to life-threatening ventricular arrhythmias than patients with a saddleback-type ST-segment elevation in the same leads.[2] A community-based study reported that subjects who displayed a spontaneous coved ST-segment elevation in the right precordial leads were not at risk for sudden death,[3] but another study, in which the mean follow-up period was >40 years, reported that an ECG with a coved ST-segment elevation was related to an increased risk of unexplained death.[4] Similar inconsistency has been found among studies conducted in hospital-based populations.[5–7] The discrepancy indicates that a distinct coved ST-segment elevation may not be the sole determinant of prognosis.

Editorial p???

To stratify prognostic risk in BS, pharmacological and electrophysiological tests are performed, but the prognostic value of these tests is yet to be settled.[8,9] In addition, mutation of the gene that encodes the cardiac sodium channel, *SCN5A*,[10] is detected only in approximately 20% of patients diagnosed with BS,[6] suggesting that it may be difficult to screen out subjects who are at high risk for lethal arrhythmia by genetic testing alone.

The large database of a university hospital containing 12-lead ECGs of more than 100,000 patients stored digitally for over 25 years, enabled us to evaluate long-term prognosis using computer-processed analysis. Since 12-lead ECG is the most convenient method of diagnosing BS in a large population, such as in health examinations, in the present

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study we focused on patients with a spontaneous coved ST-segment elevation ≥0.2 mV in the right precordial leads (ie, type 1 Brugada ECG). Our aim was to determine the quantitative traits of a spontaneous type 1 Brugada ECG that can stratify the risk for sudden cardiac death (SCD).

Methods

Database

The database comprised 102,065 consecutive patients (49,286 females and 52,779 males) who had undergone ECG recording in the university hospital between January 1983 and October 2008. A total of 308,391 ECGs were collected during this period. The 12-lead ECG was recorded at rest for 10 s at a sweep speed of 25 mm/s, calibrated to 1 mV/cm in the standard leads. The data were digitally stored in a 12-bit server computer with a sampling interval of 2 ms.

Patient Population

From the database, we chose patients who had a spontaneous coved ST-segment elevation in the right precordial leads. We enrolled patients who fulfilled the following ECG criteria: (1) J-point elevation ≥0.2 mV amplitude in lead V_1 or V_2, (2) the amplitude at the middle of the ST-segment (STM: defined as the time point after an interval of 1/16 of the average RR interval from the J point) in lead V_1 or V_2 was less than that at the J point in the same lead, (3) the amplitude at the end of the ST-segment (defined as the time point after an interval of 1/8 of the average RR interval from the J point) in lead V_1 or V_2 was less than that at the middle of the ST-segment in the same lead, and (4) the amplitude at the J point and the middle of the ST-segment was positive in leads V_1 and V_2. The J point was defined as the offset of the QRS complex that was the latest detection of ventricular depolarization. To exclude right bundle branch block, we did not include any ECG that displayed the QRS complex in lead V_1 with a decrease in amplitude of ≥0.4 mV from the J point.

ECG Analysis

The ECG analysis was performed using software (MUSE7.1, GE Marquette Medical Systems, Inc, Milwaukee, WI, USA). ECG variables, including duration, interval, amplitude, and axis, were digitally measured. A median complex was computed as follows: (1) all QRS complexes with the same morphology were aligned in time and (2) the algorithm generated a representative QRS complex from the median voltages that were found at each successive sample time. QRS duration was measured from the earliest detection of depolarization in any lead (QRS onset) to the latest detection of depolarization in any lead (QRS offset). Similarly, the QT interval was measured from the earliest detection of depolarization in any lead (QRS onset) to the latest detection of repolarization where the downsloping limb joined the baseline in any lead (T offset), while the U wave was excluded. The QTc interval was calculated after correction for heart rate with Bazett's formula. The frontal plane angle of the P wave, QRS complex, and T wave was determined from the frontal leads (I, II, III, aV_R, aV_L, and aV_F). The frontal plane QRS–T angle was defined as the absolute value of the difference between the frontal planes of the QRS axis and T axis, and was adjusted to the minimal angle using "360° minus the angle" for an angle >180° (axis measurement range; –89° to +270°). Because all variables of the 12-lead ECG were digitally measured by computer-processed analysis, neither intra-observer nor interobserver variability was taken into account.

Follow-up

The follow-up period of all patients was defined as the interval from the first day when a spontaneous type 1 Brugada ECG was recorded to the day when prognostic outcome was identified. The prognostic value was assessed for the endpoint of documented ventricular fibrillation (VF) or sustained ventricular tachycardia (VT) that was either symptomatic or revealed by an implanted device. A postal questionnaire was used to assess the prognosis of patients who were not associated with the Division of Cardiology in the hospital. Written informed consent was given by all patients enrolled in this study.

Diagnosis

When a spontaneous type 1 Brugada ECG was present, BS was diagnosed on the basis of a consensus report requiring at least one of the following criteria: documented VF, self-terminating polymorphic VT, family history of sudden death, type 1 Brugada ECG in family members, positive electrophysiological study, unexplained syncope suggestive of ventricular tachyarrhythmia, and nocturnal agonal respiration.[2] Confounding factors[1] that have been previously reported as disorders accounting for a type 1 Brugada ECG were excluded. Drug-induced Brugada-like ECG pattern[2] was also excluded.

Gene Analysis

The methods of DNA isolation and mutation analysis are described elsewhere.[11] Briefly, genomic DNA was isolated from blood lymphocytes and then screened for candidate genes using denaturing high-performance liquid chromatography with a WAVE System Model 3500 (Transgenomic, Omaha, NB, USA). Polymerase chain reaction was used to amplify abnormal conformers, and sequencing was performed on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

We explored the prognostic factors for developing life-threatening events and the endpoint was the occurrence of life-threatening events. Continuous variables are reported as mean±SD and categorical variables as observed number of patients (percentage). We compiled 2 groups: patients who were diagnosed with BS (diagnosis group) and patients who were not diagnosed with BS (no-diagnosis group). In the comparison of their clinical and ECG characteristics, we used a t-test for continuous variables and Fisher's exact test or χ^2 test for categorical variables. Receiver-operating characteristic curve was used to determine the optimal cut-off point of the prognostic factors that maximizes the sensitivity and specificity of ECG variables for the diagnosis of BS. Logistic regression was used to compare the patients with/without BS to explore the prognostic factors accounting for confounding. The forward selection procedure was applied for the selection of variables in the logistic regression and the criteria was set as $P<0.10$. For the significant variables in the model, the Kaplan-Meier curve was made to describe the event-free survival rate and the log-rank test was used to examine the difference between 2 groups. All tests were 2-tailed and the significance level was set as 0.5. The research protocol was approved by the Ethical Committee of Shiga University of Medical Science (19–75).

Results

We located 185 patients who fulfilled the ECG criteria of a

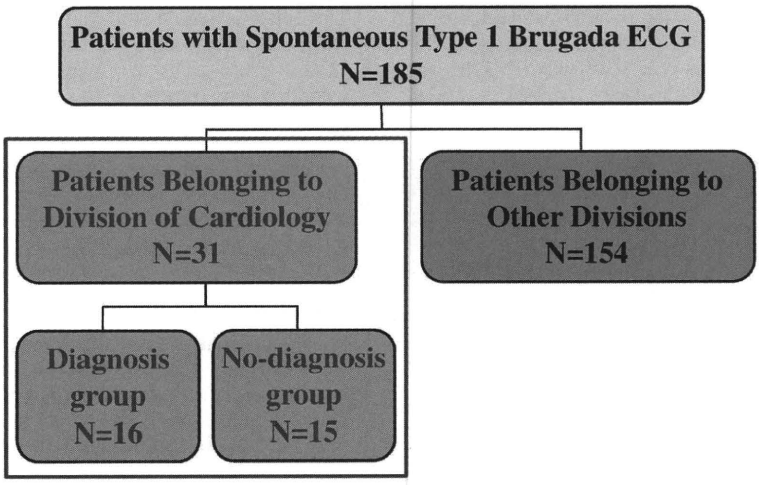

Figure 1. Flowchart of the categorization of patients with a spontaneous type 1 Brugada ECG: the 185 patients were chosen from an ECG database by virtue of a computer-processed algorithm. Of them, 31 patients were in the hospital's Division of Cardiology and 154 patients were not. The former patients were grouped: 16 patients who were diagnosed with Brugada syndrome (diagnosis group) and 15 patients who were not (no-diagnosis group). Red squares denote patients who were enrolled into this study.

Table 1. Characteristics of the Patients With Spontaneous Type 1 Brugada ECG

	Diagnosis group	No-diagnosis group	P value
No. of patients	16	15	
Age (years)	46.7±14.0	50.1±13.4	0.50
Male (n, %)	15 (93.8)	15 (100)	0.24
Follow-up period (months)	58.5±75.1	43.2±42.7	0.49
Family history of SCD (n, %)	8 (50.0)	0 (0)	0.0008
Syncope (n, %)	14 (87.5)	3 (20.0)	<0.001
Aborted SCD or documented VF (n, %)	11 (68.8)	0 (0)	<0.001

SCD, sudden cardiac death; VF, ventricular fibrillation.

spontaneous type 1 Brugada ECG in the database (**Figure 1**). The prevalence of a spontaneous type 1 Brugada ECG was 0.18% of the total patients who underwent ECG recording in the hospital. Of the 185 patients, 31 attended the Division of Cardiology: 16 patients were diagnosed as BS (diagnosis group) and 15 patients were not (no-diagnosis group). Representative ECGs are shown in **Figures S1** and **S2**. All the patients of the diagnosis and no-diagnosis groups were carefully followed in the Division of Cardiology. The remaining 154 patients who had exhibited a spontaneous type 1 Brugada ECG attended other divisions of the hospital and had undergone ECG recording irrespective of cardiovascular disease (eg, before surgery). The mean follow-up of the 2 groups was 51.1±61.1 months, ranging from 1 to 238 months.

Characteristics of the Patients

The detailed clinical characteristics of each group are shown in **Table 1**. The mean age of each group was not significantly different, the male predominance was similar between the 2 groups and the mean follow-up period was not significantly different between the 2 cohorts. In the diagnosis group, 14 of the 16 patients diagnosed with BS suffered syncope, and in 12 of the 14 patients, lethal ventricular arrhythmias (VF or VT) were documented. Although the remaining 2 patients did not have a documented episode of lethal arrhythmias, both patients had syncopal episodes considered to be of arrhythmic origin. In those 2 patients, VF was induced by programmed ventricular stimulation. An implantable cardioverter-defibrillator (ICD) was implanted in all patients but 1 patient who rejected the procedure. After ICD implantation, 6 patients experienced VF. In the diagnosis group 8 patients (50%; 1 female, 7 males) had a family history of sudden death. In the no-diagnosis group, the 15 patients, who had been referred to the Division of Cardiology, displayed a spontaneous type 1 Brugada ECG but were not diagnosed as BS because none suffered from unexplained syncope or had a family history of SCD. Therefore, it was not considered necessary to implant ICDs in these patients, but they were required to visit hospital regularly for health checks. In the no-diagnosis group, 3 patients experienced syncope that was most likely due to a neurally mediated mechanism, because precipitating events, such as severe

Table 2. Measurements of 12-Lead ECG of the Patients With Spontaneous Type 1 Brugada ECG

	Diagnosis group	No-diagnosis group	P value
Heart rate (beats/min)	64.4±9.5	68.3±14.7	0.38
PQ interval (ms)	187.4±28.3	161.2±21.5	0.0073
P axis (degrees)	63.9±13.5	58.7±19.5	0.41
QRS axis (degrees)	49.1±44.1	44.9±34.8	0.78
T axis (degrees)	52.8±20.4	59.3±14.0	0.31
QRS-T angle (degrees)	19.8±17.3	27.3±24.0	0.32
QRS-complex duration (ms)	108.4±25.5	103.2±8.6	0.46
QT interval (ms)	398.1±34.9	388.7±33.6	0.45
QTc interval (ms)	409.3±28.2	408.9±17.2	0.96

Table 3. Measurements of Individual Leads of the Patients With Spontaneous Type 1 Brugada ECG

	Diagnosis group	No-diagnosis group	P value
QRS-complex duration in lead V_1 (ms)	101.4±31.6	90.3±19.1	0.25
QRS-complex duration in lead V_2 (ms)	99.0±31.9	88.9±18.1	0.29
QRS-complex duration in lead V_5 (ms)	104.1±27.9	98.3±11.4	0.46
Dispersion of QRS-complex duration (ms)	4.7±14.3	7.9±21.9	0.63
R'-wave amplitude in lead V_1 (μV)	41.3±88.8	94.9±138.4	0.22
R'-wave amplitude in lead V_2 (μV)	90.3±188.5	188.9±248.9	0.23
J-point amplitude in lead V_1 (μV)	183.3±127.9	128.8±78.0	0.17
J-point amplitude in lead V_2 (μV)	311.8±117.6	339.0±135.7	0.55
STM amplitude in lead V_1 (μV)	55.3±66.9	81.7±73.9	0.31
STM amplitude in lead V_2 (μV)	172.1±107.5	192.9±157.8	0.67
Descending amplitude in lead V_1 (μV)	128.0±154.0	47.1±66.6	0.071
T-wave amplitude in lead V_1 (μV)	−170.2±174.6	−43.2±122.3	0.027
T-wave amplitude in lead V_2 (μV)	95.0±363.3	232.1±200.0	0.21
R-wave amplitude in lead aV_R (μV)	104.5±142.8	103.7±101.3	0.99
R/q ratio in lead aV_R	0.35±0.44	0.22±0.20	0.38
R-wave amplitude in lead aV_L (μV)	254.3±281.0	212.7±192.9	0.64
R'-wave amplitude in lead aV_L (μV)	32.2±48.4	49.9±88.4	0.49
R'/q ratio in lead aV_L	0.0±0.0	0.87±1.71	0.20

Dispersion of QRS-complex duration, absolute value of the difference between the QRS-complex duration in leads V_1 and V_5; STM, site at the middle of the ST segment (see Methods); Descending amplitude, difference in the amplitudes at the J-point and STM.

pain, emotional distress, or supine posture, were associated with syncope that was preceded by prodromal symptoms (sweating, nausea, vomiting, yawning). Cardiac transthoracic echocardiography revealed normal left ventricular function without evidence of structural heart disease in all patients of both groups. None of the family members of patients in either group was involved. We performed genetic analysis of 14 of the 16 patients in the diagnosis group, which identified a mutation in *SCN5A* in 1 patient (8.3%) who was a subject of our previous report;[12] no abnormality in *SCN5A* was determined in the remaining 13 patients.

Characteristics of the ECG

Table 2 shows the ECG characteristics. Heart rate did not significantly differ between groups. The PQ interval was significantly longer in the diagnosis group than in the no-diagnosis group; however, there was no significant difference between the 2 groups in the duration of the QRS complex, QT interval, and QTc interval. In addition, the frontal plane axis of the P-, QRS-, and T-wave did not differ between the diagnosis and no-diagnosis group. **Table 3** shows the ECG measurements in individual leads. Between the 2 groups, there was no significant difference in the duration of the QRS complex in leads V_1, V_2 and V_5, nor was there in the dispersion of the QRS-complex duration in leads V_1 and V_5, the R'-wave amplitude in leads V_1 and V_2, the J-point amplitude in leads V_1 and V_2, and the STM-amplitude in leads V_1 and V_2. The T-wave in lead V_1 was more negative in the diagnosis group than in the no-diagnosis group, but the T-wave amplitude in lead V_2 was not significantly different between the 2 groups. There was no significant difference between the 2 groups in the other ECG variables, including R-wave amplitude and R/q ratio in lead aV_R, and R-wave amplitude, R'-wave amplitude, and R'/q ratio in lead aV_L.

Risk Stratification

In the patients of the diagnosis and no-diagnosis groups, the factors predictive of life-threatening arrhythmic events were evaluated. Univariate analyses were performed to identify patients at risk of life-threatening events. The presence of a family history, and a PQ interval, and the T-wave amplitude in lead V_1 were significantly associated with BS (**Table 4**). Multivariate logistic regression analysis revealed that the PQ interval and T-wave amplitude in lead V_1 were indepen-

Table 4. Probability of Life-Threatening Arrhythmias According to Clinical and ECG Variables of the Patients With Spontaneous Type 1 Brugada ECG

	P value	Odds ratio	95%CI
Univariate analysis			
Age ≥50 years	0.36	1.93	0.47–8.42
Family history of sudden death	0.043*	10.50	1.47–216.68
Heart rate ≥66 beats/min	0.59	1.47	0.36–6.24
PQ interval ≥170 ms	0.0014*	14.0	2.62–115.18
QTc interval ≥405 ms	0.36	0.52	0.12–2.14
J-point amplitude in V_1 ≥155 μV	0.85	0.86	0.15–3.22
STM amplitude in lead V_1 ≥68 μV	0.58	0.67	0.16–2.76
T-wave amplitude <–105 μV in lead V_1	0.024*	6.05	1.36–32.09
Descending amplitude in lead V_1 ≥49 μV	0.59	1.47	0.36–6.24
Multivariate analysis			
PQ interval ≥170 ms	0.045*	11.50	1.05–268.62
T-wave amplitude <–105 μV in lead V_1	0.037*	8.98	1.14–117.28
Descending amplitude in lead V_1 ≥49 μV	0.19	0.23	0.02–1.94
Family history of sudden death	0.59	2.18	0.12–66.22

Dispersion of QRS-complex duration, absolute value of the difference between the QRS-complex duration in leads V_1 and V_5; STM, site at the middle of the ST segment (see Methods); Descending amplitude, difference in the amplitudes at the J-point and STM.
CI, confidence interval.

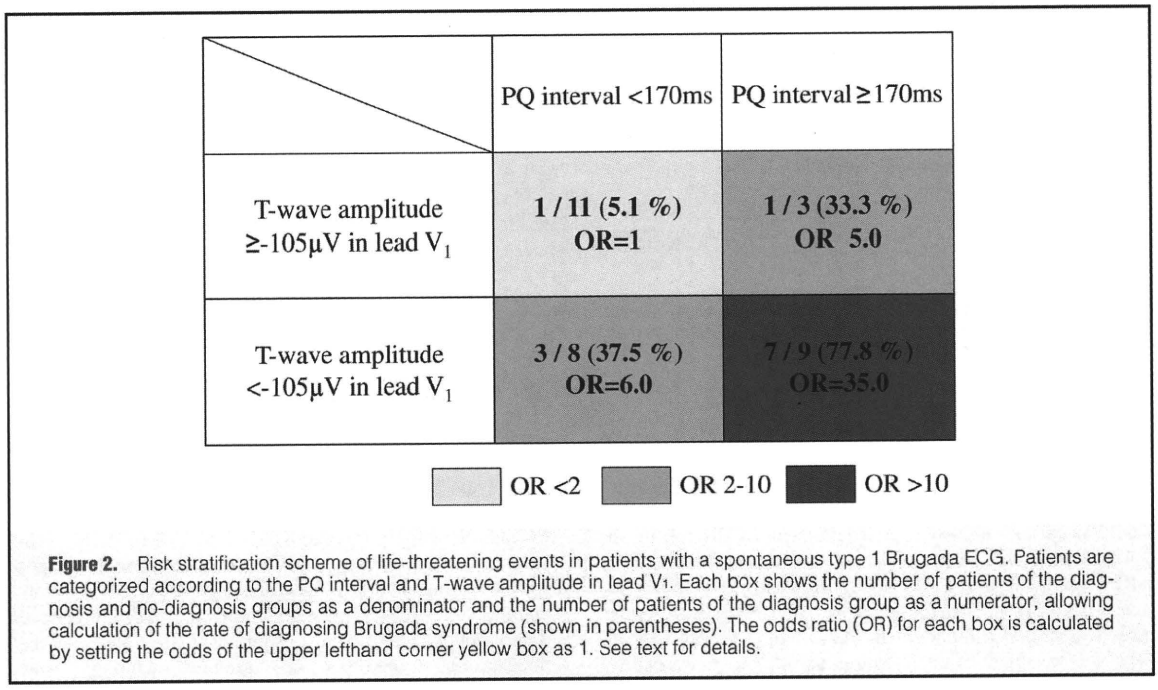

Figure 2. Risk stratification scheme of life-threatening events in patients with a spontaneous type 1 Brugada ECG. Patients are categorized according to the PQ interval and T-wave amplitude in lead V_1. Each box shows the number of patients of the diagnosis and no-diagnosis groups as a denominator and the number of patients of the diagnosis group as a numerator, allowing calculation of the rate of diagnosing Brugada syndrome (shown in parentheses). The odds ratio (OR) for each box is calculated by setting the odds of the upper lefthand corner yellow box as 1. See text for details.

dently associated with life-threatening events in patients with a spontaneous type 1 Brugada ECG (**Table 4**). **Figure 2** is a schema for risk stratification constructed according to the values of the PQ interval and T-wave amplitude in lead V_1. Using receiver operating characteristic analysis, the sensitivity and specificity of the PQ interval and T-wave amplitude in lead V_1 in response to life-threatening events were maximized by a PQ interval of 170 ms and a T-wave amplitude of –105 μV in lead V_1. The patients of the diagnosis and no-diagnosis groups were allocated to 4 categories according to the PQ interval and T-wave amplitude in lead V_1. The rate of diagnosing BS was higher in the category having a PQ interval ≥170 ms than in that of PQ interval <170 ms. The same was true for the categories dichotomized by T-wave amplitude of –105 μV in lead V_1. Furthermore, when present together, the PQ interval and T-wave amplitude in lead V_1 potentiate each other, leading to a diagnosis of BS that is substantially greater than that of its individual components.

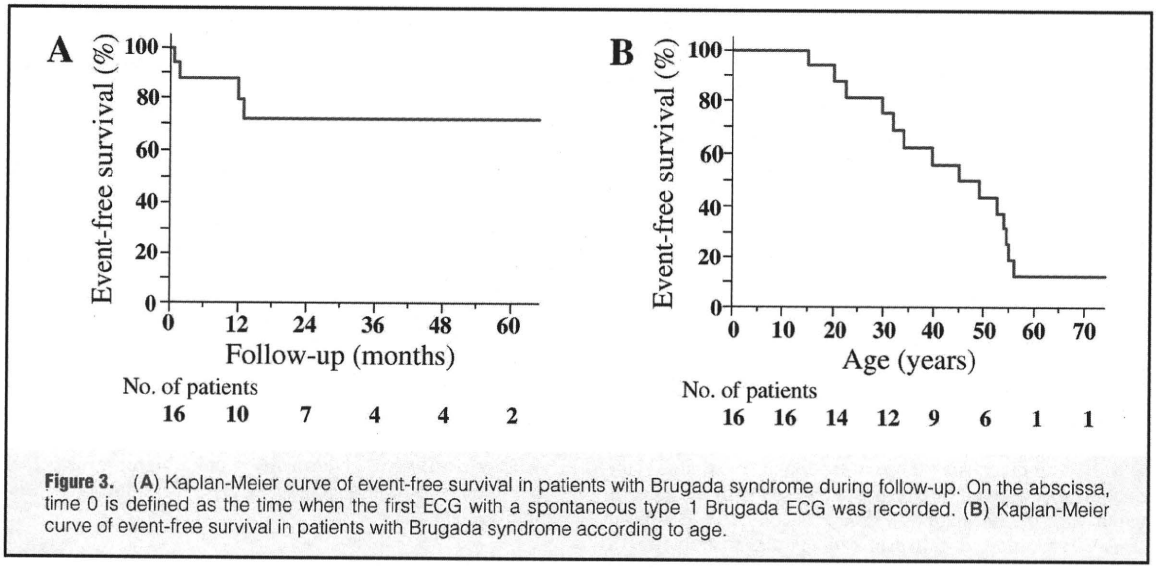

Figure 3. (**A**) Kaplan-Meier curve of event-free survival in patients with Brugada syndrome during follow-up. On the abscissa, time 0 is defined as the time when the first ECG with a spontaneous type 1 Brugada ECG was recorded. (**B**) Kaplan-Meier curve of event-free survival in patients with Brugada syndrome according to age.

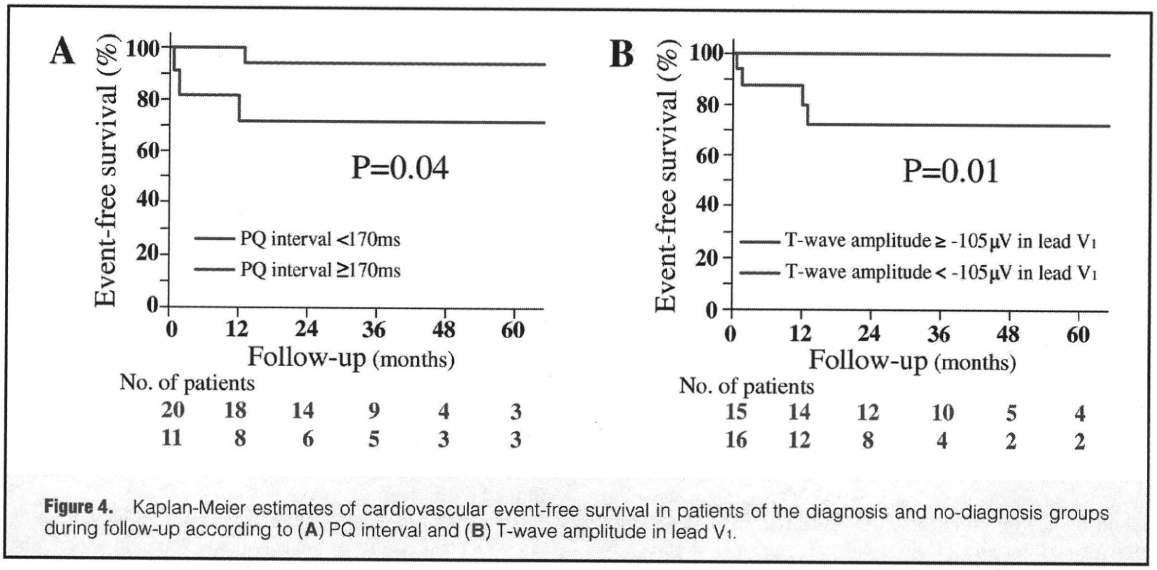

Figure 4. Kaplan-Meier estimates of cardiovascular event-free survival in patients of the diagnosis and no-diagnosis groups during follow-up according to (**A**) PQ interval and (**B**) T-wave amplitude in lead V_1.

Long-Term Outcome

Figure 3A shows the Kaplan-Meier survival curve of life-threatening events in the diagnosis group after a type 1 Brugada ECG was recorded. During the follow-up, 6 of the 15 patients (40%) who received an ICD experienced recurrence of life-threatening arrhythmic events. No patient died in the diagnosis group during follow-up. The duration from the diagnosis of BS to recurrence of life-threatening events ranged from 0.7 to 74 months (mean, 28.9±34.6 months). Approximately 30% of patients had recurrence within 1 year (**Figure 3A**). **Figure 3B** shows the age-dependent event-free survival curve of life-threatening events in the diagnosis group. Life-threatening events occurred between 15 and 60 years of age. A comparison of the ECG variables of patients with ICD intervention with those of patients without an ICD in the diagnosis group was made (**Table S1**). Heart rate was significantly faster in patients with an ICD than in those without, and the PQ interval was significantly longer in patients with an ICD than in those without. However, other ECG variables did not differ between patients with and without an ICD.

On the basis of the significant association of PQ interval and negative T wave in lead V_1 with BS, as shown in **Table 4**, the life-threatening event-free rate was estimated for the patients in both groups according to PQ interval and T-wave amplitude in lead V_1. **Figure 4** shows the Kaplan-Meier life-table analysis. The PQ interval was associated with a significant (P=0.04) difference in the life-threatening event-free rate between patients (n=11) with a PQ interval ≥170 ms and those (n=20) with a PQ interval <170 ms (haz-

ard ratio, 6.9; 95%CI, 1.1–133.5) (**Figure 4A**). In addition, the T-wave amplitude in lead V_1 was associated with a significant (P=0.013) difference in the life-threatening event-free rate between patients (n=16) with an amplitude $<-105\,\mu V$ and those (n=15) with an amplitude $\geq-105\,\mu V$ (hazard ratio, not available [because of the lack of events in patients with a T-wave amplitude in lead V_1 $\geq-105\,\mu V$]) (**Figure 3B**). Because none of the patients in the no-diagnosis group had a life-threatening event, multivariate survival analysis could not be performed.

Discussion

To utilize the convenience of the 12-lead ECG, a spontaneous type 1 Brugada ECG was used to enroll patients in this study. By conducting a computer-processed analysis, the discriminative ECG features of patients diagnosed with BS (diagnosis group), compared with patients not diagnosed with BS (no-diagnosis group), were found. First, atrial conduction was delayed in patients diagnosed with BS as compared with the no-diagnosis group. Second, the T-wave in lead V_1 was more negative in patients diagnosed with BS. Third, the duration of the QRS-complex in the right precordial leads did not show a significant difference between the diagnosed and undiagnosed patients. In addition, the amplitude of the R'-wave, J-point, and STM in the right precordial leads did not show a significant difference between the diagnosed and undiagnosed patients. These ECG findings are novel for differentiating patients at risk of developing life-threatening arrhythmia among patients who show a spontaneous type 1 Brugada ECG. Moreover, the risk-stratification schemes elaborated in this study revealed the patients who needed an ICD.

Risk Determinants

In BS, the transient outward current (I_{to})-mediated phase 1 is much more prominent in the epicardium than in the endocardium, leading to ST-segment elevation in the right precordial leads.[11] However, our data from the present study showed there was no significant difference in either the J-point amplitude or the ST-segment amplitude in the right precordial leads between patients diagnosed or not diagnosed with BS. There are several reasons for this: (1) we enrolled patients with a J-point amplitude ≥2 mm and coved-type ST-segment elevation, (2) the ST-segment elevation could be due to ion channel abnormalities such as a reduction of sodium and calcium currents, and (3) a clinically veiled histological abnormality may affect ventricular repolarization. In contrast to the amplitude of the J-point and the ST segment, the T-wave in lead V_1 was more negative in patients diagnosed with BS than in the undiagnosed patients. This finding is compatible with the inward calcium current overcoming the outward I_{to} during phases 1 and 2, causing a secondary depolarization in the epicardial action potential. Besides, the balance of the 2 ionic currents reverses as the I_{to} current overwhelms the calcium current, resulting in a loss of the action potential dome and an abbreviation of the action potential duration that lead to phase 2 reentry.[13] Consistent with these fundamental mechanisms, Nagase et al[14] demonstrated that prolongation of the epicardial action potential following I_{to}-mediated accentuation of the action potential in the right ventricular outflow tract caused the T-wave in the right precordial leads to become negative, coinciding with a type 1 Brugada ECG. In addition, dynamic instability depicted by the restitution property of the action potential duration in the right ventricular outflow tract may contribute to the occurrence of reentry.[15]

Though the patients diagnosed with BS did not show significant intraventricular conduction delay in the right ventricle, which was different from a previous report,[16] the atrial conduction delay was more pronounced in the diagnosed patients than in the undiagnosed patients. It has been reported that there is an increased atrial vulnerability to fibrillation in BS.[17,18] In those reports, the inter- and intra-atrial conduction delays were associated with atrial fibrillation. In the present study, the PQ interval was longer in the diagnosis group than in the no-diagnosis group, but the QRS-complex duration did not differ between the 2 groups. These findings suggest that a conduction disturbance occurred in the atrium and/or the atrioventricular node rather than in the ventricle. In fact, atrial fibrillation occurred only in 1 patient of each group during the follow-up. We should therefore pay close attention to examining whether atrial fibrillation develops. Furthermore, a P-wave abnormality[19] and a high prevalence of sick sinus syndrome[20] complicated by BS indicate atrial involvement.

Long-Term Prognosis

Brugada et al showed that symptomatic and asymptomatic patients with ST-segment elevation in the right precordial leads shared a similar incidence of cardiac arrest.[21] Other investigators[6,7] also reported that asymptomatic patients with such an ECG characteristic were at risk for sudden death, although the event-free survival rate in those studies was much lower than that of patients in the "Brugada" registry.[13] In contrast, sudden death did not occur in any of patients of the no-diagnosis group and asymptomatic group in our study. This result may be related to not involving family members of proband in the study.

Similar to previous reports,[6,7] we found that most patients of the diagnosis group suffered from ventricular tachyarrhythmia or syncope of unknown origin and approximately one-third patients of the diagnosis group had a recurrence of ventricular tachyarrhythmia. In contrast, none of patients not diagnosed with BS (no-diagnosis group) had sudden death or ventricular tachyarrhythmia. Thus, we emphasize again the importance of medical history-taking: syncope and family history of sudden death.

Study Limitations

First, the ST-segment elevation is not constantly observed in BS patients, because of the so-called "wax and wane" phenomenon, therefore patients with an ST-segment elevation <0.2 mV at the J-point were missed even if they had BS. Second, because of the limited follow-up, it cannot be assumed that asymptomatic patients did not develop SCD. Third, the response bias of the questionnaire should be considered. We must pay further attention to assessing the long-term prognosis in asymptomatic patients with a spontaneous type 1 Brugada ECG.

Study Implications

Despite the fact that a spontaneous type 1 Brugada ECG is diagnostic, the discriminative ECG features associated with a risk for SCD remain undetermined. From the results of the present study, we propose the PQ interval and negative T wave in lead V_1 as valuable ECG markers of BS. In addition, we underscore that medical information, including the family history, is helpful in the management of patients with a spontaneous type 1 Brugada ECG. It may be possible to deduce