Mean QT_c interval of 9 probands were 494 ± 90 ms. Although there is only one family with IVS7 +3A>G mutation, clinical features were apparently severer in families with c.1032G>A mutation.

3.2. Mutation analysis

DNA samples from 3 members of the family were subjected to a mutation screening of the KCNQ1 gene. An abnormal migration pattern was identified by DHPLC analysis (Fig. 2a; note the greater height of the left peak in II-1 and I-2 as indicated by the arrows) in KCNQ1 exon 7 of the 2 affected individuals. The control sample and the father (I-1) showed a normal pattern, as indicated by the comparable height of the left and right peaks (Fig. 2a). DNA sequencing identified a heterozygous adenine to guanine transition in KCNQ1 at nucleotide IVS7 +3 (IVS7 +3A>G) (Fig. 2b right panel), which located in the 5′ splice-site of intron 7. Fig. 2b left panel shows a schematic structure of KCNQ1 channel subunit. The exon 7 spans from part of the P-loop to part of the S6 region (indicated by red color).

3.3. Screening of KCNQ1 splicing mutation using minigene assay

Minigene assay was performed in COS7 cells to assess the effect of the IVS7 +3A>G mutation on the splicing of KCNQ1 exon 7. Fig. 3a shows the construct of minigene harboring KCNQ1 exon 7 and its flanking introns inserted into the pSPL3 vector. We also tested 3 other neighboring mutations that may affect the splicing of exon 7 (c.1022C>T, c.1032G>A, IVS7 +28T>C). c.1032G>A was used as a positive control that we and others reported to cause skipping of exon 7 [10,12].

The control KCNQ1 minigene expression in COS7 cells resulted in a production of the single mRNA band that corresponds to KCNQ1 exon 7 joined to the vector exons (Fig. 3b). The mutant minigene containing c.1022C>T or IVS7 +28T>C also showed the same single mRNA, indicating these mutations do not cause aberrant splicing (Fig. 3b). However, the mutant minigene containing IVS7 +3A>G, as well as the positive control c.1032G>A, generated 2 major mRNA bands that correspond to the normal transcript and a shorter transcript lacking KCNQ1 exon 7 respectively (Fig. 3b). The expression level of the shorter mRNA band appeared to be greater in c.1032G>A compared with IVS7 +3A>G. No band was detected in the RNA sample without reverse-transcriptase. We confirmed similar results both in CHO and HL-1cells under the same experimental conditions (data not shown).

3.4. Identification of exon-skipping KCNQ1 mRNAs in patient's blood sample

To directly confirm the minigene assay results, total RNA samples extracted from the patients' lymphocytes were subjected to RT-PCR (Fig. 4a), using primers spanning exons 5 through 10. Samples from individuals having IVS7 +3A>G and c.1032G>A showed shorter bands as well as the normal-sized WT. The direct sequencing of these short-sized transcripts revealed the existence of three kinds of exon-skipping mRNAs as indicated to the right of panel 4a (Δ7-8:399 bp, Δ7: 495 bp, Δ8: 510 bp, WT: 606 bp). Nucleotide sequence of each of the exon-skipping mRNAs is also shown. Control, c.1022C>T and IVS7 +28T>C showed normal patterns; the predominant WT and a small portion of Δ8.

3.5. Quantification of exon-skipping KCNQ1 mRNAs using real-time RT-PCR

We carried out quantitative analysis of short-sized mutant mRNAs in affected patients carrying the IVS7 +3A>G or c.1032G>A mutation, using real-time RT-PCR. Normal individuals had minor fractions of splicing variants (WT: 93.0 ± 0.7%, Δ7: 0.0 ± 0.0%, Δ7-8: 0.1 ± 0.0%, Δ8: 6.9 ± 0.7%, of total KCNQ1 transcripts; n = 4) as shown in the left bar graph of Fig. 4b. In contrast to c.1032G>A carriers who displayed a distinct exon skipping (WT: 55.2 ± 0.9%, Δ7: 23.5 ± 1.7%, Δ7-8: 16.8 ± 0.9%, Δ8: 4.5 ± 0.7%; n = 3, right bar graph in panel 4b), IVS7 +3A>G carrier showed modest but significant amount of exon skipping (WT: 81.2%, Δ7: 9.7%, Δ7-8: 5.7%, Δ8: 3.4%; n = 1, middle bar graph).

3.6. Biophysical characteristics of exon-skipping KCNQ1 proteins

Previously, we performed biophysical characterization of mutant KCNQ1 proteins (Δ7, Δ7-8, and Δ8) in *X. laevis* oocytes injected with mutant cRNAs. We demonstrated the *Xenopus* oocytes injected with Δ7, Δ7-8, or Δ8 alone displayed no time-dependent currents, indicating these mutants were non-functional. Furthermore, each exon-skipping KCNQ1 protein had the mutant-specific level of dominant-negative effect on WT channels [10].

In order to simulate the electrophysiological properties of cardiac cells of the affected patients, we injected the cRNAs (total 10 ng) with the relative ratios of WT and mutant KCNQ1 inferred from the data obtained in the real-time RT-PCR experiment (Fig. 4b). Oocytes injected at cRNA ratios comparable to those evaluated in IVS7 +3A>G showed remarkable reduction in currents compared with those of normal individuals, but less pronounced than c.1032G>A carriers; 100 ± 14.5% (n = 6) for control, 64.8 ± 4.5% (n = 7) for IVS7 +3A>G carriers ($p<0.05$), 41.4 ± 9.5% (n = 6) for c.1032G>A carriers ($p<0.05$) (Fig. 5).

3.7. Computer simulation

Finally, we performed a computer simulation study employing the 1D myocardial model (Fig. 6a) to explore the cellular mechanisms by which these splicing mutations manifest QT prolongation under exercise and induce ventricular tachyarrhythmias.

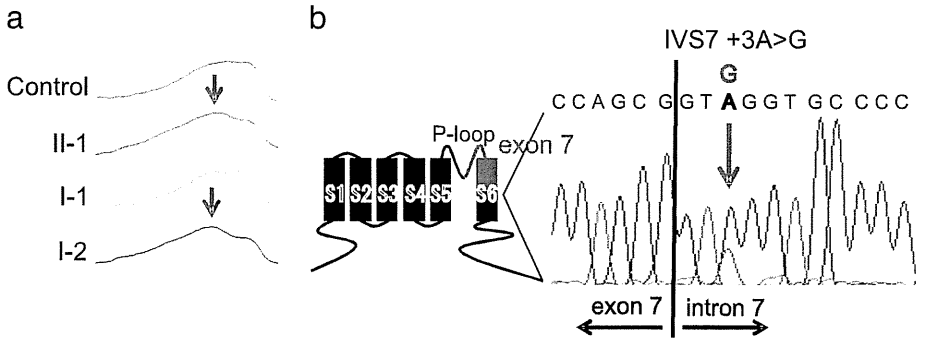

Fig. 2. Mutation analysis. (a) DHPLC revealed abnormal migration patterns in the affected individuals. (b) Left panel: scheme of the transmembrane topology of the cardiac KCNQ1 channel illustrating the location of exons 7 (red). Right panel: automated DNA sequencing electropherogram demonstrates IVS7 +3A>G mutation.

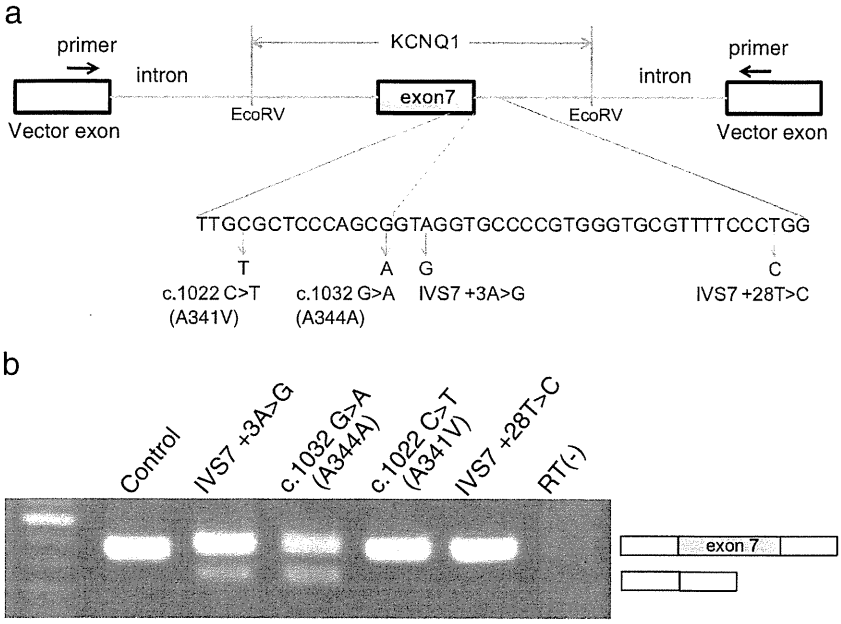

Fig. 3. Minigene analysis. (a) The structure of the minigene harboring KCNQ1 exon 7 (dark shaded box) and its flanking introns (lines between EcoRV sites) inserted into the pSPL3 vector. Open boxes and its flanking lines indicate pSLP3 vector exons and introns, respectively. Arrows upon the open boxes indicate the forward and reverse primers for RT-PCR. The locations of mutations studied in the assay are indicated. (b) RT-PCR from COS7 cells transfected with the minigene constructs. Two major bands were identified; one with KCNQ1 exon joined to vector exons, the other with vector exons only.

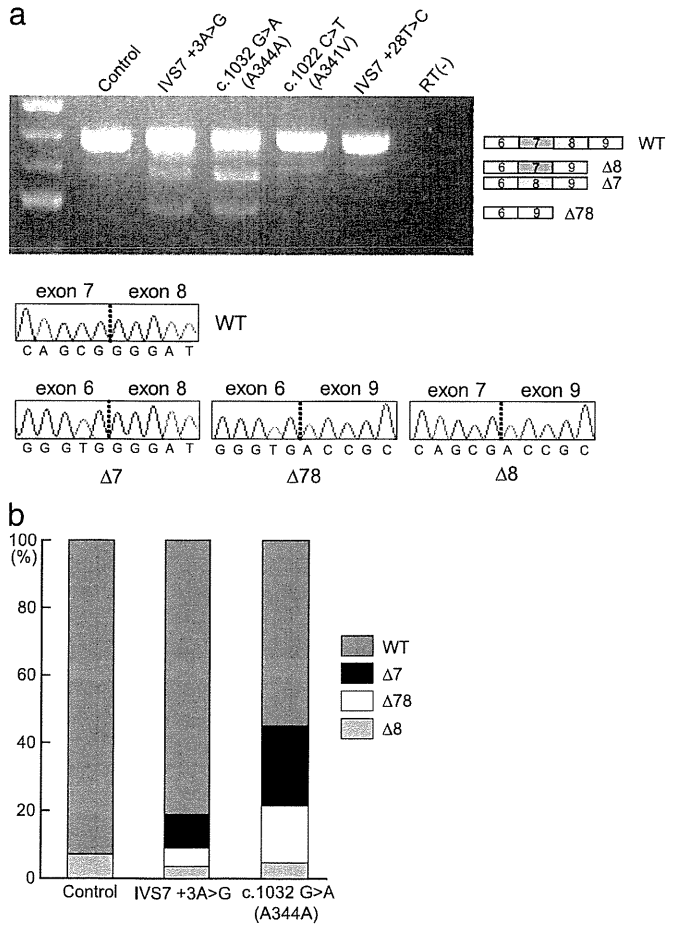

Fig. 4. RT-PCR analysis of patients' blood samples. (a) The result of RT-PCR with total RNA samples extracted from patients' lymphocytes. PCR was performed using primers spanning exons 5 though 10. Nucleotide sequence of each of the exon-skipping mRNAs is also shown. (b) The amounts of WT and mutant mRNAs expressed as a percentage of the total KCNQ1 mRNA. Controls: 4 normal healthy individuals, IVS7 +3A>G: the proband, c.1032G>A: 3 mutation carriers.

Fig. 6b presents regional action potentials of endocardial, mid-myocardial, and epicardial tissues of the 1D model during pacing at 1 Hz in the cases of control (black line), I_{Ks} 60% as a model of IVS7 +3A>G carrier (dark gray line), and I_{Ks} 60% with β-adrenergic stimulation as a model of IVS7 +3A>G carrier under exercise stress (light gray line). Simulated ECGs in the above 3 cases are also shown in the bottom. In the model of IVS7 +3A>G carrier, the β-adrenergic stimulation markedly prolonged the QTc (668 ms) while the I_{Ks} 60% alone did not (388 ms vs. 366 ms in control case).

Similar to Fig. 6b, c presents regional action potentials and simulated ECGs in the cases of control (black line), I_{Ks} 40% as a model of c.1032G>A carrier (dark gray line), and I_{Ks} 40% with β-adrenergic stimulation as a model of c.1032G>A carrier under exercise stress (light gray line). In the model of c.1032G>A carrier, the β-adrenergic stimulation markedly prolonged the QTc (741 ms) while the I_{Ks} 40% alone did not (405 ms).

Fig. 6d and e show regional action potentials and simulated ECGs for a longer period (21–40 s after the first pacing stimulus) in the cases of I_{Ks} 60% (IVS7 +3A>G carrier) with β-adrenergic stimulation and I_{Ks} 40% (c.1032G>A carrier) with β-adrenergic stimulation, respectively. Intriguingly, no arrhythmia was induced for IVS7 +3A>G carrier model (Fig. 6d) whereas tachyarrhythmia was induced for c.1032G>A carrier model (Fig. 6e). In the latter, the monomorphic ventricular tachycardia (VT) was initially derived from triggered activities due to delayed afterdepolarization (asterisks) in the epicardial region. The VT soon degenerated into the fibrillation-like activities (VF) because of marked long APD in the mid-myocardial region, causing decremental conduction (†, endocardial to mid-myocardial regions) followed by propagated graded response (mid-myocardial to endocardial regions) and phase-2 reentry (‡, epicardial activation originating from action potential plateau in the mid-myocardial region).

4. Discussion

4.1. Identification of the novel splicing mutation using minigene assay

A significant fraction of disease-causing mutations affect pre-mRNA splicing. In the present study, three potential splice mutations as well as one definite splice mutation (c.1032G>A) in the intron 7 5′ splice-site

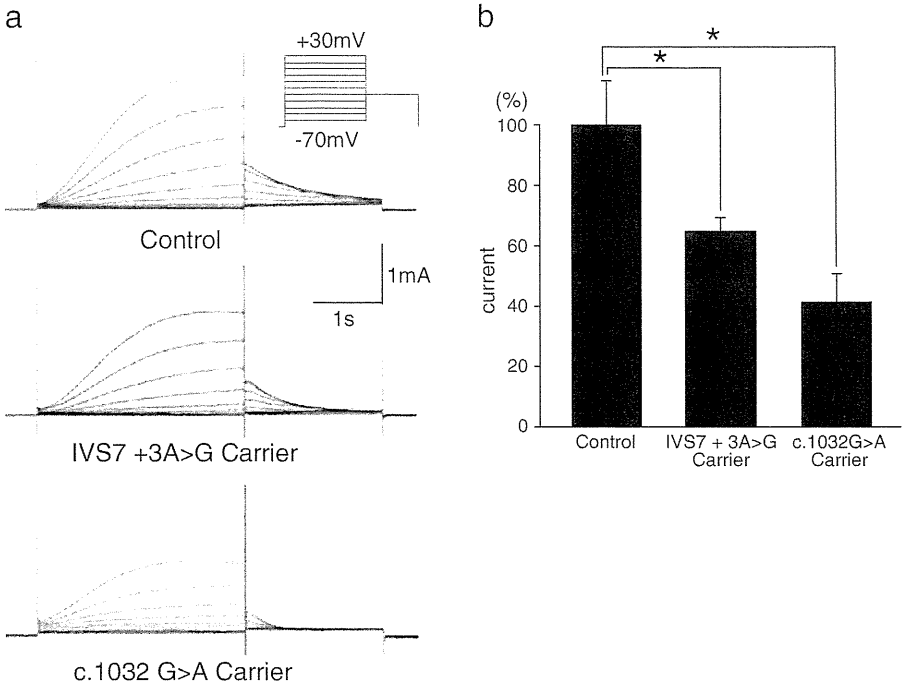

Fig. 5. Electrophysiological analysis. (a) Representative current traces recorded from two-electrode voltage-clamp of *Xenopus* oocytes simulating the proportions of mRNA of control individuals and mutation carriers. Currents were recorded at various membrane potentials from −70 to +30 mV for 3 s in 10 mV increments from a holding potential of −80 mV. A total of 10 ng of cRNA was injected with the relative ratios of WT and mutant KCNQ1 inferred from the data obtained in the real-time RT-PCR experiment. All the current recordings in the present study were performed in the presence of KCNE1 β-subunits (1 ng). (b) Pooled data of currents. Current amplitudes were measured at 1.8 s after the initiation of 3-s pulse applied to a +30 mV test potential. Background I_{Ks} current (22.9 nA) was subtracted. n = 7 for control, 8 for IVS7 +3A>G and 7 for c.1032G>A. *p<0.01 vs. control.

of KCNQ1 identified in clinically-diagnosed LQTS patients were studied. First, we assessed the effects exerted by these mutations on splicing of the KCNQ1 transcript using a hybrid minigene in transient transfection experiments, and found that c.1032G>A and IVS7 +3A>G resulted in the skipping of KCNQ1 exon 7 (Fig. 3). In good agreement with the minigene assay results, we confirmed the presence of exon-skipping transcripts in the blood samples of the mutation carriers (Fig. 4a). The minigene assay is a useful tool to screen for potential splicing mutations in clinically-diagnosed LQTS patients with no mutation in the KCNQ1 coding sequence.

4.2. Mechanistic basis of splicing abnormality

Splice sites are conserved sequences at both ends of an intron that are recognized during the initial steps of splicing [24]. Mutations at 5′ splice-site are frequent among mutations that cause human diseases [25,26]. The human 5′ splice-site consensus sequence is MAG/GT***R***AGT (M is A or C; R is A or G), spanning from position −3 to position +6 relative to the exon–intron junction. A (59%) and G (35%) are conserved at position +3 [27], but it has been shown that 5′ splice-site with disease-causing +3A>G mutations are frequently associated with non-consensus nucleotides at positions +4 and +5 [28]. Indeed, the KCNQ1 intron7 5′ splice-site sequence, GCG/GTA***G***GT, has non-consensus G at position +4, which presumably facilitates the skipping of exon under +3A>G mutation. These dependencies between +3 (A/G) and +4/+5 were demonstrated by other investigators'in in vitro experiment; the splicing defect in the +3A>G mutant was successfully fixed by converting either +4 or +5 independently to the consensus [28]. In contrast, G at position-1 is more strictly conserved, and mutations at this position, as c.1032G>A, cause robust splicing defects [28]. Hence, these differences in the strength of 5′ splice-site sequence dictate the extent to which splicing is disrupted, as indicated by the greater amount of exon-skipped transcripts in c.1032G>A compared with IVS7 +3A>G (Fig. 4b).

4.3. Significance of quantitative assessment of splicing abnormality in risk stratification

The family with IVS7 +3A>G splicing mutation showed mild LQTS phenotype; asymptomatic and had no history of sudden cardiac death, despite an exercise-induced QT prolongation (Fig. 1). Meanwhile, c.1032G>A mutation, a similar KCNQ1 splicing mutation, was more malignant; 8 families out of 9 were symptomatic with episodes of syncope (mostly during exercise or swimming) or sudden death, as we previously reported [10]. These observations strongly suggest the possible correlation between genotypes and clinical phenotypes in LQTS caused by aberrant splicing of KCNQ1. This profound suppression in I_{Ks} currents may underlie the pathophysiology of these patients. Moreover, the level of aberrant proteins may parallel the clinical severity.

We previously showed that the exon-skipping mutant channel subunits (Δ7, Δ7-8, or Δ8) are non-functional, and they have mutant-specific degree of dominant-negative effect on WT channels, by trapping WT intracellularly and thereby interfering its translocation to the plasma membrane [10]. On the assumption that as the result of splicing error the reduction in potassium current would occur in the mutant carriers with similar degrees evaluated by the real-time RT-PCR (Fig. 4b), we estimated functional consequences of these splicing mutation (IVS7 +3A>G and c.1032G>A). Ratios simulating the proportions of various transcripts of KCNQ1 in affected individuals resulted in a pronounced reduction in the whole-cell potassium current in *Xenopus* oocytes, compared with ratios simulating those in normal individuals; 64.8% for IVS7 +3A>G, and 41.4% for c.1032G>A (Fig. 5b).

The computer simulation study (Fig. 6) incorporating these quantitative results demonstrated the pronounced QT prolongation under beta-stimulation in both IVS7 +3A>G and c.1032G>A, and the occurrence of tachyarrhythmias only in c.1032G>A. Our computer model was human ventricular model [15] and the parameters for beta-stimulation were based on physiological data reported

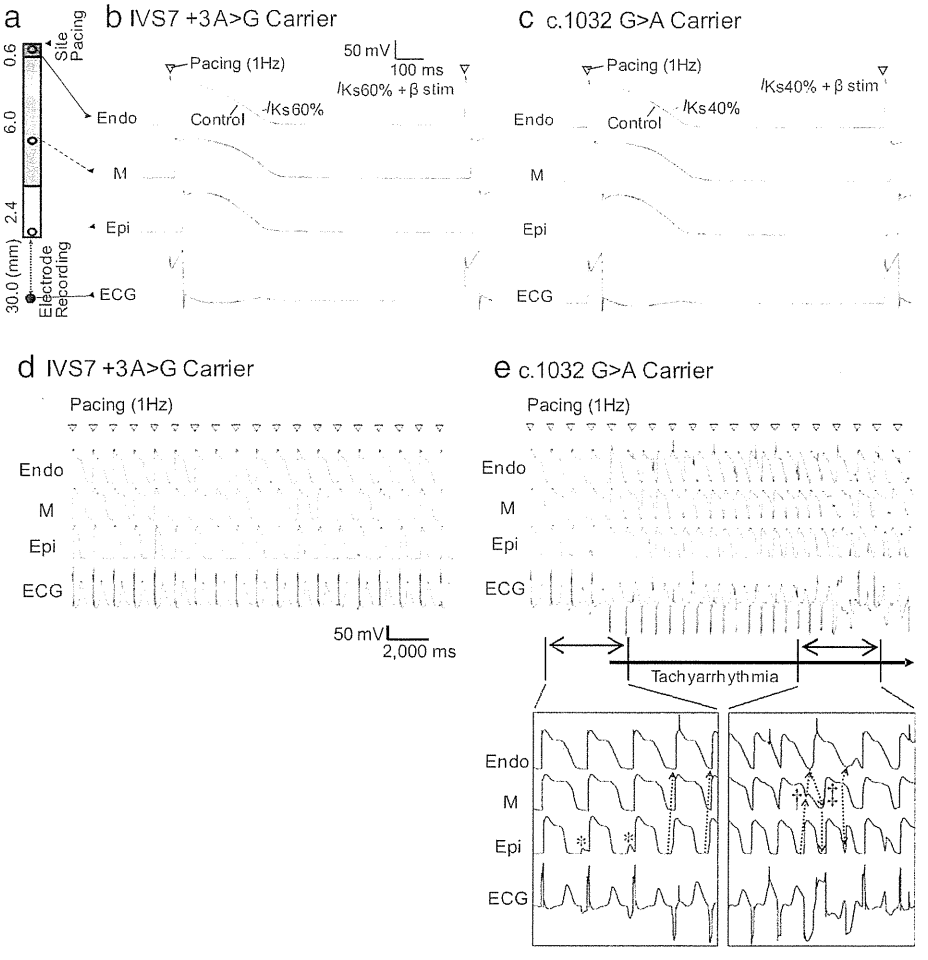

Fig. 6. Computer simulation. (a) One-dimensional myocardial simulated tissue, representing the electrical behaviors of left ventricular free wall. Endo, endocardium; M, mid-myocardium; and Epi, epicardium. Open circles indicate recording sites for regional action potentials, whereas the filled circle represents a unipolar recording electrode for ECG. (b and c) Regional action potentials and simulated ECG during 1 Hz pacing for IVS7 +3A>G carrier and c.1032G>A carrier, respectively. Open triangles denote timings of 1 Hz pacing. (d and e) No arrhythmia was induced for IVS7 +3A>G carrier, whereas tachyarrhythmia was induced for c.1032G>A carrier. See text for details.

previously [20–22]. These observations appear to parallel with clinical phenotypes of the mutation carriers; syncopal episodes mainly during exercise in c.1032G>A but not IVS7 + 3A>G patients, whereas asymptomatic IVS7 +3A>G carriers. Thus, the quantitative assessments of splicing abnormality could be useful in the risk stratification of LQTS patients associated with KCNQ1 splicing mutations.

4.4. Study limitations

Compared to the clinical data on c.1032G>A, those on IVS7 + 3A>G were gathered from two patients, because we failed to conduct the genetic test and clinical tests in their remaining relatives. Because of a relative mildness of their phenotypes, carriers of IVS7 + 3A>G mutation may be less frequently recommended for the genetic test, and this may partially explain why the number of identified IVS7 + 3A>G family was small.

4.5. Conclusions

A novel KCNQ1 splicing mutation IVS7 +3A>G generates exon-skipping mRNAs, and manifests a mild phenotype of LQTS. The amount of these mRNAs and its functional consequences may determine the clinical severity of the disease.

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Original Article

Carvedilol, a Non-Selective β-with α_1-Blocker is Effective in Long QT Syndrome Type 2

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Background: β-blockers offer the first line therapy in congenital long QT syndrome (LQTS), and are more effective to prevent the cardiac event in LQTS type 1 than in type 2 or 3. In contrast, left cardiac sympathetic denervation (LCSD) was shown to be highly effective in patients refractory to β-blockers. Total sympathetic ablation by LCSD indicates the addititional involvement of α-adrenoceptor-mediated pathway. In genotyped LQT2 patients, we therefore hypothesized that blockade of α-adrenoceptor in addition to β-adrenoceptor by carvedilol could reduce cardiac events more efficiently than other types of β-blockers.

Methods and Results: The study population consisted of 51 genotyped LQT2 patients (18 males, 23 ± 11 years old). They were divided into 2 groups (group 1: 43 patients treated with selective β-blockers, group 2: 8 patients with carvedilol) and retrospectively analyzed the efficacy of the respective β-blocker therapy in suppressing cardiac events. Cardiac events were observed in 11 patients of group 1 (26%) but none in group 2 during a follow-up period of 83 ± 80 months (P = 0.098).

Conclusions: Carvedilol may be a potentially beneficial therapy for genotyped LQT2 patients who are refractory to other β selective blockers.
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Key words: Long QT syndrome, β-blocker therapy, carvedilol, α-adrenoceptor

Introduction

Long QT syndromes (LQTS) are heterogeneous inherited ion channelopathies characterized by prolonged ventricular repolarization, syncope, ventricular arrhythmias, and sudden cardiac death with normal cardiac structure.[1)] Sympathetic activation and arrhythmogenesis are uniquely associated with

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LQTS. For example, in LQTS type 1 (LQT1), exercise produces the QT prolongation and subsequent arrhythmic episodes.[2,3] β-blockers are therefore routinely prescribed in symptomatic LQT1 patients and reported to prevent first cardiac events in 74–80% of patients.[4,5] In contrast, in LQT2, cardiac symptoms mainly develop at rest, while sleeping or by auditory stimuli,[6] and the efficacy of β-blockers is limited compared to that in LQT1. In a recent report[7] with large cohort of LQT2, β-blocker use reduced the risk of first cardiac events by 63%, however it was associated with less protection (29%) in the prevention of lethal cardiac events.

As to the additional therapy for patients refractory to β-blockers, left cardiac sympathetic denervation (LCSD) has been shown to be highly effective.[8,9] Total sympathetic ablation by LCSD indicates the additional involvement of α-adrenoceptor-mediated pathway. Labetalol, an α_1-and non selective β-blocker, was reported to be effective to suppress cardiac events in LQTS, although patients in this study were not genotyped.[10] As an anecdotal case, we experienced that a LQT2 patient (S871fs+31X) whose repetitive syncope due to TdP was not suppressed by propranolol (30 mg per day), but subsequent carvedilol (10 mg per day), an α_1-and non selective β-blocker, was fully effective.

Indeed, the difference in response to β-blockers may result from the distinct sympathetic response of I_{Ks} currents (encoded by *KCNQ1* gene that underlines LQT1[11,12]) and I_{Kr} currents (encoded by *KCNH2* gene that underlines LQT2[13]), which are both critically responsible for ventricular repolarization. We recently demonstrated both in CHO cells and HL-1 cardiomyocytes that Kv 11.1 channels encoded by *KCNH2* are acutely downregulated by α_1-adrenergic stimulation.[14] In the presence of reduced function with Kv11.1 mutants (Y43D and K595E), additional α_1-adrenergic stimulation led to a further decrease in residual channel currents and thereby producing an extreme delay in repolarization. Thus, α_1-adrenoceptor blockade might serve as a promising medication and improve the symptoms in LQT2 patients. We therefore retrospectively surveyed the efficacy of carvedilol for suppressing cardiac events compared to other β-selective blockers in genotyped LQT2 patients.

Methods

Study population (Figure 1) and genetic analysis

The study cohort consisted of 82 LQT2 probands and their 51 family members, who were referred as inherited cardiac arrhythmia subjects from 36 institutes in Japan, and genotyped from June 1996 to December 2009 in Shiga University of Medical Science (Otsu) or Kyoto University Graduate School of Medicine (Kyoto). The patients with LQT1, 3, 5, 6, 7 or compound mutations[15] were excluded. In the total of 133 LQT2 patients, the β-blocker therapy was introduced in 51 patients, which consisted of our final study population (**Figure 1**).

DNA sequence analyses of *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ2* were performed as described previously.[16,17] Mutation screening was performed using polymerase chain reaction (PCR) or denatured high-performance liquid chromatography analyses (dHPLC, WAVE system; Transgenomic Inc., Omaha, NE, USA).[18] For aberrant PCR products, DNA sequencing was then conducted with a DNA sequencer (ABI 3130 DNA Sequencer; Perkin Elmer, Foster City, CA, USA). When a mutation was detected, the result was

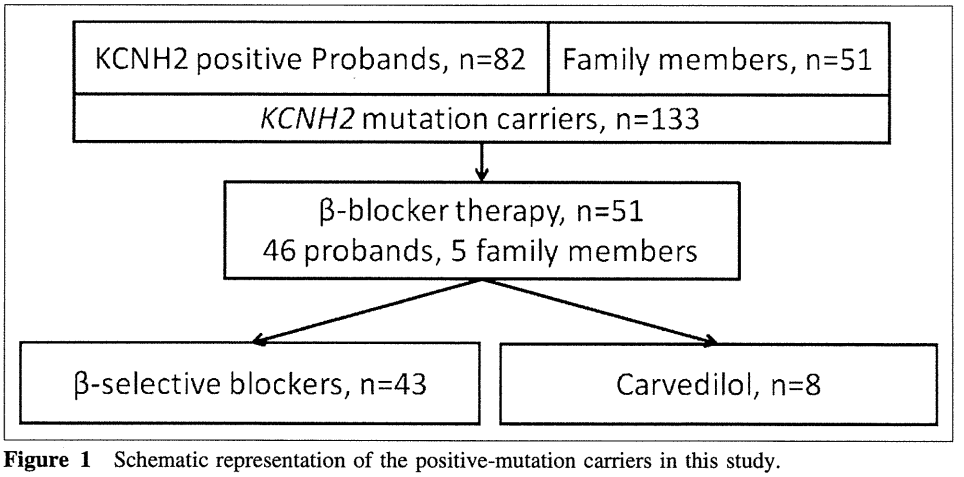

Figure 1 Schematic representation of the positive-mutation carriers in this study.

compared to >200 Japanese control subjects, and single nucleotide polymorphisms were excluded from this study.

The protocol for genetic analysis complied with the Declaration of Helsinki and was approved by the institutional ethics committees and performed under their guidelines. All individuals or their guardians gave written informed consent to genetic and clinical data analyses.

Clinical characteristics

In 51 LQT2 patients receiving the β-blocker therapy, baseline clinical characteristics were collected including age at diagnosis, age at the first cardiac event, age at β-blocker therapy started, and Schwartz score.[19] With regard to family history, we defined it as positive if a subject had a family member who had Schwartz score of ≥4. Triggers of symptom were defined as follows: 1) at rest/during sleep, 2) arousal, 3) auditory stimuli, 4) pregnancy/post delivery and 5) during exercise.

ECG parameters used for analyses were baseline heart rate (HR) and QT intervals. Measurements were performed in 3 successive sinus beats in lead II (if not possible, in lead V5) and averaged. QT was manually measured as the time interval between QRS onset (Q) and the point at which the isoelectric line intersected a tangential line drawn at the maximal down slope of the positive T wave or the upslope of the negative T wave (QT), and corrected using Bazett's formula.[20]

LQTS-related cardiac events included unexplained syncope, aborted cardiac arrest requiring cardiac resuscitation, appropriate ICD shock and unexpected sudden death exclusive of a known cause before age 45 years. Those data were compared between the group treated with β-selective blockers and the group with carvedilol.

Treatment including β-blocker therapy

The specific β-blocker used, as well as dose, was at the discretion of the treating physician. The type and dose of β-blocker used, cardiac event rate on each β-blocker, adjunctive therapy and follow-up period were collected and compared between the β-selective blockers group and the carvedilol group. If patients were treated with β-selective blockers and thereafter carvedilol due to ineffective β-blocker therapy, they were included in the carvedilol group. Follow-up period was calculated from the starting date of β-blocker to the day of a cardiac event. We excluded patients from analysis who were non-compliant to a prescribed β-blocker.

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for continuous variables. Comparisons were performed by chi-square test for categorical variables and *t*-test for continuous variables. The Kaplan-Meier estimator was used to assess the time to a first event and the cumulative event rates by groups were compared using the log-rank test. $P < 0.05$ was considered statistically significant. The statistical software used for the analyses was JMP version 7.0.1 (SAS institute Inc., NC, USA).

Results

Characteristics of the study population

In our LQTS cohort, 133 patients from 82 unrelated families were identified as sole *KCNH2* mutation carriers. In those LQT2 patients, 51 patients (46 probands and 5 family members) received the β-blocker therapy. To compare the clinical characteristics, we divided the study population into 2 groups: the β-selective blockers group (43 patients, mean age at diagnosis 22 ± 11 years) and the carvedilol group (8 patients, mean age at diagnosis 26 ± 11 years) (**Figure 1**).

Table 1 shows the clinical characteristics of the two groups. Regarding triggers of symptoms, sudden arousal was more frequent in the carvedilol group ($P = 0.0313$). There was no statistical difference between the two groups including age, gender, Schwartz scores, ECG parameters and cardiac events before therapy. Distribution of mutations was not significantly different between the two groups.

Treatment

As an adjunctive therapy, β-selective group received mexiletine more frequently than the carvedilol group (10/43 β-selective group vs. 0/8 carvedilol group, $P = 0.0473$) (**Table 1**). Ten patients (9/43 in the β-selective group, 1/8 in the carvedilol group) were implanted with an implantable cardioverter defibrillator (ICD). No patients underwent LCSD.

β-Blocker therapy (Table 2)

The β-blocker therapy was started at age of 19 ± 12 (0–63) years in total, 17 ± 11 years in the β-selective group and 26 ± 12 years in the carvedilol group, respectively (**Table 1**). The mean age of carvedilol start was significantly ($P = 0.0372$) older than that of β-selective blockers. We experienced three patients in whom β-selective blockers first failed but subsequent carvedilol was successful to prevent cardiac events. Those cases were included

Table 1 Clinical characteristics

	All patients n = 51	β-selective blockers n = 43	Carvedilol n = 8	*P* value
Demographics				
Proband, n (%)	46 (90)	40 (93)	6 (75)	N.S.
Male, n (%)	18 (35)	16 (37)	2 (25)	N.S.
Age at diagnosis, mean ± SD, years	23 ± 11	22 ± 11	26 ± 12	N.S.
Age at first cardiac event, mean ± SD, years	15 ± 10	14 ± 9	18 ± 10	N.S.
Age at β-blocker started	19 ± 12	17 ± 11	26 ± 12	0.0372*
Schwartz score, mean ± SD	5.2 ± 1.7	5.2 ± 1.7	5.6 ± 1.3	N.S.
Electrocardiogram				
HR, mean ± SD, bpm	63 ± 10	61 ± 7	64 ± 11	N.S.
QTc, mean ± SD, ms	515 ± 63	513 ± 62	525 ± 65	N.S.
Location of mutation, n (%)				
N terminal	10 (17)	8 (17)	2 (25)	N.S.
C terminal	18 (35)	16 (37)	2 (25)	N.S.
Pore	11 (22)	10 (23)	1 (13)	N.S.
Trans membrane	12 (24)	9 (21)	3 (38)	N.S.
Non-missense mutation, n (%)	22 (43)	17 (40)	5 (62)	N.S.
Cardiac events before β-blocker therapy				
Syncope, presyncope, n (%)	48 (94)	40 (93)	8 (100)	N.S.
Documented TdP, n (%)	26 (51)	22 (51)	4 (50)	N.S.
Documented VF, n (%)	5 (10)	5 (12)	0 (0)	N.S.
Trigger of symptoms				
At rest, during sleep, n (%)	24 (50)	22 (55)	2 (25)	N.S.
Arousal, n (%)	10 (20)	6 (15)	4 (50)	0.0313*
Auditory stimuli, n (%)	7 (14)	6 (15)	1 (12)	N.S.
Pregnancy, post delivery, n (%)	3 (6)	2 (5)	1 (13)	N.S.
Exercise, n (%)	2 (4)	2 (5)	0 (0)	N.S.
Adjunctive LQTS therapy (any time)				
Mexiletine, n (%)	10 (20)	10 (23)	0 (0)	0.0473*
Pacemaker, n (%)	0	0	0	N.S.
LCSD, n (%)	0	0	0	N.S.
ICD, n (%)	10 (20)	9 (21)	1 (13)	N.S.
Follow-up duration after β-blocker, month ± SD	83 ± 80	84 ± 86	73 ± 43	N.S.

*P < 0.05 vs β-selective blocker group

TdP: Torsade de pointes, VF: ventricular fibrillation, LCSD: left cardiac sympathetic denervation, ICD: implantable cardioverter defibrillator

into the carvedilol group because they recurred shortly after β-selective blocker treatment and the period for carvedilol therapy was sufficiently long. β-selective blockers used in 43 LQT2 patients were atenolol in 7 patients (75.0 ± 61.2 mg, 25–200 mg), bisoprolol in 3 (4.2 ± 1.2 mg, 2.5–5.0 mg), carteolol in 1 (20 mg), metoprolol in 5 (68.0 ± 33.1 mg, 30–120 mg), nadolol in 1 (60 mg), and propranolol in 26 (34.0 ± 13.3 mg, 10–60 mg). Eight patients were treated with carvedilol (16.9 ± 10.3 mg, 5–40 mg). Unfortunately, we have no data of patients' body weight, and the data of mg per kg were not shown.

Cardiac events during follow-up

Mean follow-up durations without any cardiac events after drug introduction were 84 ± 86 months in the β-selective blocker group and 73 ± 43 months in the carvedilol group. Eleven patients (26%) had cardiac events while receiving β-blocker therapy, but no one (0%) experienced arrhythmic episodes in the carvedilol group (**Table 3**). Kaplan-Meier survival curve showed a tendency of superior efficacy of carvedilol (P = 0.098, log-rank test) (**Figure 2**). Three of 11 recurrence cases had pore mutations (27%), and four had non-missense mutations (36%),

Table 2 Dose and variety of β-blockers

	n, (%)	Dose per day, mean ± SD (range)
β-selective blockers, n = 43		
Atenolol	7 (14)	75.0 ± 61.2 (25–200) mg
Bisoprolol	3 (6)	4.2 ± 1.2 (2.5–5) mg
Carteolol	1 (2)	20 ± 0 mg
Metoprolol	5 (10)	68.0 ± 33.1 (30–120) mg
Nadolol	1 (2)	60 ± 0 mg
Propranolol	26 (51)	34.0 ± 13.3 (10–60) mg
Non-selective β- and α_1-blocker, n = 8		
Carvedilol	8 (16)	16.9 ± 10.3 (5–40) mg

Table 3 Cardiac events on β-blocker therapy

	β-selective blocker, n = 43	Carvedilol n = 8
Total of cardiac events, pt n, (%)	11 (26)	0
Syncope, n, (%)	11 (26)	0
Documented TdP, n, (%)	4 (9)	0
Aborted sudden cardiac arrest, n, (%)	1 (2)	0
Appropriate ICD shock, n, (%)	2 (5)	0

TdP: Torsade de pointes, ICD: implantable cardioverter defibrillator

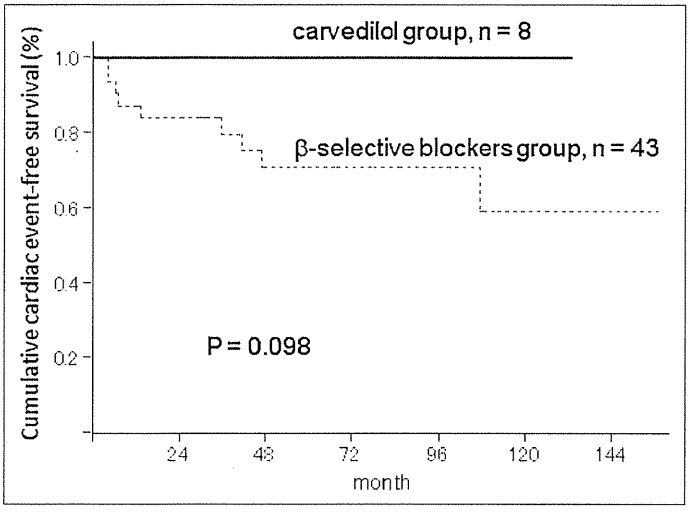

Figure 2 Kaplan-Meier analysis of cumulative cardiac event-free survival from each β-blocker therapy started to 150 months.

and therefore these incidences were not significantly different irrespective of β-selective blocker effectiveness.

Two of eleven patients (18%) in the β-selective blocker group experienced appropriate ICD shocks: one patient treated with propranolol (30 mg per day) and another treated by nadolol (60 mg per day), who showed an electrical storm. In two patients with ineffective propranolol therapy, recurrent syncope was controlled by diazepam as an adjunctive therapy.

Three of eight patients in the carvedilol group were first treated with propranolol, but experienced recurrent cardiac events, and the treatment was changed to carvedilol. In two cases, carvedilol was introduced because it was longer acting than propranolol. In the one remaining case, carvedilol was prescribed because of its additional α-blocking action.

Figure 3A shows a family tree showing a typical case that was refractory to propranolol but successfully controlled by carvedilol. The proband was a 9-year-old girl (indicated by arrow in **Figure 3A**), and genetic test identified a heterozygous *KCNH2* mutation (S871fs+31X). She was diagnosed as long QT syndrome at age of 6 years (12-lead ECG in **Figure 3B**, QTc = 500 ms) but remained asymptomatic. At age 9, she had loss of consciousness twice just after waking to an alarm clock in the morning. Her Holter ECG (**Figure 3C**) revealed repetitive TdP triggered by auditory stimuli, and she complained of uncomfortable chest distress. Propranolol (30 mg per day) was then prescribed, but her clinical complaints continued. Medication was finally switched to carvedilol (10 mg per day), which completely relieved her symptoms though the drug did not alter her QTc and HR. Her mother also collapsed at the delivery of the proband at age of 32 and genetic test revealed the same *KCNH2* mutation. Carvedilol was also started to prescribe for this proband's mother.

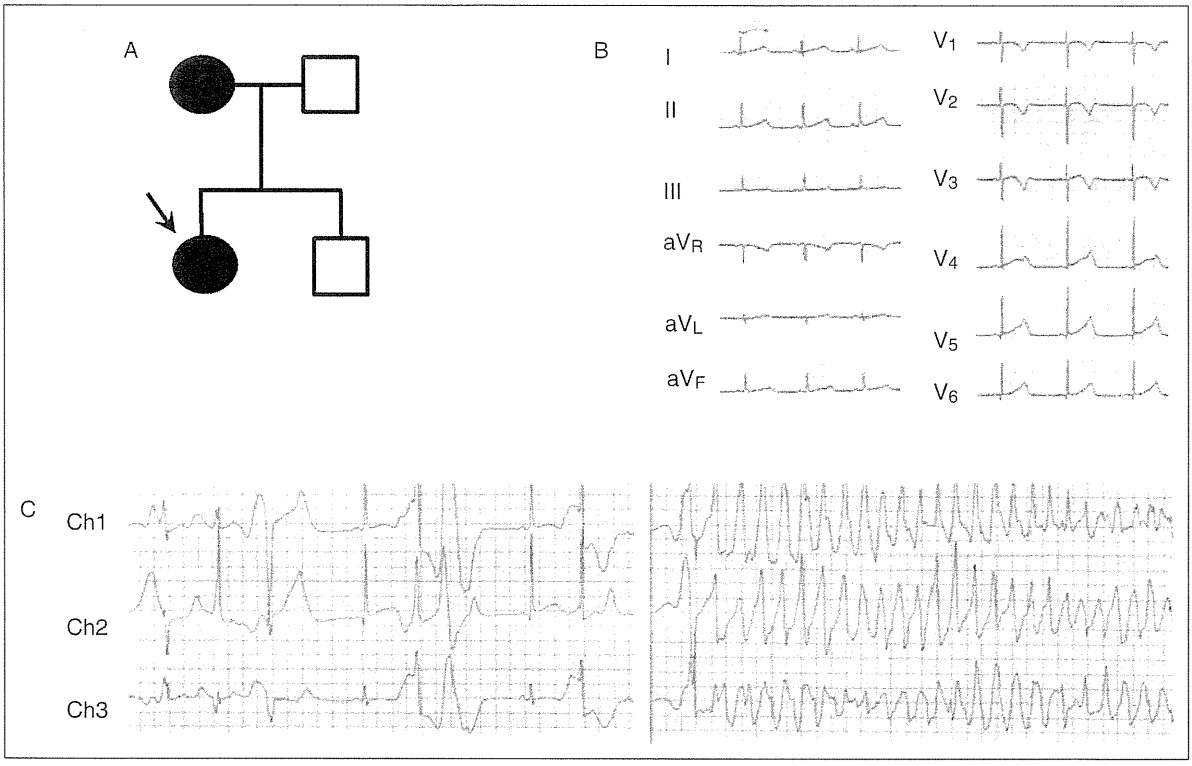

Figure 3 A typical case that was refractory to propranolol but controlled by carvedilol.
A. A pedigree of *KCNH2*-S871fs+31X. Circle indicates female, square indicates male. Closed symbol indicates mutation positive subject. Black arrow indicates proband of this family, a 9-year-old girl. **B.** 12 lead ECG at resting state of proband. QTc = 500 msec (V5). **C.** A Holter ECG of proband. TdP was detected during an episode of syncope triggered by alarm clock early in the morning. Left panel: just before the TdP. Right panel: TdP following left panel.

Discussion

In our study cohort of LQT2 patients, carvedilol, which blocks both α_1-and β-adrenoceptors, was more effective than other β-selective blockers in suppressing cardiac events without major complications. As widely recognized, β-blockers are the first line therapy for the prevention of cardiac events in the long QT syndrome. The studies on genotype-phenotype relationships of the syndrome in the last decade, however, have confirmed that β-blockers are most effective in LQT1, but less in LQT2 or LQT3 patients.[4] For example in 2004, Priori and colleagues[21] reported nearly a 3-fold increase in the risk of cardiac events during β-blocker treatment in LQT2 as compared to LQT1 patients, although there is a conflicting report by Goldenberg and colleagues.[22] They demonstrated that a β-blocker was sufficiently effective in high risk patients with both LQT1 and LQT2. As to α_1-and β-blockers, there are a few reports which demonstrated that α_1-and β-adorenoceptor blockade was effective to shorten QTc interval in the upright position before exercise and early recovery phase after exercise,[23] or suppress arrhythmic events in LQTS with unknown genotype.[10] There is another suggestive study of Khositseth et al[24] that phenylephrine-induced bradycardia increased transmural dispersion of repolarization (TDR) in symptomatic LQT2 but not in LQT1 patients. Therefore, α_1-adrenoceptor blockade (in addition to β-adrenoceptor blockade) in LQT2 patients may actually lead to suppression of QT prolongation and/or TDR, and eventually TdP. The results in the present study were in line with the above-mentioned hypothesis, and carvedilol actually appeared to be beneficial in suppressing cardiac events in LQT2.

As Moss et al. reported in 1971,[25] LCSD offers an alternative therapy to block sympathetic activation for LQTS patients without reducing heart rate. Antiarrhythmic action of LCSD is due largely to the electrophysiological consequences of reduced release of norepinephrine at the ventricular level and includes prevention/suppression of early afterdepolarization (EAD). Although it does not entirely control all cardiac events, the operation significantly reduces the number of symptoms and ICD shocks.[9] As the LCSD suppresses both β-and α-adrenergic

contribution of sympathetic control, blockade of α_1-adrenoceptors in addition to β-adrenoceptors by medication would also be useful to suppress the severe form of cardiac events.

More recently, we reported that α_1-adrenergic stimulation acutely reduced Kv 11.1 channel activities via membrane PIP_2 pathway.[14] Sudden α_1-adrenoceptor-mediated reduction in I_{Kr} at a lower HR (for example, during sleep) would act additionally to prolong action potential durations and may enhance inward current through Na/Ca exchanger, both contributing to the occurrence of EAD.[26] These observations may partially explain why sudden auditory stimulation by an alarm clock induces cardiac events in LQT2 patients.[6] In this connection, more recently Kim and colleagues[27] demonstrated in a large cohort of genotyped LQT2 patients that β-blockers were less effective in patients with arousal or non-exercise triggered events than those with exercise triggered events to prevent the recurrence. Although they did not mention the detailed species of β-blockers used for their patients, in our cohort, 11 recurrence cases in spite of β-selective blockers were all triggered by arousal or in a non-exercise resting state, and carvedilol prevented the cardiac event in three who were initially refractory to propranolol.

Study limitations

Because this study was conducted in a retrospective manner, we were not able to adjust the selection of patients between the two groups. Our study cohort consisted of a relatively small number of LQT2 patients. In the carvedilol group, there were no recurrent cases, which made further statistical analyses, such as multivariate correlation study, difficult. A further study with a larger number of patients will be awaited. In conclusion, in our genotyped LQT2 cohort, carvedilol was effective to suppress cardiac events, whereas 26% of the patients treated with other β-selective blockers experienced cardiac events, suggesting that the simultaneous blockade of α_1-adrenoceptors may offer an additional therapy for LQT2 patient with non-exercise triggers.

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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.

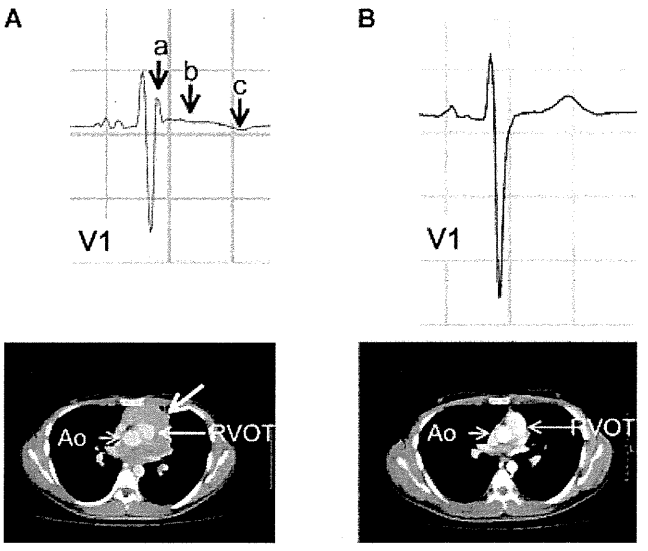

Figure 1. *A and B: ECG recording in lead V_1 and contrast-enhanced computed tomography scan before and after chemotherapy, respectively. Ao = Aorta; RVOT = right ventricular outflow tract.*

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No disclosures.

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左室心外膜側にて著明なJ波とOut of QRS Potentialが記録されたBrugada症候群(J Wave Syndrome)の1例

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心臓病センター榊原病院

大江透

要旨：症例は39歳，男性。心室細動自然発作を認めるBrugada症候群との診断で当院入院。心電図ではV2でのsaddle back型ST上昇とI，II，aVl，V3-6でのJ波を認めた。心臓電気生理学的検査では，経冠静脈的に左室側壁心外膜側に多極カテーテルを挿入し電位の記録を行ったところ，単極誘導では明瞭なJ波が記録され，双極誘導ではJ波の成分に伴いout of QRS potentialが記録された。単極誘導でのJ波は心房ペーシング，およびisoproterenol投与で減高し，pilsicainide投与で増高した。また双極誘導でのout of QRS電位は心房ペーシングおよびisoproterenol投与で短縮しpilsicainide投与で延長した。再分極異常の性質を示す左室心外膜側J波がout of QRS potentialとしても記録され，J wave syndromeの成因を考えるうえで興味深い症例と考えられここに報告する。

Key Words：Brugada syndrome, J wave syndrome, late potential

J wave and out of QRS potential at left ventricular epicardium in a patient with Brugada syndrome (J wave syndrome)

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はじめに

近年，J wave syndromeという概念が提唱され，Antzelevitchらは，心外膜側心筋において一過性外向き電流（Ito）の亢進により活動電位に深いnotchが形成され，心外膜側と心内膜側間に生じた電位勾配が心電図上著明なJ波を形成し，心室性不整脈を発生すると報告している[1,2]。またBrugada症候群はその一型であるとも報告している。

以前我々はBrugada症候群患者において右室流出路心外膜側および心内膜側での単極誘導での電位を同時記録し，pilsicainideで増大するout of QRS potentialおよびcoved型ST上昇との関連を報告した[3,4]。またBrugada症候群には，下壁および側壁誘導にJ波を合併する症例が存在し，致死的心室性不整脈発生との関連も報告されている[5,6]。しかしこれまでにJ wave

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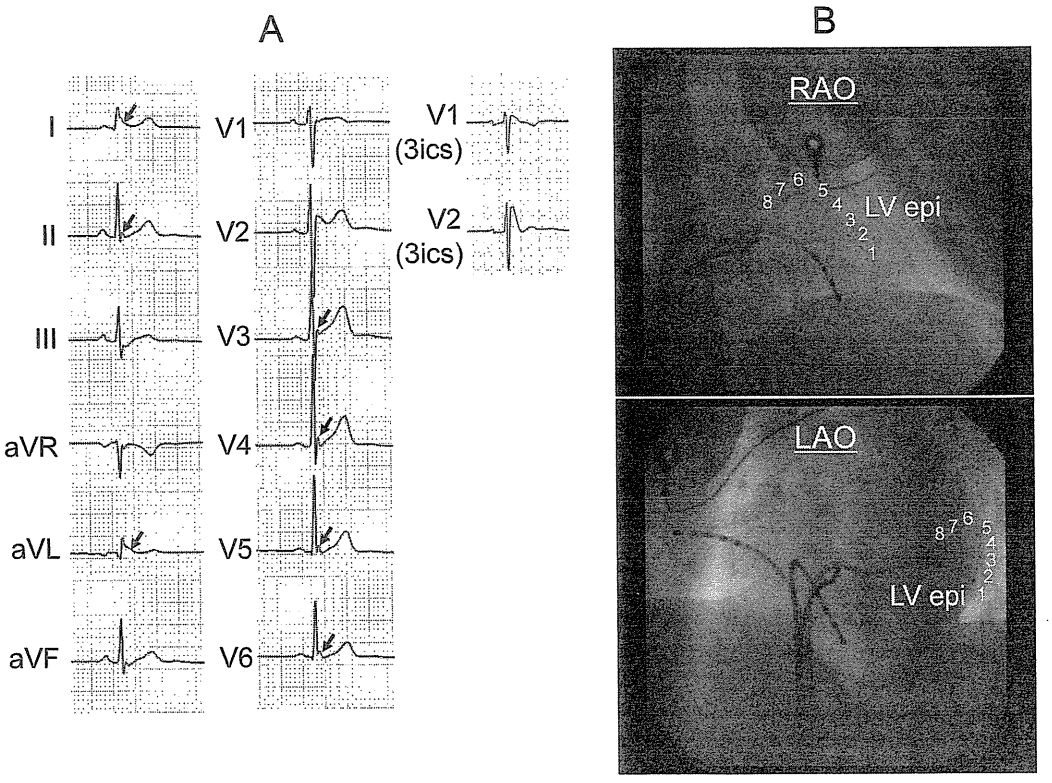

図1　A：入院時体表面心電図，B：カテーテル配置（右前斜位 RAO，左前斜位 LAO）
A：V2 誘導での saddle back 型 ST 上昇と I，II，aVl，V3-6 誘導での J 波を認めた。
B：経冠静脈的に左室側壁心外膜側（LV epi）に多極カテーテルを挿入し，電位の記録を行った。

syndrome の症例において左室心外膜側電位を直接記録し検討した報告はない。今回我々は，左室心外膜側において，著明な J 波と out of QRS potential が記録された Brugada 症候群（J wave syndrome）患者を経験したので報告する。

症　例

症例：39 歳，男性。

既往歴：特記すべきものなし。

家族歴：祖父の兄弟が突然死。

現病歴：生来健康であったが，2009 年 5 月朝 4 時ごろ，呼吸停止に家族が気付き，救急要請した。救急隊の AED にて心室細動が確認され除細動施行された。体表面心電図にて Brugada 症候群が疑われ当院紹介となった。入院時心電図では，V2 誘導での saddle back 型 ST 上昇と I，II，aVl，V3-6 誘導での J 波を認めた（図 1A）。胸部 X 線，心臓超音波検査，冠動脈造影検査では器質的異常は認めなかった。加算平均心電図では心室遅延電位（late potential; LP）の有無は陽性であった。

心臓電気生理学的検査

心臓電気生理学的検査（解析は EP-Worok-Mate ver 3.7.1; St. Jude Medical 社製）では，まず右心室心内膜側マッピングを行ったが明らかな異常電位は認めなかった。また右室心尖部および右室流出路にて高頻度刺激および 3 連早期刺激まで行ったが持続する心室頻拍／心室細動は誘発されなかった。Edrophonium 10 mg 投与

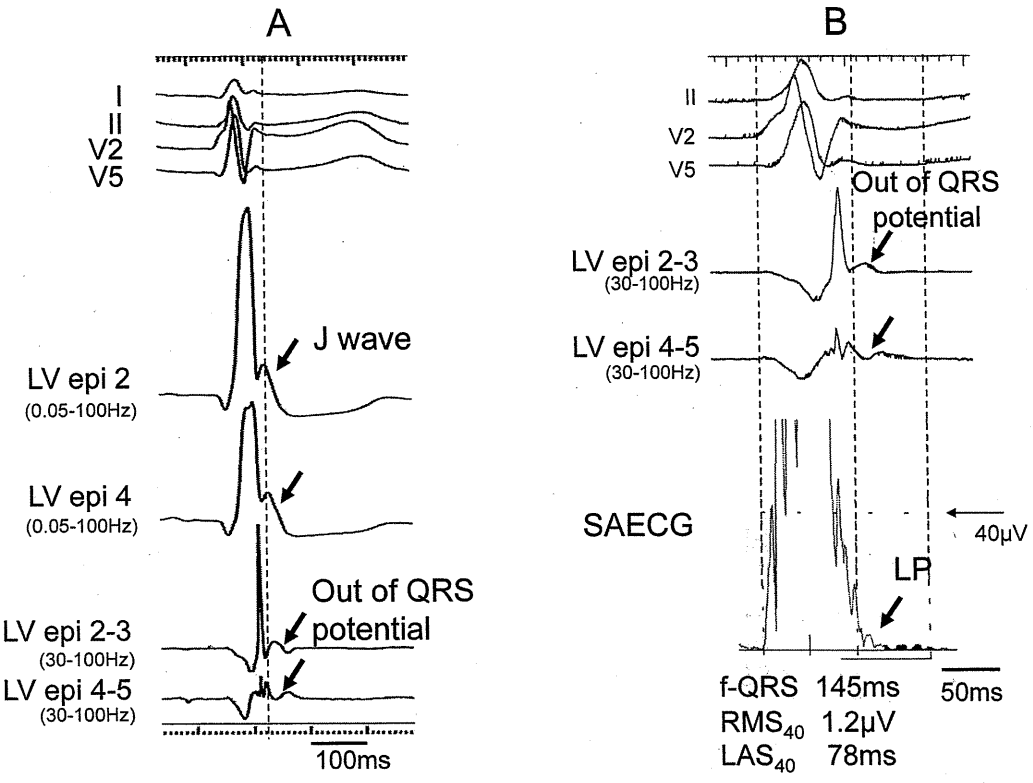

図２　A：左室側壁心外膜側での電位記録，B：加算平均心電図と左室心外膜側電位との関係
A：単極誘導（0.05 ～ 100 Hz，LV epi 2，4）では明瞭なＪ波が記録され，双極誘導（30 ～ 100 Hz，LV epi 2-3，4-5）ではＪ波の時相に一致して out of QRS potential が記録された。
B：左室側壁心外膜側単極誘導で記録された out of QRS potential は加算平均心電図での LP とも時相はほぼ一致していた。

後に右室心尖部から３連早期刺激（400/230/190/180）を行うと心室細動が誘発され除細動を行った。

経冠静脈的に左室側壁心外膜側に多極カテーテル（アンサンブル FX1820 8 極電極長先端 1.6 mm，他 1.3 mm，電極間 5 mm；日本ライフライン社製）を挿入し（図 1B）電位の記録を行ったところ，単極誘導（0.05 ～ 100 Hz, LV epi 2, 4）では明瞭なＪ波が記録され，双極誘導（30 ～ 100 Hz，LV epi 2-3，4-5）ではＪ波の時相に一致して out of QRS potential が記録された（図 2A）。この際，QRS 終末部は V5 誘導にて決定し，out of QRS potential は QRS 終末部以降に記録される電位と定義した。この out of QRS potential は加算平均心電図での LP とも時相はほぼ一致していた（図 2B）。左室側壁心外膜側の電極と対側の心内膜側の電位も同時に記録すると，心外膜側で記録された単極誘導でのＪ波，双極誘導での out of QRS potential は心内膜側では明瞭に認められなかった（図 3）。Edrophonium 投与前後では体表面心電図（図 4A），左室側壁心外膜側でのＪ波と out of QRS potential（図 4B）は著変を認めなかった。次に心房高頻度刺激を行うと，単極誘導でのＪ波は減高し，双極誘導での out of QRS potential は短縮した（図 5）。次に pilsicainide（1mg/kg）を投与すると，体表面心電図では V2 誘導で saddle back 型 ST 上昇が，そして第３肋間の V2 誘導では coved 型

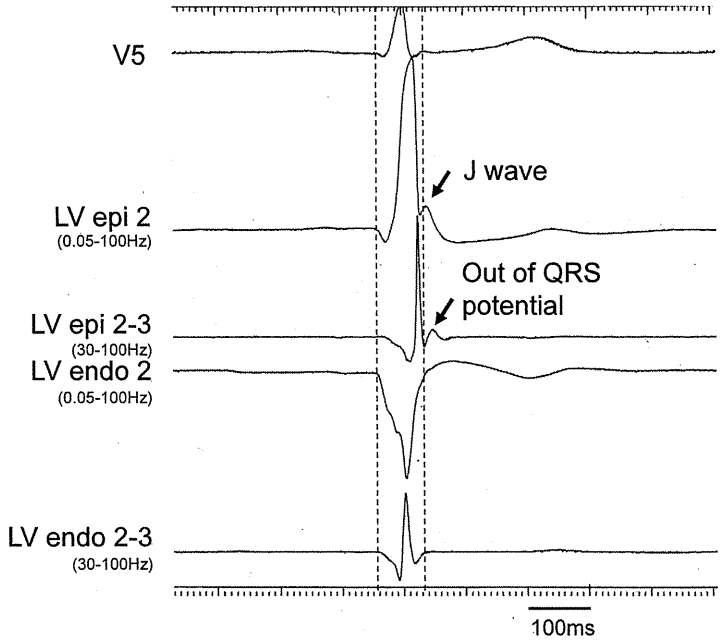

図３　左室側壁心外膜側（LV epi）と，対側の左室側壁心内膜側（LV endo）の
単極誘導電位記録（0.05 ～ 100 Hz）
左室心外膜に挿入した電極の対側となる左室心内膜側に電極を挿入し同時に記録を行った。心外膜側で記録される単極誘導での J 波，双極誘導での out of QRS potential は心内膜側では明瞭に認められなかった。

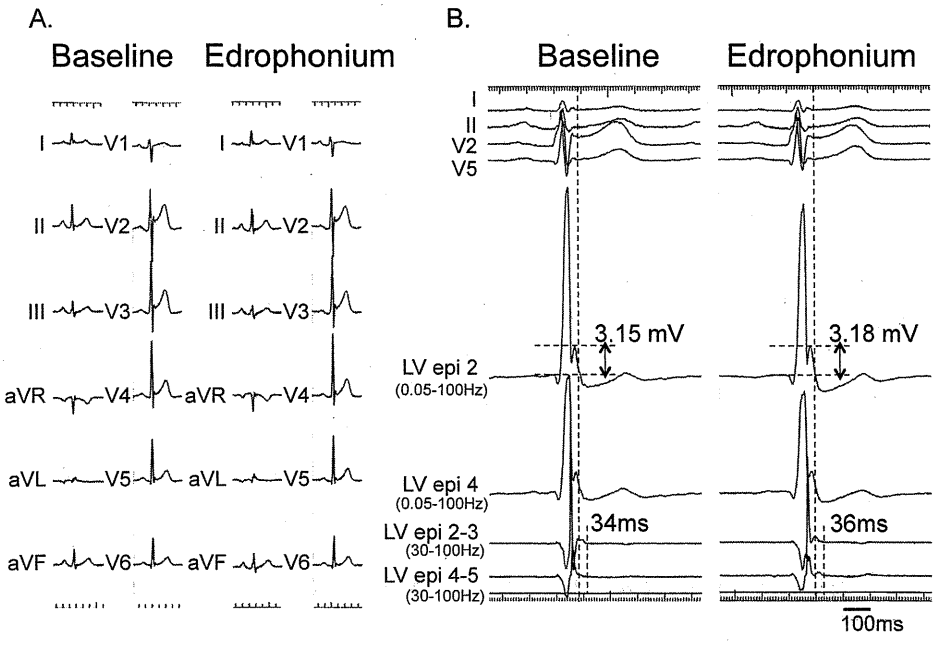

図４　Edrophonium 投与前後の A：体表面心電図，B：心内心電図
体表面心電図，心内心電図ともに edrophonium 投与で J 波に著明な変化は認めなかった。

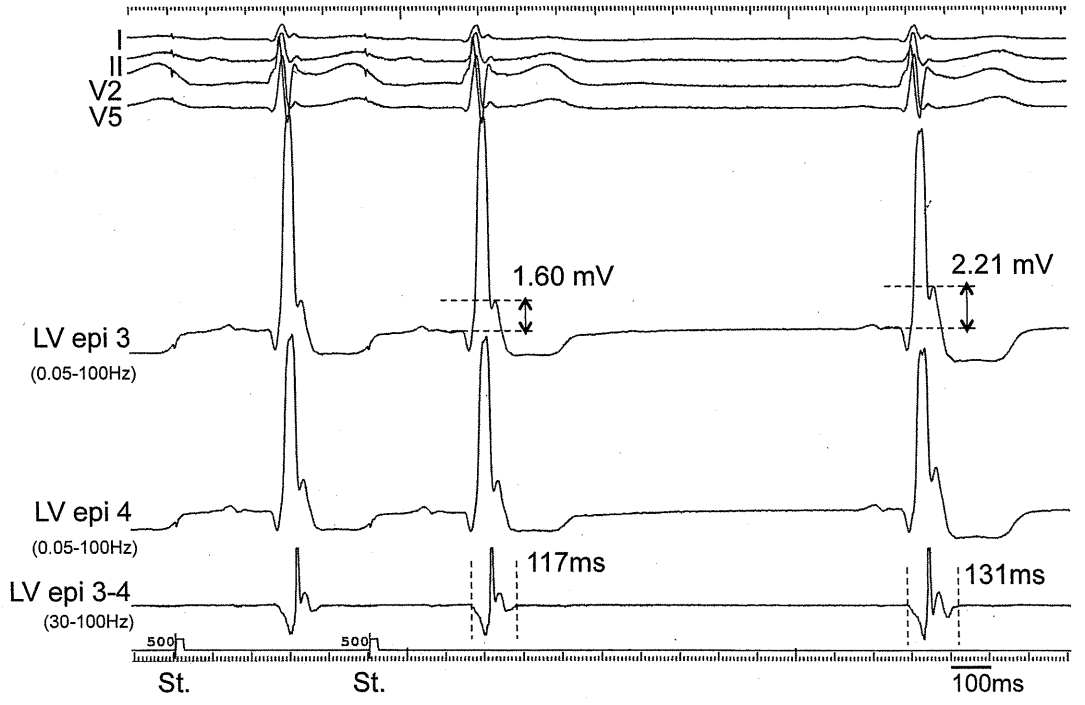

図5　右房高頻度ペーシング時の心内心電図
周期500 msecで右房高頻度ペーシングを行うと，J波は減高し（LV epi 3, 4），out of QRS potentialは短縮する所見を認めた（LV epi 3-4）。

ST上昇が記録された。I，II，aVl，V3-6誘導で記録されていたJ波はST部分の上昇によって不明瞭となった（図6）。また左室心外膜側の記録では，単極誘導でのJ波は増高し，双極誘導でのout of QRS potentialは延長した（図7）。続いてisoproterenolを1 μg単回静注すると，単極誘導でのJ波は減高し，双極誘導でのout of QRS電位は短縮した（図8）。また体表面心電図では上昇していたST部分の減高を認めた。

入院後経過

植込み型除細動器（ICD）植え込み術を施行し退院となったが，心室細動によるICD頻回作動を認め再入院となった。Quinidine 200 mg/日の内服を開始したところ，体表面心電図のJ波は減高し，加算平均心電図でのLPも減少した（図略）。退院後はICDの作動を認めていない。

考　察

体表面心電図の下壁・側壁誘導でJ波を認めるBrugada症候群患者において，左室側壁心外膜側にて著明なJ波（単極誘導，0.05 ～ 100 Hz）とout of QRS potential（双極誘導，30 ～ 100 Hz）が記録された。単極誘導でのJ波は心房ペーシング，およびisoproterenol投与で減高し，pilsicainide投与で増高した。また双極誘導でのout of QRS電位は心房ペーシングおよびisoproterenol投与で短縮しpilsicainide投与で延長した。

Aizawaらは，Brugada症候群患者において加算平均心電図で記録されるLPがlow-cut filterを40 Hzから100 Hzに変更すると著明に減少するが不整脈源性右室心筋症ではこのような現象を認めないこと，そしてquinidine投与によりcoved型がsaddle back型のST上昇に改善するとともにLPが消失することから，LPが再

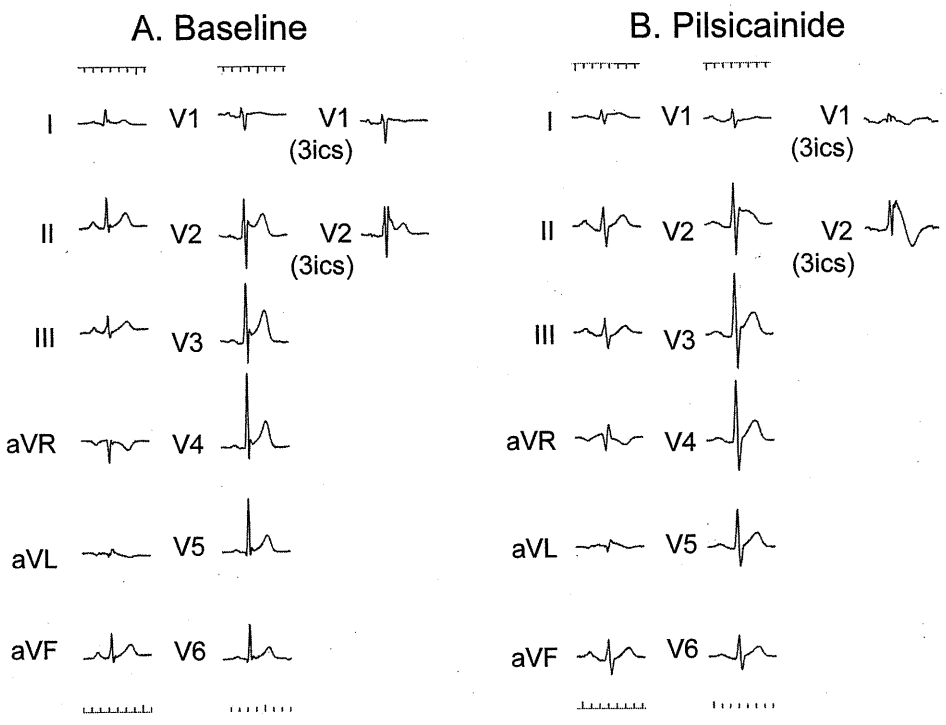

図6　体表面心電図　A：Pilsicainide 投与前，B：Pilsicainide 投与後

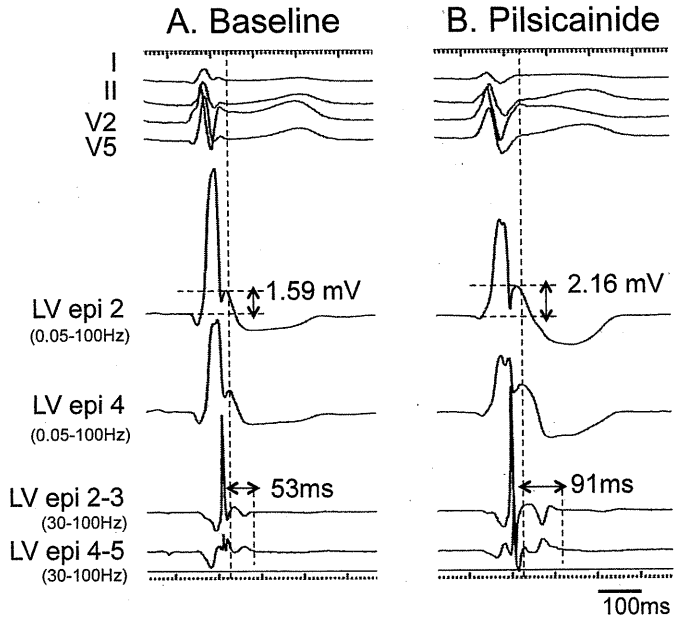

図7　心内心電図　A：Pilsicainide 投与前，B：Pilsicainide 投与後
Pilsicainide 投与にて，J 波は増高し（LV epi 2, 4），out of QRS potential は延長する所見を認めた（LV epi 2-3, 4-5）。