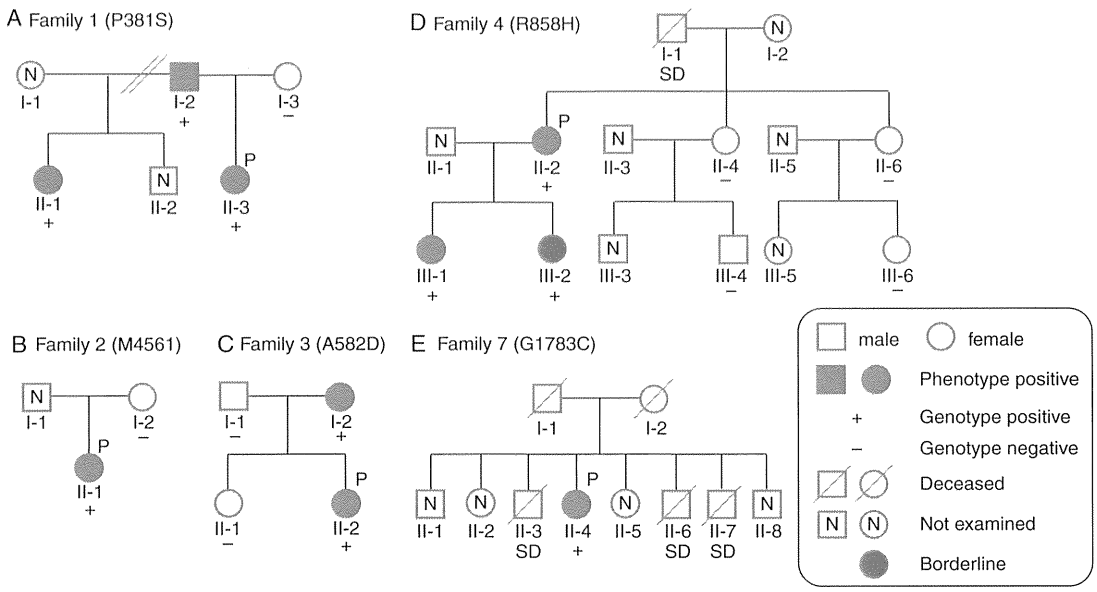

Figure 2 Pedigrees of the available families for *CACNA1C* mutations. Pedigrees of (*A*) P387S, (*B*) M456I, (*C*) A582D, (*D*) R858H, and (*E*) G1783C families. Squares indicate males; circles, females. Solid symbols depict phenotype positive and grey solid symbols depict borderline (QTc = 440−460 ms). +, genotype positive; −, negative. *P* marks indicate probands. N, not examined.

school at ~9 AM and naturally recovered while waiting for an ambulance. His 12-lead ECG revealed a prolonged QT interval and severe bradycardia (44 b.p.m.), which is not typical of children of his age (*Figure 3F*).[9] His minimum HR recorded by Holter ECG was 36 b.p.m. at midnight. Unfortunately, we could not get informed consent for gene screening from family members.

Family 7

A heterozygous G1783C (c.5347 g>t) was identified in a 58-year-old woman with syncope (*Figure 1F*, II-4 in *Figure 2E*). Her 12-lead ECG at rest revealed notched T-waves (*Figure 3G*). In an exercise stress test, the QTc interval was prolonged to 725 ms with a notched T-wave (data not shown). Her three brothers suddenly died at the ages of 6, 12, and 44 (II-3, II-6, and II-7 in *Figure 3E*). Her elder brother (II-3 in *Figure 3E*) died during exercise at the age of 12; ventricular tachycardia was documented in one of her younger brothers (II-7 in *Figure 3E*, who died at the age of 44); however, more information and genomic DNAs of family members were not available because we could not get informed consent.

All probands showed no findings of Timothy syndrome, such as structural cardiac abnormalities that originated from congenital disease, syndactyly, immune deficiency, and central nervous system.

We applied three analytical methods to obtain an indication of the potential impact of sequence variants on protein function. Three of the variants (P381S, A582D, and R858H) were judged using the PolyPhen-2 analysis to be probably damaging (*Table 2*).

Electrophysiology

Using a heterologous expression system, we evaluated the I_{Ca} characteristics of WT and five mutant channels. *Table 3* summarizes the biophysical parameters measured in multiple cells (the number of experiments is indicated in parentheses). *Figure 4A* depicts representative whole-cell current traces from six different CHO cells expressing *CACNA1C*-WT and five mutant channels. The current densities of cells transfected with *CACNA1C*-R858H were significantly larger than those of WT. In *Figure 4B*, peak inward current densities are plotted as a function of test potential. Compared with WT (open circles), peak inward current densities were significantly larger in R858H (squares) at potentials between −10 and +30 mV, with a maximum of 52% increase at +10 mV (−51.5 ± 8.8 vs. −78.3 ± 6.6 pA/pF, $P < 0.05$) (*Figure 4B*).

Figure 4C shows overlapping traces of two representative I_{Ca} currents recorded from WT and A582D (dotted trace) mutant channels. The mutant A582D current indicated a slower decay, and time constants (τ) for inactivation calculated as described in the Patient cohort and methods section are summarized as a function of test potentials in *Figure 4D*. Time constants for A582D (blue circles) were significantly larger than those for WT (open circles) at +10 and +20 mV test potentials. There were no significant differences between WT and other mutations.

Figure 4E depicts conductance–voltage activation and inactivation curves for WT and five mutants. Smooth lines were drawn by fitting to the Boltzmann equation. Activation of R858H channels showed an ~2 mV negative shift compared with WT. In contrast, inactivation of

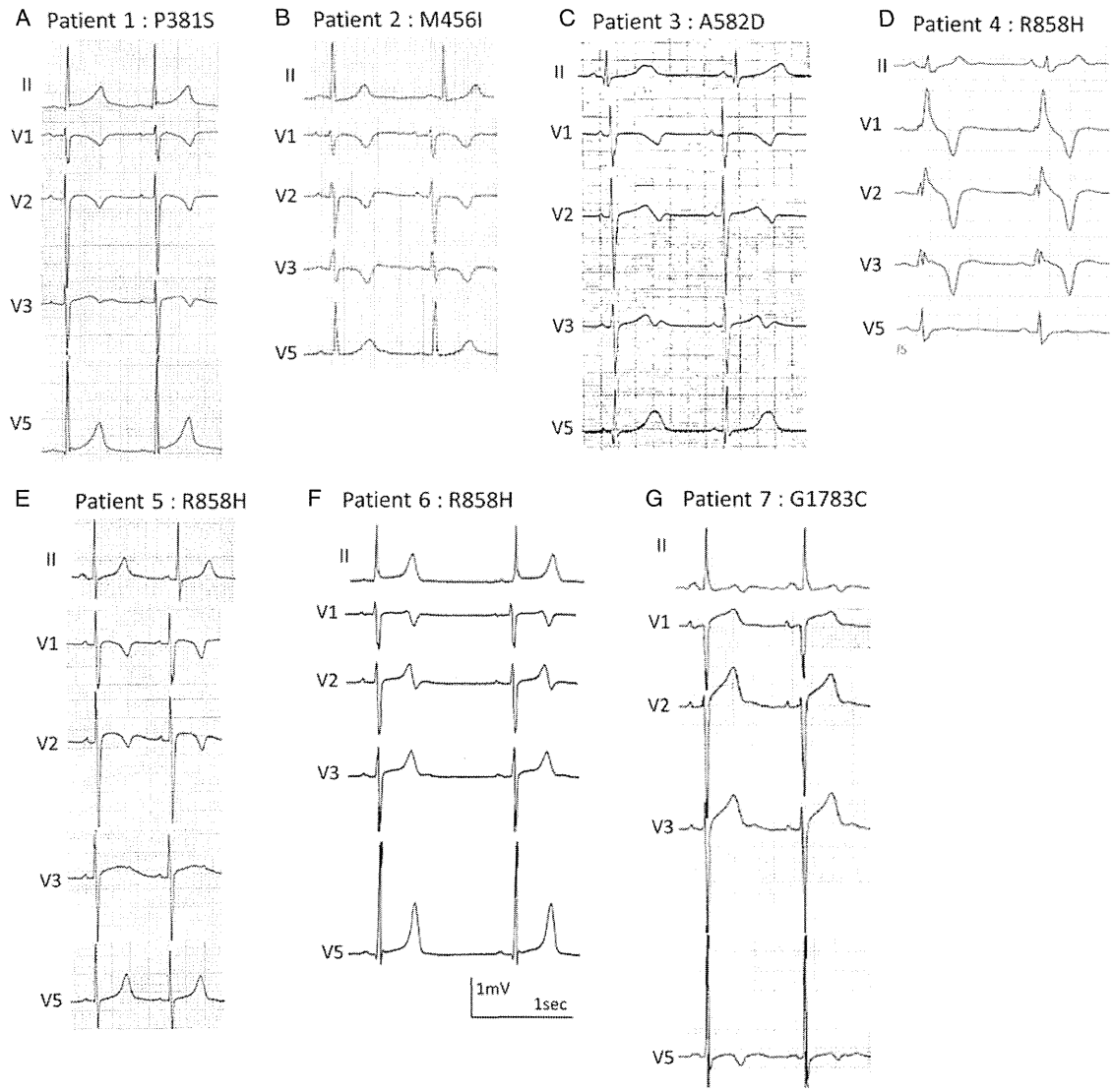

Figure 3 Electrocardiograms of *CACNA1C* mutation carriers. (*A*) An 8-year-old girl, p. P381S; (*B*) a 12-year-old girl, p. M456I; (*C*) a 12-year-old girl, p. A582D; (*D*) a 54-year-old woman, p.R858H; (*E*) a 7-year-old boy, p. R858H; (*F*) a 15-year-old boy, p.R858H; (*G*) a 58-year-old woman, p.G1783C.

A582D and R858H channels showed an ~2 mV positive shift. Thus, both R858H and A582D mutations exerted gain-of-function effects, and eventually their window currents were larger than those of the WT. The P381S, M456I, and G1783C mutations demonstrated no significant differences in gating of I_{Ca} current.

Discussion

The present study is the first comprehensive attempt to associate genetic and phenotypic variations in *CACNA1C* with LQTS, including detailed functional analysis for all variants and clinical information. We identified five novel *CACNA1C* mutations in seven unrelated probands in our cohort (*Table 1*). This mutation frequency (2.5%) is higher than that of LQT8 previously reported. Furthermore, the frequency of rare variants with unknown significance (VUS) in *CACNA1C* in our healthy control samples was only 0.5% (in this study), and the frequency of VUS in Japanese subjects has not been established. Although the number of mutation carriers was small, mutations were predominantly found in females. Most of our carriers tended to have bradycardia at their age, and more than half of them were young, consistent with a previous report;[9] particularly, young age patients with LQTS often showed bradycardia. Two of three

Table 2 Prediction of the impact of mutations

	PolyPhen-2 analysis	MutPred analysis	Align GVGD
P381S	0.998 (Probably damaging)	0.722 (Probably damaging)	73.35 (Class C65) (Probably damaging)
M456I	0.001 (Benign)	0.283 (Benign)	10.12 (Class C0) (Benign)
A582D	0.998 (Probably damaging)	0.686 (Probably damaging)	125.75 (Class C65) (Probably damaging)
R858H	0.997 (Probably damaging)	0.372 (Benign)	28.82 (Class C25) (Possibly damaging)
G1783C	0.002 (Benign)	0.337 (Benign)	158.23 (Class C65) (Probably damaging)

Table 3 Biophysical parameters on *CACNA1C*-WT and mutant channels

Biophysical parameters	WT ($n = 22$)	P381S ($n = 22$)	M456I ($n = 13$)	A582D ($n = 18$)	R858H ($n = 22$)	G1783C ($n = 14$)
Activation						
Peak density (pA/pF)	−51.5 ± 8.8	−53.5 ± 5.4	−54.9 ± 8.6	−61.1 ± 12.8	−78.3 ± 6.6*	−41.6 ± 7.7
V_h (mV)	−2.1 ± 1.3	−2.7 ± 1.0	−2.7 ± 1.3	−0.03 ± 1.3	−4.4 ± 0.7	−2.1 ± 1.3
k	7.7 ± 0.5	7.1 ± 0.5	8.1 ± 0.9	8.9 ± 0.3*	7.0 ± 0.3	7.6 ± 0.3
Conductance						
V_{rev} (mV)	72.5 ± 2.8	73.4 ± 2.3	72.0 ± 3.8	67.4 ± 1.7	70.0 ± 2.0	72.9 ± 2.7
Peak density (pS)	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	0.8 ± 0.1
Inactivation						
V_h (mV)	−22.3 ± 2.1	−21.5 ± 0.9	−23.9 ± 2.5	−21.8 ± 1.7	−21.5 ± 1.8	−28.6 ± 4.8
k	−7.6 ± 1.0	−7.3 ± 0.6	−9.6 ± 0.9	−9.0 ± 1.4	−11.2 ± 1.1*	−9.7 ± 1.2
Time constant (ms)						
0 mV	17.9 ± 1.5	19.4 ± 1.6	18.8 ± 1.9	20.6 ± 1.5	14.8 ± 1.1	19.7 ± 1.6
+10 mV	16.7 ± 1.1	16.7 ± 1.3	16.2 ± 1.2	24.6 ± 1.1**	14.5 ± 0.7	18.4 ± 1.2
+20 mV	17.5 ± 1.0	17.7 ± 1.4	17.0 ± 1.1	29.1 ± 1.3**	16.4 ± 0.9	19.7 ± 1.2

Peak current densities of activation were measured at +10 mV, peak densities of conductance were measured at +50 mV, and time constants were measured at 0–20 mV.
*$P < 0.05$ vs. WT.
**$P < 0.0001$ vs. WT.
Plus–minus values are means ± SEM.

symptomatic patients experienced cardiac events in their 50s, indicating that careful observation of the clinical time course of asymptomatic young carriers was also required.

There were three probands with Schwarz score ≤3.0 (*Table 1*). Two of them were indeed asymptomatic and showed a modest QT interval prolongation. Remaining one proband had syncope, nature unknown, without family history, and his QTc (420 ms) was totally normal. The score defined them as 'intermediate probability of LQTS', which is very different from defining as 'low probability of LQTS'. In addition, biophysical assay of the R858H variant (two of the probands, *Table 1*) clearly demonstrated that the mutation exerted a gain-of-function effect on LTCCs. Therefore, we should carefully observe the clinical course of these probands.

In the pedigrees of probands' families which were available (*Figure 2*), each mutation had completely penetrated in three families. Family 7 displayed a severe history of sudden death, but unfortunately, no further clinical information was available due to the lack of informed consent. Regarding three families carrying the heterozygous R858H, we considered that these families were unrelated because three probands had no overlapped SNP near the mutation, and there were no kinships in three generations of the family members.

Topologically (*Figure 5*), we observed alternative locations of mutation. Four of the five identified mutations were located in the intracellular region and one (P381S) in the transmembrane region. Two mutations reported in Timothy syndrome (p. G402S and p. G406R; *Figure 5*) are close to P381S and M456I. A582D is the mutation first found in the segment 2–3 linker of the domain II.[17] R858H is located in next to *CACNA1C*-P857R, which was recently reported in long QT patients.[7]

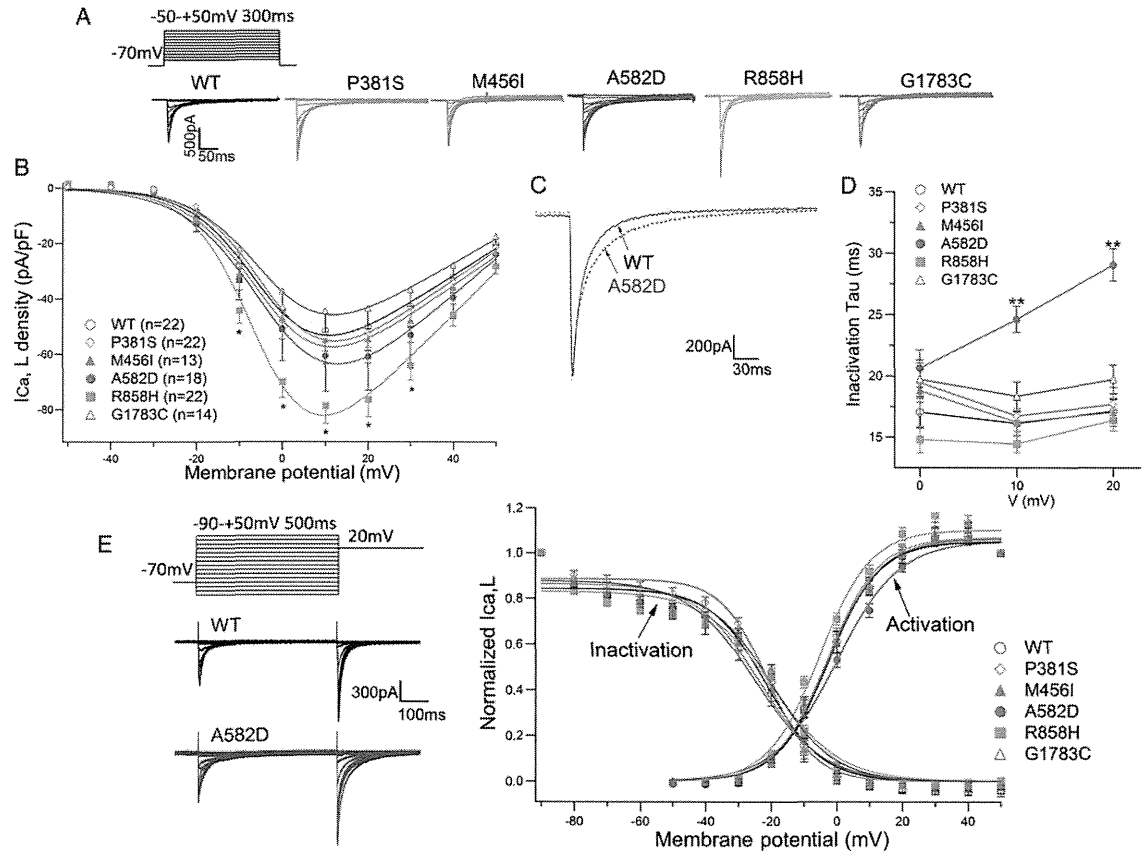

Figure 4 Functional analysis of the mutant Ca^{2+} channels. (*A*) Whole-cell current recordings of WT and mutant Ca^{2+} channels. Peak inward current–voltage relationships were constructed applying 300 ms pulses from a holding potential of −70 mV to potentials ranging from −50 to +50 mV. (*B*) Peak inward current–voltage relationship. Peak inward currents were normalized to cell capacitance to give a measure of Ca^{2+} current density. Symbols indicate means of data obtained from 13 to 22 cells. $*P < 0.05$. (*C*) Overlapping I_{Ca} traces expressing WT and A582D. Currents were elicited from a holding potential of −70 to +10 mV. (*D*) Time constants for the voltage dependence of inactivation in WT and mutant channels. $**P < 0.0001$. (*E*) Inset to the left of graph shows the two-step voltage-clamp protocol for measuring voltage dependence of inactivation. Voltage dependence of steady-state inactivation and activation for WT and mutant channels are plotted against test potentials.

In the functional analysis, both A582D and R858H were shown to be gain-of-function mutations. A582D peak current was not significantly different from that of WT. However, A582D-I_{Ca} inactivation was significantly slower than that of WT, and the *k* value of its activation was significantly larger. The functional analysis on A582D mutation was a slow I_{Ca} inactivation and resembled that found on *CACNA1C*-G406R, although our carrier showed QT prolongation alone without extracardiac phenotypes of Timothy syndrome.

In contrast, R858H-I_{Ca} showed a significantly larger current density than WT. Mutant channels showed peak I_{Ca} density similar to that of P857R channels at +10 mV.[7] Despite the increased I_{Ca} current, two out of three R858H carriers did not show significant QT prolongation but suffered from severe arrhythmic attacks. Different results of functional analyses are in accordance with the difference in QT and QRS shapes between A582D and R858H mutation carriers.

The M456I and G1783C mutations, which were predicted to be 'benign' by the PolyPhen-2 analysis, revealed no significant differences in the functional analysis. Although P381S-I_{Ca} showed no significant differences in our functional analysis, a damaging effect of this mutation was predicted by all three software packages. According to the previous report,[14] P381S was considered to be a causal mutation. The probands carrying these mutations showed QTc intervals <480 ms at rest (*Table 1*), but in all of them, exercise prolonged QTc intervals >500 ms. Approximately 20–25% of genotype-confirmed LQTS patients have a normal range of QTc[18] and exercise stress testing would be useful to unmask patients with concealed LQTS.[19] Therefore, there still remain some unexplained mechanism(s) underlying the discrepancy between genotype and phenotype, such as interactions between specific proteins, trafficking, or adrenergic stimulation, in the difference between *in vivo* and *in vitro*.

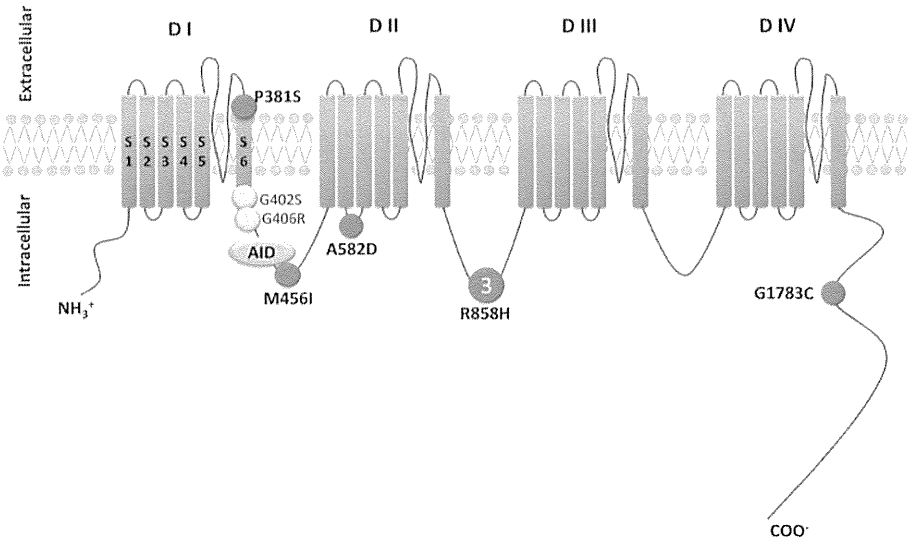

Figure 5 Location of the *CACNA1C* mutations. Predicted topology of the $Ca_V1.2$ (α) subunit. AID, α subunit interaction domain; S, segment; D, domain. Pink filled circles indicate positions of presented mutation. Yellow filled circles denote mutations causing Timothy syndrome. Larger symbols with numbers denote multiple probands with the same mutation.

Study limitations

Although we extensively explored the families' clinical and genetic background, we could not identify mutation carriers in four out of seven families, particularly in families with M456I or G1783C mutations, which are equivocal for the aetiology of the disease. The genotype–phenotype correlations are indispensable to elucidate the mechanism of LQT8.

Conclusions

Calcium channelopathy causes various phenotypes in inherited arrhythmia syndromes; LQT8 as gain-of-function and Brugada syndrome (BrS3) and short QT syndrome as loss-of-function.

CACNA1C mutations also cause LQTS without extracardiac phenotypes observed in Timothy syndrome, suggesting that their frequency is higher than reported. Therefore, gene screening of *CACNA1C* may be of clinical importance in patients with LQTS in whom no mutations have been identified.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

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INTERNAL MEDICINE

□ CASE REPORT □

Long-term Pharmacological Therapy of Brugada Syndrome: Is J-wave Attenuation a Marker of Drug Efficacy?

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Abstract

We herein describe two patients with Brugada syndrome in whom J-waves were successfully modified by drugs. Case 1 was a 54-year-old man who presented with repeated ventricular fibrillations (VF) and J-point elevation in the right precordial and lateral leads. After administration of cilostazol (200 mg/d), J-waves disappeared and coved-type ST-segment elevation changed to a saddleback-type for 25 months. Case 2 was a 31-year-old man who presented with a VF storm and J-point elevation in the lateral leads. After administration of quinidine (300 mg/d), J-waves and coved-type ST-segment elevation disappeared for 20 months. J-wave disappearance and coved-type ST-segment elevation were followed by VF suppression, probably due to transient outward potassium current (I_{to}) suppression.

Key words: Brugada syndrome, J-wave syndrome, pharmacological therapy, ventricular fibrillation, cilostazol, quinidine

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Introduction

The concept of J-wave syndrome has recently been proposed because the presence of electrocardiographic J-waves (J-point elevation) plays a crucial role in ventricular fibrillation (VF) pathogenesis (1). The J-wave is thought to be the consequence of a transmural voltage gradient caused by a predominant expression of transient outward potassium currents (I_{to}) in the epicardium, as observed in Brugada syndrome (BrS) and J-wave-prominent idiopathic VF (IVF) (2). The J-wave may be augmented prior to VF episodes, and its pharmacological suppression controls these VF episodes (3-6). Quinidine is effective in suppressing VF storms and recurrences, but its availability is limited (7, 8). Cilostazol has been shown to be effective in preventing VF recurrence in BrS and IVF (9, 10); however, the long-term effect of these drugs on J-waves and VF recurrence is poorly understood in BrS.

We herein describe two cases from unrelated families who were diagnosed as BrS. Oral cilostazol or quinidine effectively abolished J-wave and coved-type ST-segment elevation in both patients and prevented VF recurrence during their long-term follow-up.

Case Reports

Case 1

A 54-year-old man was admitted to our hospital because of nocturnal syncope. He had no family history of syncope or sudden death. The cardiovascular system examination was normal. The presence of structural heart disease was excluded, and his standard 12-lead electrocardiogram (ECG) showed normal sinus rhythm and normal QT interval (Fig. 1A). However, he exhibited coved-type ST-segment elevation in leads V1 and V2, and J-waves with ascending ST segment in leads V3-V6, with a maximum amplitude of 0.3 mV (arrows in Fig. 1A).

During the electrophysiological study, VF was repeatedly

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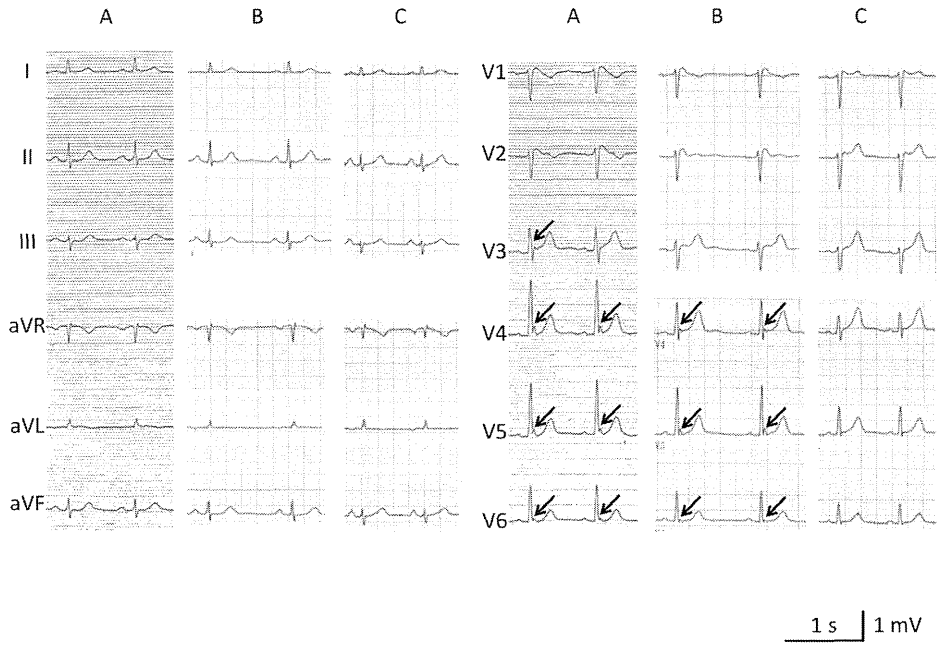

Figure 1. **Twelve-lead ECGs after the first syncopal episode (A), during the administration of clopidogrel sulfate (B), and during the administration of cilostazol (C).**

induced by triple extra-stimuli from the right ventricular outflow tract. Following a diagnosis of BrS, the patient was implanted with an implantable cardioverter-defibrillator (ICD) and discharged without administration of an anti-arrhythmic drug. Because his brain magnetic resonance imaging revealed an old lacunar infarction, clopidogrel sulfate (75 mg/d) was administered for secondary prevention.

Twelve months later, he experienced two consecutive ICD shocks due to VF episodes during sleep. An ECG showed both coved-type ST-segment elevation in the V1 lead and prominent J-waves in leads V4-V6 (arrows in Fig. 1B) as previously observed. His heart rate was relatively slow (57 beats/min), and we switched from clopidogrel sulfate to cilostazol (200 mg/d). On cilostazol, the patient's heart rate increased to 76 beats/min, and coved-type ST-segment elevation and J-waves were no longer observed (Fig. 1C). He has been followed on the drug and has not experienced VF for 25 months.

Case 2

A 31-year-old man was admitted because of syncope immediately after waking up in the early morning. His father had died suddenly at the age of 36 in his sleep, and his grandfather had died suddenly in his 60s after an early morning walk. DNA screening for an *SCN5A* mutation was negative.

The patient's cardiovascular system was normal. His 12-lead ECG showed normal sinus rhythm (53 beats/min) and J-waves with an ascending ST segment in leads I and V3-V6 and a horizontal ST segment in the aVL lead (arrows in Fig. 2A). ECG recorded at higher intercostal spaces did not fill the criterion of a typical ECG pattern for BrS (arrow head in Fig. 2A). The pure sodium channel blocker pilsicainide was given at a dose of 1 mg/kg and failed to induce the characteristic ECG pattern of BrS. VF was not induced by one to three extrastimuli applied to the right ventricle apex and outflow tract. He underwent an ICD implantation and was discharged without any anti-arrhythmic agent.

Forty-one months after discharge, he experienced two ICD shock deliveries during sleep. A month later, an electrical storm (≥3 VF episodes/24 h) occurred at night. His ECG recorded immediately after the storm showed J-waves in leads I and V6 and ST-segment elevation in leads V1 and V2 (arrows in Fig. 2B). Recordings from the higher intercostal spaces showed coved-type ST-segment elevation in the V2 lead, which was compatible with BrS (arrow head in Fig. 2B).

Quinidine was started at 300 mg per day, which increased the patient's heart rate from 53 to 62 beats/min, and VF was prevented. Both J-waves and ST-segment elevation were abolished by the drug (Fig. 2C). With quinidine, late potentials on the signal-averaged ECG were normalized: LAS_{40} from 60 to 35 μV, and RMS_{40} from 3 to 22 ms. He was discharged on quinidine and has not experienced VF recurrence for the past 20 months.

Discussion

In both BrS and J-wave-associated IVF, electrical storms are known to be controlled by isoproterenol or quini-

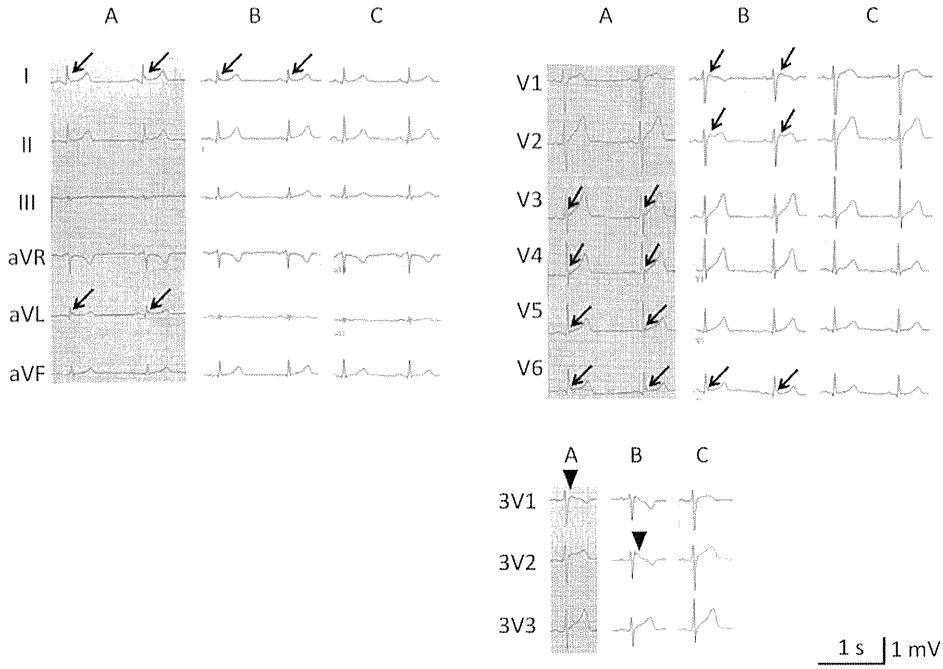

Figure 2. Twelve-lead ECGs after the first syncopal episode (A), after the VF storm (B), and during the administration of quinidine (C). The bottom panel shows the upper third intercostal ECGs.

dine (3-6, 11-13). As alternatives, cilostazol has been shown to be effective in preventing VF recurrence (9, 10). We described two cases of BrS associated with J-waves. Either cilostazol or quinidine was able to suppress VF recurrence and abolish any ECG signs of early repolarization.

Mechanistically, quinidine inhibits I_{to} currents and diminishes the transmural voltage gradient, thus reducing I_{to}-mediated J-waves (2). Cilostazol, a selective inhibitor of phosphodiesterase III, is an antiplatelet drug with vasodilatory action. This drug increases cellular cAMP levels and increases L-type calcium currents (I_{Ca}), and like isoproterenol, it counteracts I_{to}, resulting in attenuation or abolishment of the electrical inhomogeneity of action potentials. The reduced electrical inhomogeneity of action potentials would prevent phase-2 re-entry and subsequent VF, thereby leading to the diminution or disappearance of coved-type ST-segment elevation or J-waves. However, it still remains poorly understood whether the attenuation of J-wave amplitude is a hallmark of drug efficacy on a long-term basis.

It has been reported that some BrS patients exhibit J-waves and that their presence is a predictor of arrhythmic events or poor outcomes in BrS (14, 15). In addition, the fact that electrical storms can be controlled by isoproterenol or quinidine with concomitant J-wave attenuation in J-wave-associated IVF implies an important role of J-waves for VF occurrence (6). In the present study, VF recurred when the patients showed prominent J-wave augmentation, suggesting their pro-arrhythmic roles. Although both cilostazol and quinidine were effective in preventing VF in BrS and J-wave-associated IVF, the details of the underlying mechanism of action remain to be elucidated.

The authors state that they have no Conflict of Interest (COI).

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Long-term prognosis in patients with Brugada syndrome based on Class II indication for implantable cardioverter-defibrillator in the HRS/EHRA/APHRS Expert Consensus Statement: Multicenter study in Japan

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BACKGROUND The HRS/EHRA/APHRS Expert Consensus Statement for implantable cardioverter-defibrillator (ICD) in Brugada syndrome (BrS) has recently been published. However, the validity of the Class II indication for ICD in BrS patients is still unknown.

OBJECTIVE The purpose of this study was to evaluate the validity of the Class II indication for ICD implantation in the Consensus Statement with a large Japanese cohort of BrS.

METHODS Among 410 patients with BrS, a total of 213 consecutive BrS patients with the Class II indication for ICD implantation (mean age 53 ± 14 years, 199 men) were enrolled. Clinical outcomes were compared between patients with Class IIa (n = 66) and those with Class IIb (n = 147) indication according to the Consensus Statement.

RESULTS The incidence of cardiac events (documented ventricular tachyarrhythmias or sudden cardiac death) during follow-up of 62 ± 34 months was significantly higher in patients with Class IIa (n = 8, 2.2% per year) than those with Class IIb indication (n = 4, 0.5% per year; $P = .01$).

CONCLUSION We confirmed that Class IIa indication identified a group of patients with increased risk compared to Class IIb indication for ICD in the Consensus Statement of 2013. In patients with Class II indication, the combination of a history of syncope and spontaneous type 1 ECG may be an important factor in distinguishing intermediate- from low-risk patients with BrS in Japan.

KEYWORDS Brugada syndrome; Implantable cardioverter-defibrillator; Class II indication; HRS/EHRA/APHRS Expert Consensus Recommendation; Prognosis

ABBREVIATIONS **BrS** = Brugada syndrome; **ECG** = electrocardiogram; **ICD** = implantable cardioverter-defibrillator; **J-IVFS** = Japan Idiopathic Ventricular Fibrillation Study; **PES** = programmed electrical stimulation; **SCD** = sudden cardiac death; **VF** = ventricular fibrillation; **VT** = ventricular tachycardia

(Heart Rhythm 2014;11:1716–1720)

Introduction

Brugada syndrome (BrS) is known to be a high risk for sudden cardiac death (SCD) in the absence of major structural heart disease. Patients with BrS and a previous history of documented ventricular fibrillation (VF) or aborted sudden cardiac arrest are known to be at special risk for repeat cardiac events and have a poor prognosis. Therefore, implantation of an implantable cardioverter-defibrillator (ICD) is recommended for these patients.[1,2] However, risk stratification and indications for ICD implantation in BrS patients without previous cardiac arrest have not been fully established.

Recently, expert consensus recommendations for therapeutic interventions for BrS were proposed in the HRS/EHRA/APHRS Expert Consensus Statement.[3] The Class IIa indication for ICD implantation in this statement included patients with a spontaneous type 1 electrocardiogram (ECG) and a history of syncope judged likely to be caused by ventricular arrhythmias. Patients with a spontaneous or drug-induced type 1 ECG and inducible VF by programmed electrical stimulation (PES) may be considered for ICD

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implantation as a Class IIb indication. However, the validity of the Class II indications for ICD are still unknown in patients with BrS.

The Japan Idiopathic Ventricular Fibrillation Study (J-IVFS) is a multicenter study involving a prospective survey started in 2002 with the aim of exploring the prospective survival of patients with BrS.[4,5] In the present study, the J-IVFS database was used to validate Class II indications for ICD implantation in the Consensus Statement with a large Japanese cohort of BrS.

Methods

Study population

Four hundred ten consecutive individuals (mean age 52 ± 14 years, 382 men) with spontaneous or sodium channel blocker induced type 1 BrS ECG but without previous cardiac arrest were enrolled in J-IVFS between February 2002 and May 2012 according to the proposed diagnostic criteria for BrS.[6,7] Of these patients, the data from 188 patients enrolled in J-IVFS before November 2006 and those from 376 patients enrolled in J-IVFS before February 2011 were already included in a previous publication.[4,5] In the present study we added 34 patients to the cohort of the last publication.[5] All patients were probands from different families who were followed for a period longer than 1 year and met the following criteria: (1) normal findings on physical examination, chest radiography, and echocardiography; (2) no administered antiarrhythmic drugs; and (3) no electrolyte abnormalities at the time of ECG recording and other examinations.[4,5]

Of these patients, we enrolled 213 patients who met Class II indications according to the HRS/EHRA/APHRS Expert Consensus Statement (mean age 53 ± 14 years, 199 men).[3] The study was approved by the ethical review committee of each participating institution. Written informed consent was obtained from all patients.

We divided the enrolled patients into 2 groups (Table 1): patients who had an implanted ICD with Class IIa (n = 66) and those with a Class IIb indication (n = 147).

Clinical course

Enrolled patients were followed for clinical events every 12 months, and the incidence of cardiac events (appropriate ICD therapy for fast ventricular tachycardia [VT] or VF >200 bpm in patients with ICD, or documented fast VT, VF, or SCD in patients without ICD) was determined. Each cardiac event in patients with an ICD was evaluated by analyzing intracardiac ECG stored in the ICD and confirmed as appropriate therapy for fast VT or VF. Clinical outcomes were compared between the groups of patients with Class IIa and Class IIb indication.

Statistical analysis

Values are given as mean ± SD. Nested analysis of variance with the Scheffe test was used to compare consecutive data between patients with Class IIa and those with Class IIb indication. Other categorical data were examined using the Fisher exact test. Event–rate curves were generated using the Kaplan–Meier method. For all tests, $P < .05$ was considered significant.

Results

Clinical profile of patients

Of the 213 enrolled patients, a family history of SCD was noted in 63 (30%), past history of documented AF in 28 (13%), and spontaneous type 1 ECG in 136 (64%).

The clinical, ECG, and electrophysiologic characteristics of the patients in the 2 groups are presented in Table 1. Based on the HRS/EHRA/APHRS Expert Consensus Statement, all patients with Class IIa indication had a history of syncope determined to be likely caused by ventricular arrhythmias and showed spontaneous type 1 ECG, and all patients with Class IIb indication had spontaneous or drug-induced type 1 ECG and inducible VF by PES. There were no differences among the 2 groups with regard to age, incidence of family history of SCD, past history of AF, positive late potentials (positive for 2 of following 3 parameters: filtered QRS duration >114 ms, RMS_{40} <20 μV, and LAS_{40} >38 ms), QRS duration in leads V_2 and V_6, QTc interval in lead V_2,

Table 1 Clinical and electrocardiographic characteristics

	Class IIa indication (n = 66)	Class IIb indication (n = 147)	*P* value
Gender (male)	61 (92%)	138 (94%)	.70
Age (years)	55 ± 15	52 ± 14	.26
History of syncope	66 (100%)	27 (18%)	<.0001
Family history of SCD	16 (24%)	47 (32%)	.25
History of AF	8 (12%)	20 (14%)	.77
Spontaneous type 1 ECG	66 (100%)	70 (48%)	<.0001
Inducible VT/VF [n/N (%)]	46/60 (77%)	147/147 (100%)	<.0001
Positive late potentials [n/N (%)]	36/45 (80%)	91/117 (78%)	.76
QRS duration in lead V_2 (ms)	98 ± 17	97 ± 13	.88
QTc interval in lead V_2 ($ms^{1/2}$)	414 ± 56	406 ± 40	.52
QRS duration in lead V_6 (ms)	97 ± 13	94 ± 14	.32
Presence of J wave	4 (6%)	13 (9%)	.49
Follow-up period (months)	67 ± 41	60 ± 31	.20

Values are given as no. (%) or mean ± SD unless otherwise indicated.

AF = atrial fibrillation; ECG = electrocardiogram; SCD = sudden cardiac death; VF = ventricular fibrillation; VT = ventricular tachycardia.

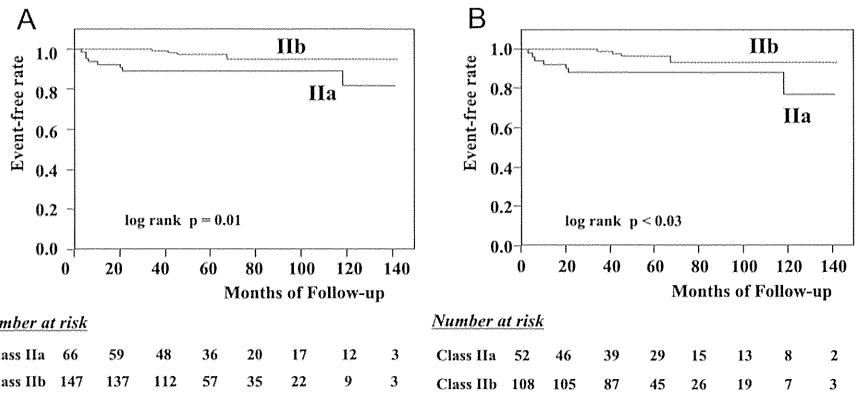

Figure 1 Kaplan–Meier curves of cardiac events during follow-up in patients with Class IIa and Class IIb indication for ICD. **A:** Kaplan-Meier curves of cardiac events in the total 213 patients with and without ICD implantation. **B:** Kaplan–Meier curves of cardiac events in 160 patients with ICD implantation.

and prevalence of inferolateral J wave defined as elevation of the QRS-ST junction (J point) of at least 0.1 mV in at least 2 inferior (II, III, aVF) and/or lateral (I, aVL, V_4– V_6) leads.[5,8]

Clinical outcome

All 213 patients were followed for a mean of 62 ± 34 months. The follow-up periods were not significantly different between the 2 groups (67 ± 41 months in Class IIa and 60 ± 31 months in Class IIb indication; $P = .20$). During follow-up, 8 of 66 patients (12%) with Class IIa indication and 4 of 147 patients (3%) with Class IIb indication had cardiac events, with event rates significantly higher in patients in Class IIa compared to Class IIb indication ($P = .01$; Figure 1A). The incidences of cardiac events per year were 2.2% and 0.5%, respectively. Among the 160 patients with ICD who met Class II indications (mean age 52 ± 13 years, 147 men, Class IIa [n = 52] and Class IIb [n = 108] indication), the clinical outcome was very similar to that of all 213 patients (Figure 1B).

When other proposed risk factors were evaluated in all patients with Class IIa indication, there were 5 incidences of cardiac events in 40 patients with induced VF, 1 event in 8 patients with family history of SCD, and 2 in 12 patients without other risk factors (Figure 2). In patients with Class IIb indication, the incidences of cardiac events were 1 in 20 in patients with syncope, 2 in 48 in those with spontaneous type 1 ECG, 1 in 22 in those with spontaneous type 1 ECG and family history of SCD, and no events in those with additional factors. None of the patients with induced VF alone experienced cardiac events (Figure 3).

Discussion

The present study is the largest survey of patients with BrS in Japan and enrolled representative patient population of BrS as reported previously.[5] Our cohort used the same inclusion criteria for BrS as other European studies.[9–12] This suggests our findings are applicable to general patient populations with BrS.

We confirmed the validity of Class II indications for ICD in the Consensus Statement with a large Japanese cohort of BrS patients without previous cardiac arrest. The incidence of cardiac events was significantly higher in patients with Class IIa indication than in those with Class IIb indication during a long follow-up period. Our findings support Class II indications for ICD in the Consensus Statement, which are useful for distinguishing intermediate- from low-risk patients with BrS, and clarify that Class IIa indication identifies a group of patients with increased risk compared to Class IIb indication. The combination of a history of syncope and spontaneous type 1 ECG may be important clinical variables for risk stratification of intermediate-risk BrS patients without previous cardiac arrest.

Previous history of syncope judged to be likely caused by ventricular arrhythmias has been recognized as an important risk factor for fast VT, VF, and SCD in most large-scale prospective studies,[9–13] except for a single Japanese study.[14] Spontaneous type 1 ECG has also been recognized as a risk factor for cardiac events and SCD in recent European prospective studies[9–13] but not in Japanese prospective

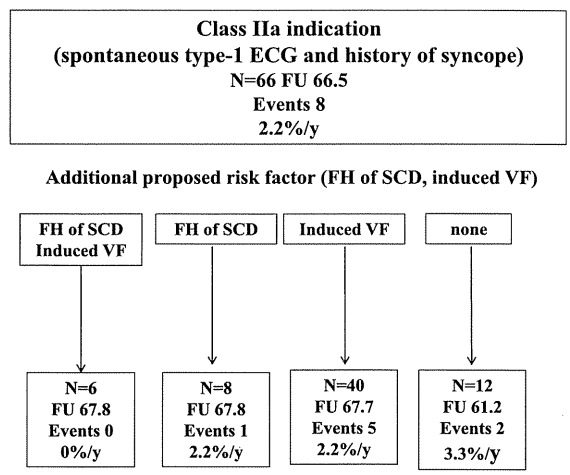

Figure 2 Mean event rate per year according to additional proposed risk factors during follow-up in patients with Class IIa indication for implantable cardioverter-defibrillator. FH = family history; FU = mean follow-up in months; SCD = sudden cardiac death; VF = ventricular fibrillation.

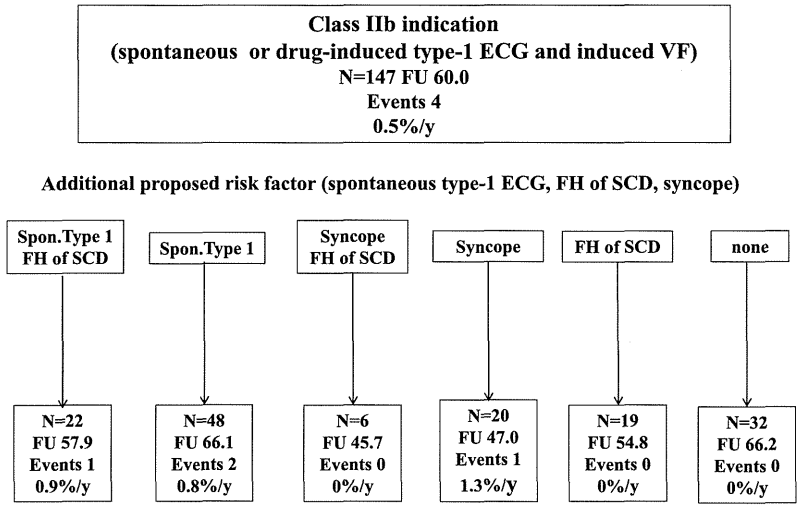

Figure 3 Mean event rate per year according to additional proposed risk factors during follow-up in patients with Class IIb indication for implantable cardioverter-defibrillator. FH = family history; FU = mean follow-up in months; SCD = sudden cardiac death; Spon. Type 1 = spontaneous type 1 ECG; VF = ventricular fibrillation.

studies.[5,14] These studies included patients with previous cardiac arrest. In European large-scale prospective studies of patients with BrS and no previous cardiac arrest,[12,13] previous history of syncope or spontaneous type 1 ECG has been acceptable as a risk factor for cardiac events and SCD. Recently, Delise et al[15] concluded the limited clinical value of any single risk factor (spontaneous type 1 ECG, familiar juvenile SCD, and positive PES) in predicting cardiac events in patients without previous cardiac arrest. Our study indicated that the combination of spontaneous type 1 ECG and previous history of syncope rather than either one of them individually was useful for identifying intermediate risk for cardiac events (2.2% per year) as well as ICD indication in patients without previous cardiac arrest.

The prognostic value of inducible VT/VF by PES has been controversial, either supporting[10,12] or disregarding a prognostic value in large-scale studies.[4,5,9,11,13,14] Although negative results were attributed to be caused, at least in part, by methodologic variations used by different institutions, the PRELUDE study, using a fixed stimulation protocol and end-point determination, demonstrated that positive PES was not proven to be a significant risk factor.[13] In our study, 5 cardiac events occurred in 40 patients with induced VT/VF (Figure 2), which appeared to be a rate comparable to that of patients with syncope and spontaneous type 1 ECG. However, inducible VT/VF was not associated with cardiac events in patients with Class IIb indication for ICD.

It should be kept in mind that the decision to implant an ICD in patients with Class IIb indication for ICD should be carefully weighed against the low rate of cardiac events (0.5% per year) and the high rate of inappropriate shocks.[16,17] It should be noted, however, that even in patients with Class IIb indication, some patients experienced cardiac events. The number of patients with cardiac events was still too small to evaluate predictors of cardiac events in the present study. Identification of patients at risk with Class IIb indication may be the primary objective of future studies.

Study limitations

In the present study, we enrolled a small number of patients without an ICD (n = 53) who met Class II indications for ICD implantation according to the HRS/EHRA/APHRS Expert Consensus Statement. Although the clinical outcome in patients with an ICD was very similar to that in all patients in the present study, a large prospective study would be necessary to evaluate the incidence of SCD in patients without ICD who met Class II indications.

In this study, the number of patients with cardiac events during follow-up was too small, so each individual risk factor, such as spontaneous type 1 ECG and previous history of syncope, might show a low positive predictive value and a high negative predictive value for event risk. Further continuing study to accumulate a larger number of patients is needed to improve our understanding of other proposed risk factors in BrS patients with Class II indication for ICD implantation.

Conclusion

This largest survey of Japanese patients with BrS demonstrated the usefulness of the Class II indication for ICD in the HRS/EHRA/APHRS Expert Consensus Statement for distinguishing intermediate- from low-risk Japanese patients with BrS. The combination of a history of syncope and spontaneous type 1 ECG may be important in identifying intermediate-risk BrS patients without previous cardiac arrest.

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Appendix

The following executive committee participated in the J-IVFS: Tachikawa Medical Center, Nagaoka: Y. Aizawa; Nippon Medical School Tama Nagayama Hospital, Tama: H. Atarashi; University of Toyama, Toyama: H. Inoue; The Sakakibara Heart Institute of Okayama, Okayama: T. Ohe; International University of Health and Welfare, Mita Hospital, Tokyo: S. Ogawa; Hirosaki University School of Medicine, Hirosaki: K. Okumura; National Cerebral and Cardiovascular Center, Suita: S. Kamakura; Yamagata University Faculty of Medicine, Yamagata: I. Kubota; Nagoya University School of Medicine, Nagoya: I. Kodama; Showa University School of Medicine, Tokyo: Y. Kobayashi; Hokko Memorial Hospital, Sapporo: M. Sakurai; Ohkubo Hospital, Tokyo: H. Sakurada; Yamaguchi University School of Medicine, Ube: A. Shimizu; Nippon Medical School, Tokyo: W. Shimizu; Toho University, Tokyo: K. Sugi; University of Fukui, Fukui: H. Tada; Chukyo Hospital, Nagoya: N. Tsuboi; Gunma Cardiovascular Center, Maebashi: S. Naito; Yokohama Minami Kyousai Hospital, Yokohama: M. Nishizaki; University of Tsukuba, Tsukuba: A. Nogami; Tokyo Women's Medical University, Tokyo: N. Hagiwara; Chikamori Hospital, Kochi: M. Fukatani; Shiga University of Medical Science, Otsu: M. Horie; St. Marianna University School of Medicine, Kawasaki: N. Matsumoto; Nagasaki International University, Sasebo: K. Yano; Sumiyoshi Clinic, Mito: I. Yamaguchi; Nagoya Daini Red Cross Hospital , Nagoya: Y. Yoshida; Fujita Health University, Toyoake: E. Watanabe.

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CLINICAL PERSPECTIVES

Indication for ICD implantation in BrS patients without previous cardiac arrest poses a difficult clinical judgment because the effective therapeutic option for preventing SCD in BrS is the ICD, but the device is expensive and is not free from complications. The recent HRS/EHRA/APHRS Expert Consensus Statement proposed Class IIa and IIb indications for ICD in BrS patients. Previous cardiac arrest of documented VT/VF or aborted SCD is a Class I indication for an ICD because of the high risk for recurrence of cardiac events in such patients. ICD indication for patients without previous cardiac arrest can be judged by Class II and III indications. We validated the Class II indication in the HRS/EHRA/APHRS Expert Consensus Statement to distinguish intermediate- from low-risk patients with BrS having no history of cardiac arrest in a Japanese multicenter study. Patients having Class IIa indication, with spontaneous type 1 ECG and a history of syncope due to ventricular arrhythmias, were shown to be at higher risk than those having Class IIb indication of induced VT/VF by PES. Class IIa indication for ICD implantation could be considered a therapeutic option because of the intermediate risk of cardiac events. The results further suggest that careful attention must be given to repeated ECG examinations to detect spontaneous type 1 ECG and to explore the nature of syncope caused by ventricular tachyarrhythmias in the management of BrS patients without previous cardiac arrest.

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Review

Molecular mechanisms of heart failure progression associated with implantable cardioverter-defibrillator shocks for ventricular tachyarrhythmias

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ABSTRACT

Implantable cardioverter-defibrillators (ICDs) are highly effective in reducing mortality related to ventricular tachyarrhythmias (VTAs). Despite this benefit, the occurrence of ICD shocks for VTAs in patients with heart failure (HF) and depressed left ventricular function has been associated with adverse outcomes. Patients with shocked VTAs are at an elevated risk of HF and death. While VTAs may be markers for high-risk patients, it is possible that the harmful effects of electrical shocks and VTAs are involved in HF progression and associated mortality. Some investigators have speculated that shocked VTAs may activate signaling pathways in the molecular cascade of HF. We recently reported in an experimental model of ventricular fibrillation storm that multiple ICD shocks for recurrent ventricular fibrillation caused striking activation of Ca^{2+}/calmodulin-dependent protein kinase II, a validated signaling molecule for HF. This review article describes the harmful effects of shocks and VTAs and proposes that Ca^{2+}/calmodulin-dependent protein kinase II could connect shocked VTAs to adverse outcomes.

Contents

1. Introduction

The implantable cardioverter-defibrillator (ICD) improves survival in patients with a history of ventricular tachyarrhythmias (VTAs) or those with heart failure (HF) and depressed left ventricular function [1–4]. Despite this survival advantage, an important prognostic association between ICD shocks for VTAs and adverse outcomes has been demonstrated. Patients receiving an ICD shock for a spontaneous VTA are at increased risk of death [5,6]. Patients with more ICD-shocked VTA episodes have a higher risk of death than patients with less [6]. Electrical storm, a syndrome characterized by frequent ICD shocks for multiple VTA episodes over a short period, has more

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serious prognostic consequences than VTAs unrelated to electrical storm [7]. Electrical storm survivors are at high risk of early death during the first 3 months after the phenomenon [8,9]. These deaths after ICD shocks for VTAs are commonly related to progressive HF [5,6,8–11]. The association of shocked VTAs, HF, and mortality is clear, although the underlying mechanisms remain uncertain. While VTA episodes may be a marker for high-risk patients, there is the possibility that harmful effects of VTAs, shocks, and their combination are involved in HF progression and associated mortality. Some investigators have speculated that ICD shocks for VTAs may activate signaling pathways in the molecular cascade of HF [7,8,12,13]. We recently created a chronic animal model of ventricular fibrillation (VF) storm in which left ventricular function deteriorated along with striking activation of the intracellular Ca^{2+}-sensitive phosphorylating enzyme Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [14], a validated signaling molecule causing HF. The aim of this paper is to discuss the potential roles of shocks and VTAs in HF progression and to present our proposal that CaMKII could connect shocked VTAs to adverse outcomes.

2. Relationship among ICD shocks, substrate, and mortality

There is a strong correlation between ICD-shocked spontaneous VTAs and subsequent mortality [5,6]. Patients who receive inappropriate shocks are also at an increased risk of mortality, although the magnitude of this increase is smaller [5,6]. These findings, together with the fact that the electrical shock may cause myocardial damage and dysfunction, suggest that the ICD shock itself is harmful, adversely affecting prognosis. However, this is uncertain because the significant correlation between shocks and prognosis was not proved in subsequent clinical studies [15,16]. Whether shocks play an independent causal role or whether this correlation is due solely to the underlying disease and arrhythmia is strongly debated [17–19]. Recently, a retrospective study enrolling a large number of ICD recipients, which was expected to resolve this conflict, has been published. The ALTITUDE Survival by Rhythm Study [20] demonstrated that patients who received shocks for VTAs and atrial fibrillation had an increased risk of death and that there was no increased risk of mortality for those with inappropriate shocks for sinus tachycardia or lead noise/artifact/oversensing. This indicated that the adverse outcomes after ICD shocks are more closely related to the underlying disease and arrhythmia than to a harmful effect from the shock. Conversely, the importance of shocks on the prognosis has been suggested by another pivotal clinical trial. The MADIT-RIT study demonstrated that the programming of ICD therapies for VTAs of a high rate or those with a prolonged delay in therapy was associated with reductions in inappropriate therapy and mortality [21]. Reductions in the number of inappropriate shocks and the associated reductions in total shock energy contributed in part to the mortality benefit in the high rate and delayed therapy group. The potential roles of shocks and VTAs in HF progression, based on currently available information obtained in clinical and experimental studies, are discussed in the following sections.

2.1. Adverse effects of shocks

There is extensive literature on the harmful effects of electrical shocks. Transient impairment of cardiac function, mild elevation of cardiac troponin I levels, pathological changes (inflammation, fibrosis, calcification, macrophage infiltration, myocyte necrosis, and interstitial edema), and ultrastructural alterations (mitochondrial swelling, loss of membrane integrity, and mitochondrial crest disruption) have been demonstrated in human and animal studies [22,23]. Many of these changes are linked to electroporation, the disruption of cell membranes by an electrical shock [24]. Electroporation recovers over seconds to minutes, but cell membranes are unable to recover following severe electroporation injury, leading to cell death [24]. There is an assumption that irreversible electroporation may be an important contributor to worsening HF and poor outcomes [18]. However, this is unlikely because of conflicting observations that electroporation caused by the shock occurs at limited regions. Wang et al. examined the spatial distribution and extent of electroporation by assessing propidium iodide uptake in normal and infarcted rabbit hearts subjected to a clinically relevant internal shock for sustained VF [25]. The majority of propidium iodide staining was observed near the shock electrode positioned at the right ventricular apex. The total amount of propidium iodide staining was only ~4% of the entire ventricular mass, comparable to that between normal and infarcted hearts. The hearts that were allowed to recover for 45 min after a defibrillation shock showed substantial reduction in propidium iodide staining. Nevertheless, the possibility that electroporation may be more likely induced in failing myocardium cannot be excluded. A study in patients with structural heart disease, HF, and implanted ICD for primary and secondary prevention of sudden cardiac death found that the instantaneous emergence of local injury current on intracardiac right ventricular electrograms, which was probably caused by electroporation, during defibrillation threshold testing was a predictor of HF hospitalization and death [26]. Moreover, the local injury current has recently been shown to appear after spontaneous appropriate and inappropriate ICD shocks in some individuals [13]. More studies are needed to clarify the effects of electroporation on cardiac function in intact failing hearts, as well as the significance of local injury current following spontaneous ICD shocks on prognosis in randomized clinical studies.

An ICD shock activates the sympathetic nervous system. Systemic catecholamine levels increase three-fold and persist for 10 min following an ICD shock for induced VF in patients, which is similar to an external shock for atrial fibrillation [27]. It remains uncertain whether transient increases in sympathetic activity are associated with long-term effects, but it is clear that this is harmful in some patients. Sympathetic stimulation can cause vasoconstriction and amplify myocardial ischemia in the setting of decreased coronary reserve [28]. Adrenergic surge plays a role in an electrical storm [7]. Acute β-adrenergic receptor stimulation activates CaMKII, a validated signal for HF (see Section 4) in cardiomyocytes [29]. Additionally, a recent study using cardiomyocyte cultures reported that electric shock caused an elevation of diastolic Ca^{2+}-levels, probably via electroporation [30].

ICD shocks often cause psychiatric disorders. Patients have decreased quality of life, including emotional dysfunction, during the month following an ICD shock. Patients with anxiety and depression have an activated hypothalamus–hypophysis–adrenal axis and increased sympathetic activity [22]. Chronic sympathetic activation could directly affect the myocardium and worsen cardiac dysfunction. Chronic daily isoproterenol injection for 2 weeks causes cardiac dysfunction and dilatation in mice [31]. Mice overexpressing protein kinase A (PKA), β-adrenergic receptor signaling molecule, develop dilated cardiomyopathy with reduced cardiac contractility in association with aberrant intracellular Ca^{2+}-homeostasis [32].

2.2. Adverse effects of ventricular tachyarrhythmias

The condition of myocardium at the time of VTA termination may be an important determinant for patient outcome. As VF accompanies myocardial ischemia, global ischemic stunning contributes to myocardial depression after defibrillation. Oxygen-derived free radical formation and cytosolic Ca^{2+}-overload because

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of ischemia/reperfusion [33] might be involved in post-defibrillation stunning. Activation of Ca^{2+}-activated proteolytic enzyme calpains, proteolysis of troponin I and α-actinin, acidosis-induced reduction in myofilament Ca^{2+} responsiveness, and tissue injury by activation of nuclear factor-κB (NF-κB), a transcription factor of proinflammatory response genes underlie contractile dysfunction associated with ischemia/reperfusion [33,34].

Contractile dysfunction following defibrillation occurs even in the absence of ischemia or acidosis. Studies in perfused hearts reported that VF caused contractile dysfunction without acidosis or ATP depletion [35,36]. Electromechanical dissociation, the most common cause of death during defibrillation testing [37], occurs even under the conditions of anesthesia and oxygenation. These findings suggest that excess cytosolic Ca^{2+} during VF affects contractile function, independent of ischemia/reperfusion, and allows us to hypothesize that VTAs-induced Ca^{2+}-overload worsens the substrate for HF by augmenting disease pathways. This may be supported by clinical evidence that the association between shocked VTAs and mortality is influenced by VTA subtype. Increased mortality risk is greatest for VF and lowest for slow ventricular tachycardia [18].

Experimental and clinical findings from the literature suggest that activation of signaling and effector molecules by Ca^{2+}-overload resulting from VTAs in combination with shocks and associated adrenergic surge may be responsible for HF progression and adverse outcomes in patients with shocked VTAs.

3. Calcium signaling in an experimental model of VF storm

Intracellular Ca^{2+}-cycling is controlled by ion channels and transporters, including L-type Ca^{2+} channel, sarcoplasmic reticulum (SR) Ca^{2+}-release channel ryanodine receptor (RyR2), SR Ca^{2+}-pump (SERCA2a), its regulatory protein phospholamban (PLB), and Na^{+}–Ca^{2+} exchanger (NCX). Protein phosphorylation is a major regulatory mechanism for contractility and relaxation (Fig. 1). Phosphorylating mediators include PKA, CaMKII, and protein kinase Cα (PKCα). Under physiological conditions, CaMKII is activated by a fast heart rate and CaMKII-mediated phosphorylation of these Ca^{2+}-handling proteins increases Ca^{2+} influx and SR Ca^{2+}-storage, leading to increased intracellular systolic Ca^{2+} and increased contractility. PKA is activated by β-adrenergic receptor agonists and catalyzes phosphorylation of the same Ca^{2+}-handling proteins modified by CaMKII, but at different amino acids. PKCα is activated downstream to a variety of G-protein-coupled receptors such as angiotensin II receptor, α-adrenoreceptor and endothelin receptor, and is activated by increased intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$). PKCα activation leads to decreased activity of SERCA2a by phosphorylating inhibitor-1 (I-1). PKCα -mediated phosphorylation of I-1 leads to PLB dephosphorylation, resulting in reduced SR Ca^{2+} load and Ca^{2+} release, causing reduced contractility [38]. While cardiac function is regulated by these protein kinases, excess activity of PKA [32], CaMKII [39–41], and PKCα [42] and the resulting hyperphosphorylation of these Ca^{2+}-handling proteins are linked to mechanical dysfunction and arrhythmias.

We examined the alterations in Ca^{2+}-signals and protein phosphorylation in a rabbit model of VF storm (Fig. 2) [14]. This animal model was developed from a rabbit model of chronic complete atrioventricular block (CAVB). CAVB rabbits show biventricular hypertrophy and electrophysiological features similar to long QT syndrome including torsades de pointes-like arrhythmia, VF, and sudden death [43,44]. CAVB rabbits equipped with ICDs followed up for ~80 days all showed cardiac hypertrophy, long QT,

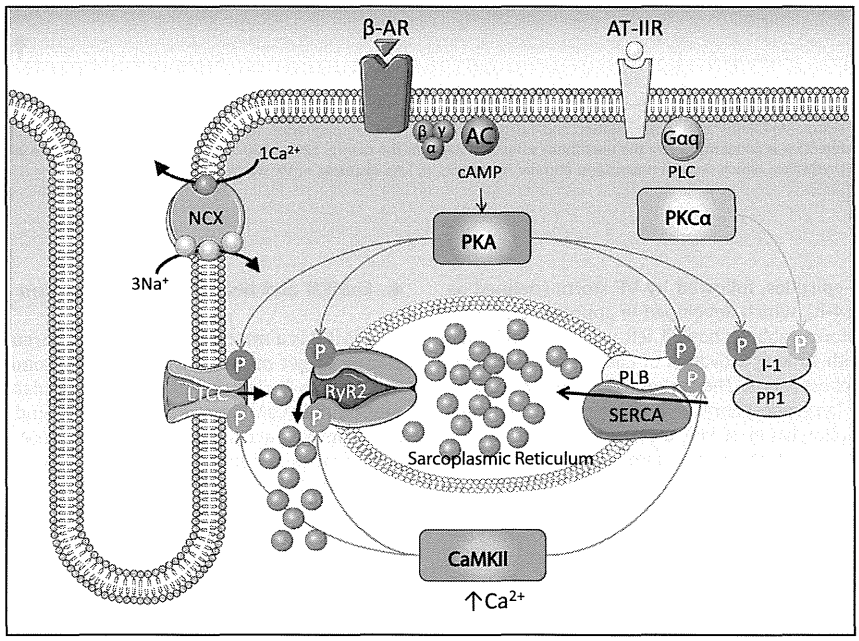

Fig. 1. Regulation of intracellular Ca^{2+} cycling. β-Adrenergic activation of the cAMP-dependent protein kinase (PKA) directly phosphorylates L-type Ca^{2+} channel (LTCC), Ca^{2+}-release channel (RyR2), and phospholamban (PLB), increasing the gain of excitation-contraction coupling. Faster heart rates increase the average $[Ca^{2+}]_i$, which activates Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). CaMKII can phosphorylate LTCC, RyR2, and PLB. Activation of the angiotensin-II receptor (AT-IIR) or α-adrenoceptor (α-AR) activates phospholipase C (PLC), which, in turn, activates protein kinase Cα (PKCα). PKCα can directly phosphorylate protein phosphatase inhibitor-1 (I-1), augmenting the activity of the protein phosphatase-1 (PP1) and causing hypophosphorylation of PLB. PLB hypophosphorylation inhibits SERCA2a function. PKA-phosphorylated I-1 inhibits PP1, augmenting the PLB phosphorylation resulting from direct PKA action. The expression and activity of CaMKII and PKCα are upregulated in human and animals with HF.

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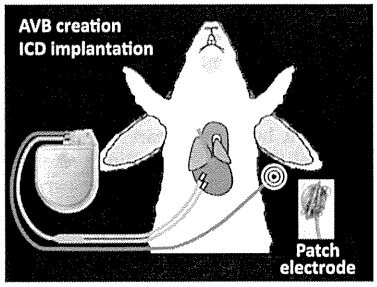
A



B

ICD parameter in rabbits

	Enable	Initial	Redetect	V Interval (Rate)
VF	On	120/160	30/40	240 ms (250 bpm)
FVT	Off			
VT	Off			

Therapy	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6
VF	3 J	6 J	10 J	12 J	13 J	14 J

C

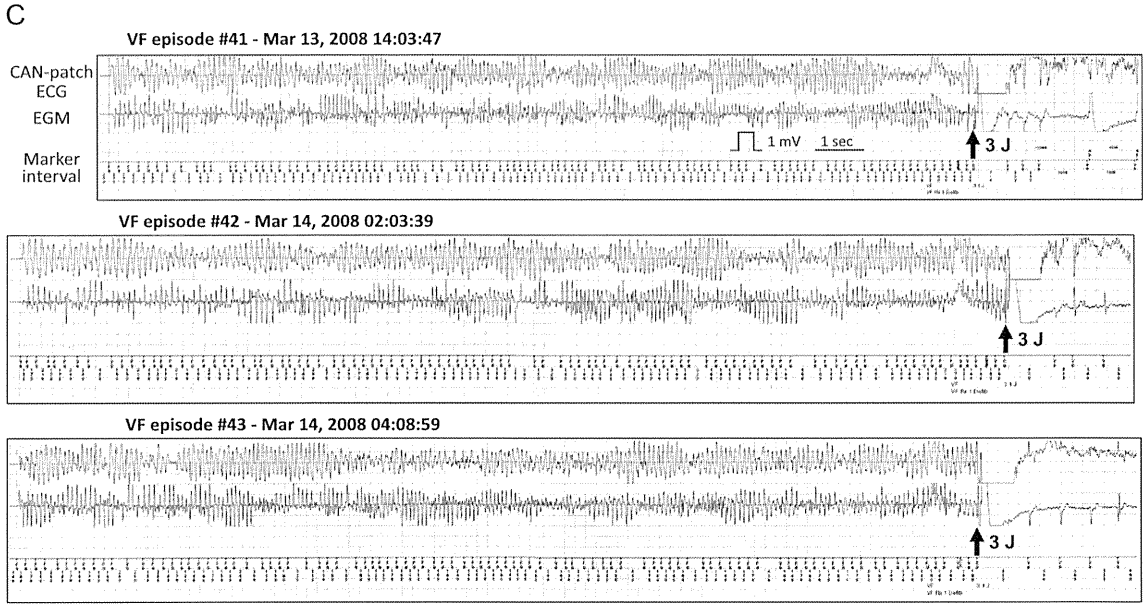


Fig. 2. Rabbit model of VF storm. (A) Chemical AV node ablation and extracardiac ICD system implantation. (B) ICD parameters. The number of intervals to detect VF (NID) and to redetect VF (NID redetect) was programmed to the maximum values available in the device, 120 of 160 and 30 of 40, respectively, to avoid inappropriate shocks for torsades de pointes-like arrhythmias, which occur frequently in chronic AV rabbits. (C) An example of VF storm (modified from Tsuji et al. [14]).

and ICD-detected VF-episodes, followed by VF storm (defined as ≥ 3 VF episodes per 24-h), which developed in approximately 50% of CAVB rabbits. VF storm rabbits showed left-ventricular function deterioration, along with striking activation of CaMKII and enhanced expression of protein phosphatase. These alterations were associated with notable changes: hyperphosphorylation of L-type Ca^{2+} channel and RyR2 and dephosphorylation of PLB, a phosphorylation pattern similar to findings in failing human and animal hearts [45]. These prominent changes in VF storm rabbits were attributed in part to the direct effects of repeated VF and defibrillation. Indeed, VF induction and defibrillation 10 times over 1 h in baseline rabbits reproduced the changes observed in CaMKII activation and PLB phosphorylation in VF storm rabbits, but 10 shock pulses without VF induction did not. PKAα catalytic subunit was decreased similarly in VF storm and non-storm rabbits. PKA-dependent phosphorylation of myosin-binding protein C and troponin I did reduce, but there was no difference between the VF storm and non-storm rabbit groups. Neither PKCα catalytic subunit expression nor PKCα activity were altered. These results suggest that CaMKII, but not PKA or PKCα, plays a central pathophysiological role in left ventricular dysfunction associated with VF storm in this animal model.

4. CaMKII and heart failure progression

CaMKII is a multifunctional serine–threonine kinase, phosphorylating target proteins. Under resting conditions, CaMKII is inactive. A rise in intracellular Ca^{2+} stimulates binding of Ca^{2+} to calmodulin (CaM), a ubiquitous Ca^{2+}-binding protein. The Ca^{2+}/CaM complex activates CaMKII (Ca^{2+}/CaM-dependent activation). With a sustained increase in Ca^{2+}/CaM or reactive oxygen species (ROS), CaMKII transitions into a Ca^{2+}/CaM-autonomous active enzyme after autophosphorylation at Thr286 or oxidation at Met281/282 (Ca^{2+}/CaM-independent activation) [46,47]. This activation is maintained under normal Ca^{2+} concentrations, a feature that has been regarded to be similar to a "memory" molecule [47]. Autophosphorylated and/or oxidized CaMKII that become constitutively active participate in the disease pathway by phosphorylating protein targets for excitation–contraction coupling and cell survival, including ion channels and Ca^{2+}-handling proteins, and transcription factors that drive hypertrophic and inflammatory gene expression. CaMKII is now linked to the pathophysiology of a variety of cardiac diseases including HF, myocardial infarction, ischemia/reperfusion and cardiac arrhythmias, most of which have

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been demonstrated by Anderson et al. and reviewed in their excellent articles [45,47–49].

The expression and activity of CaMKII are increased in human and animals with HF. CaMKII-overexpressing mice died prematurely with HF phenotype [39–41]. Deletion of CaMKIIδ, a predominant cardiac isoform, prevented transition from pressure overload-induced hypertrophy to HF in mice [50].

CaMKII triggers diverse, maladaptive cellular events essential to HF development, including hypertrophy, inflammation, and cell death. Excessive CaMKII activates hypertrophic genes by phosphorylating class II histone deacetylases and by derepressing myocyte enhancer factor 2-dependent transcription [51]. CaMKII mediates NF-κB activation to activate proinflammatory genes after myocardial infarction [31] and ischemia/reperfusion [34]. Genetic CaMKII inhibition attenuates adverse remodeling and protects against HF after myocardial infarction in mice [31].

Excessive CaMKII also causes mitochondrial Ca^{2+} dysregulation and activates the cell death pathway [49]. An increase in mitochondrial Ca^{2+} concentrations secondary to cytosolic Ca^{2+} overload depolarizes the membrane potential across the inner membrane potential ($\Delta\psi_m$), produces ROS, and activates mitochondrial permeability transition pore (mPTP) opening, followed by the subsequent release of apoptosis-related proteins into the cytosol [49]. CaMKII promotes $\Delta\psi_m$ depolarization and mPTP opening in permeabilized rat myocytes [52]. A recent study has found that CaMKII phosphorylates mitochondrial Ca^{2+} uniporter, a mitochondrial Ca^{2+} entry channel, resulting in increased inward current into the mitochondria. Mitochondrion-delimited CaMKII inhibition prevented mPTP opening and $\Delta\psi_m$ depolarization and diminished mitochondrial disruption and cell death in response to ischemic/reperfusion injury in mice [53].

The effect of CaMKII on ion channels contributes to aberrant intracellular Ca^{2+} homeostasis in HF. CaMKII-dependent hyperphosphorylation of RyR2 increases inappropriate diastolic Ca^{2+} release from SR that promotes HF (because of loss of SR Ca^{2+} load) and arrhythmogenic delayed afterdepolarizations. Pharmacological CaMKII inhibition reduces the phosphorylation levels of RyR2 at both Ser2809 (PKA/CaMKII phosphorylation site) and Ser2815 (CaMKII site) and improves contractility along with reduced SR Ca^{2+} leak and increased SR Ca^{2+} stores in failing human myocardium [54]. Mice with constitutively phosphorylated RyR2 that can be attributed to mutant S2814D have RyR2-mediated SR Ca^{2+} leak and develop late onset cardiomyopathy [55]. Moreover, knock-in mice with an inactivated Ser2814 phosphorylation site are protected from HF development after transverse aortic constriction [56].

CaMKII phosphorylation of L-type Ca^{2+} channels alters channel gating by increasing open probability. CaMKII increases the window current of $I_{Ca\text{-}L}$, increasing the probability of the channels reopening during phase 2 action potential and predisposing to early afterdepolarizations. Recently identified phosphorylation sites of L-type Ca^{2+} channels include Ser1512 and Ser1570 of the α-subunit $Ca_V1.2$ and Thr498 of the accessory β_{2a}-subunit [57,58]. However, the relative relationship between these phosphorylation sites and $I_{Ca\text{-}L}$ changes remains unclear. Interestingly, a study using rabbit ventricular myocytes overexpressing β_{2a} demonstrated that Thr498 phosphorylation reduced cell survival [59]. The authors suggested that CaMKII phosphorylation of β_{2a} could initiate a pathological cascade of enhanced cellular Ca^{2+} entry causing excessive SR Ca^{2+} release, leading to reduced cell survival.

CaMKII action on voltage-gated Na^+ channel subunit $Na_V1.5$ has also been implicated as a potent contributor to HF progression. HF is associated with increased intracellular Na^+ concentrations ($[Na^+]_i$). The rise in $[Na^+]_i$ increases Ca^{2+} influx via reverse model NCX during systole and limits Ca^{2+} efflux via forward NCX during diastole, leading to intracellular Ca^{2+} overload. Increases in diastolic Ca^{2+} levels cause abnormal left ventricular relaxation and diastolic dysfunction. The major pathway for increased Na^+ influx is through Na^+ channels. Although Na^+ channels open and close rapidly, a non-inactivated, persistent component of Na^+ current ($I_{Na\text{-}L}$) is enhanced in HF. In tachypacing-induced HF dogs, the difference in $I_{Na\text{-}L}$ before and after CaMKII inhibitor KN93 was greater in failing myocytes than in the control [60]. CaMKII associates with and phosphorylates $Na_V1.5$ at the linker between domain I and II [61,62]. A recent study in rat ventricular myocytes reported that an increase in $I_{Na\text{-}L}$ caused an elevation in the intracellular Ca^{2+} levels and activation of CaMKII, which, in turn, phosphorylated $Na_V1.5$, further promoting $I_{Na\text{-}L}$ and subsequent sodium-dependent Ca^{2+} overload [63].

5. Therapeutic consideration

From the results of larger-scale clinical trials demonstrating a high frequency of HF and non-sudden cardiac death in patients with shocked VTAs, optimization of HF therapy is strongly recommended [5,7]. Because β-blockers, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, and spironolactone reduce cellular Ca^{2+} load and ROS production, these HF medications are likely to possess CaMKII inhibitory actions. This notion, although not proven, might be supported by some experimental studies. Metoprolol reverses the hyperphosphorylation of RyR2, restores the stoichiometry of the RyR2 macromolecular complex, and normalizes single-channel function [64]. The angiotensin-receptor blocker valsartan restores SR function along with attenuation of RyR2 hyperphosphorylation and a decrease in abnormal SR Ca^{2+}-leak [65].

Therapeutic approaches targeting leaky RyR2 and $I_{Na\text{-}L}$ have recently emerged. RyR2 stabilizers JTV-519 and the more specific analog S107 reduce SR Ca^{2+} leak by increasing the binding affinity for FKBP12.6 to RyR2 [66,67], whereas dantrolene, a therapeutic agent for malignant hyperthermia, reduces SR Ca^{2+} leak by correcting domain unzipping [68]. RyR2 stabilization with these agents improves contractile function in dogs with HF [69]. Carvedilol [70] and $I_{Na\text{-}L}$ blocker ranolazine (not available in Japan) [71] have been demonstrated to reduce RyR2 single-channel open probability. Low-dose carvedilol prevents Ca^{2+} leak via RyR2 stabilization in HF dogs [72]. Inhibition of $I_{Na\text{-}L}$ with ranolazine exerts beneficial effects on systolic and diastolic HF in experimental models [73]. These agents might also mitigate CaMKII overactivity by improving aberrant Ca^{2+} homeostasis. In clinical situations where interventions targeting CaMKII are not available, maximization of both conventional and newly developed HF regimes should be underscored for the management of ICD patients at risk.

6. Conclusions

Based on our findings using a chronic animal model *in vivo* that repeated VF and defibrillation, not shocks, are associated with striking CaMKII activation, together with accumulating evidence that CaMKII is crucially involved in cellular processes for HF, it is conceivable that CaMKII activation contributes to HF progression and associated mortality in patients with shocked VTAs. Development of cardiospecific CaMKII inhibitory molecules with safe drug-like properties is desirable and necessary for confirming the efficacy on the HF phenotype, as well as clarifying this notion in diseased human hearts in further studies.

Conflict of interest

The authors have no conflicts of interest to declare.

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