Adrenergic regulation of the rapid component of delayed rectifier K^+ current: Implications for arrhythmogenesis in LQT2 patients

Dimitar P. Zankov, MD, PhD,* Hidetada Yoshida, MD, PhD,† Keiko Tsuji, MS,* Futoshi Toyoda, PhD,‡ Wei-Guang Ding, MD, PhD,‡ Hiroshi Matsuura, MD, PhD,‡ Minoru Horie, MD, PhD*

*From the *Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan; †Department of Cardiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; ‡Department of Physiology, Shiga University of Medical Science, Otsu, Shiga, Japan.*

BACKGROUND KCNH2 gene mutations disrupting rapid component of I_K (I_{Kr}) underlie type 2 congenital long QT syndrome (LQT2). Startled auditory stimuli are specific symptomatic triggers in LQT2, thus suggesting fast arrhythmogenic mechanism.

OBJECTIVE We investigated acute α_{1A}- and cyclic adenosine monophosphate (cAMP)–related β-adrenergic modulation of I_{Kr} in HL-1 cardiomyocytes, wild type (WT)– and 2 LQT2-associated mutant Kv11.1 channels (Y43D- and K595E-Kv11.1) reconstituted in Chinese hamster ovary (CHO) cells.

METHODS I_{Kr} and Kv11.1 currents were recorded using the whole-cell patch-clamp technique and confocal microscopy of HL-1 cardiomyocytes transfected with green fluorescent protein (GFP)-tagged pleckstrin homology domain of phospholipase C-δ_1 visualized fluctuations of membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) content.

RESULTS In HL-1 cardiomyocytes expressing human α_{1A}-adrenoceptor, superfusion with phenylephrine significantly reduced I_{Kr} amplitude, shifted current activation to more positive potentials, and accelerated kinetics of deactivation. Confocal images showed a decline of membrane PIP_2 content during phenylephrine exposure. Simultaneous application of adenylyl cyclase activator forskolin and phosphodiesterase inhibitor 3-isobutyl-1-methylxantine (IBMX) shifted I_{Kr} activation to more negative potentials and decreased tail current amplitudes after depolarizations between +10 and +50 mV. In CHO cells, α_{1A}-adrenoceptor activation downregulated WT-Kv11.1 channels and forskolin/IBMX produced a dual effect. Expressed alone, the Y43D-Kv11.1 or K595E-Kv11.1 channel had no measurable function. However, co-expression of WT-Kv11.1 and each mutant protein evoked currents with loss-of-function alterations but identical to WT-Kv11.1 α_{1A}- and forskolin/IBMX-induced regulation.

CONCLUSION Acute adrenergic regulation of at least 2 Kv11.1 mutant channels is preserved as in WT-Kv11.1 and native I_{Kr}. Suppression of α_{1A}-adrenoceptor-related transduction might have therapeutic implications in some cases of LQT2.

KEYWORDS Long QT syndrome; I_{Kr}; KCNH2 mutation; α_{1A}-adrenoceptor; β_1- and β_2-adrenoceptor.

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Introduction

Sympathetic activation and arrhythmogenesis are uniquely associated in long QT (LQT) syndrome, the disease that prolongs cardiac repolarization and leads to syncope and sudden cardiac death.[1] This is particularly the case in the most frequent variants, LQT type 1 (LQT1) and type 2 (LQT2), designating malfunction of slowly (I_{Ks}) and rapidly (I_{Kr}) activating components of I_K, respectively. Symptomatic triggers in congenital LQT are gene specific: often after continuous physical exercise (swimming) in LQT1 and startled auditory stimuli in LQT2.[2,3]

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Efficiency of β-blocker therapy in LQT tends to divert attention from possible α_{1A}-adrenoceptor (α_{1A}AR)-mediated arrhythmogenic mechanisms. Increase of action potential duration (APD) in Purkinje fibers by α_{1A}AR stimulation is well-documented phenomenon that would promote arrhythmogenesis in LQT.[4,5] Moreover, between 36% and 41% of patients with LQT2 (focus of this report) are resistant to β-blockers,[2,6] and the efficacy of combined α_1- and β-blockers (e.g., labetalol) in some of the LQT cases has been reported.[7]

I_{Kr} or Kv11.1 (reconstituted α-subunit of human I_{Kr} channel) current inhibition by the selective α_{1A}AR agonist phenylephrine was found in Xenopus oocyte[8,9] mammalian cell lines and rabbit ventricular myocytes.[10,11] β_1- and β_2 adrenoceptors ($\beta_{1,2}$AR) modulate I_{Kr} through the dual action of cyclic adenosine monophosphate (cAMP): direct binding to the I_{Kr} channel produces a hyperpolarizing shift

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of current activation, and stimulation of protein kinase A (PKA) results in I_{Kr} inhibition.[12,13] The net effect varies according to different reports.[14,15]

Startled auditory stimuli-triggered syncope in LQT2 suggests involvement of fast transduction. Therefore, we studied immediate α_{1A} and $\beta_{1,2}$AR regulation of I_{Kr} in HL-1 cardiomyocytes,[16] wild-type (WT)-Kv11.1 current in Chinese hamster ovary (CHO) cells, and 2 Kv11.1 mutant channels (Y43D-Kv11.1 and K595E-Kv11.1) found in LQT2 probands with loud noise–triggered syncope.

Methods

Cell lines

HL-1 cardiomyocytes, a generous gift from Dr W. C. Claycomb, Louisiana State University Health Sciences Center, New Orleans, Louisiana, derived from AT-1 cells.[16] They retain differentiated cardiac phenotype during indefinite passages in culture, generate spontaneous action potentials, and express various ion channels characteristic for mouse atrium.[16,17]

The cells were cultured as originally described[16] and transiently co-transfected with 1 μg human α_{1A}AR/pcDNA3.1 + (Missouri S&T cDNA Resource Center, Rolla, Missouri) and 0.5 μg pEGFP (Invitrogen, Carlsbad, California) or 1 μg PH-GFP/pCI (Promega, Madison, Wisconsin), using Lipofectamin 2000 Reagent (Invitrogen). After transfection with α_{1A}AR, HL-1 cardiomyocytes were kept in norepinephrine-free medium to avoid desensitization of reconstituted α_{1A}AR.

CHO cells were cultured as reported previously.[18] WT-Kv11.1/pRC (Invitrogen), Y43D-Kv11.1 or K595E-Kv11.1/pRC, each 1 μg, were co-transfected with 0.5 μg pEGFP and 1 μg human α_{1A}AR using Lipofectamin Reagent (Invitrogen). In the subset of experiments, 1 μg Y43D-Kv11.1 or K595E-Kv11.1 were co-transfected with WT-Kv11.1 (1 μg), human α_{1A}AR (1 μg), and pEGFP (0.5 μg) cDNA. WT-Kv11.1 cDNA was a generous gift from Dr M. C. Sanguinetti, Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah.

Kv11.1 mutations

Among the LQT2-associated mutations identified in our institution, we selected 2 novel heterozygous KCNH2 mutations from probands with auditory stimuli-induced symptoms.

Y43D-Kv11.1.

A 30-year-old woman had experienced repetitive syncope since the age of 13. The attacks had been provoked by sudden arousal, e.g., ringing telephone during sleep and never by exercise. Electrocardiogram at rest showed corrected QT interval (QTc) of 0.60 seconds (Bazett formula). α_1- and β-adrenergic blocker carvedilol (20 mg/day) terminated the syncope completely.

K595E-Kv11.1.

A 35-year-old woman has had syncopal attacks since her infancy. Torsades de pointes was documented at the age of 18, and propranolol (40 mg/day) was administered but ineffective. Multiple episodes of syncope recurred after sudden arousal or auditory stimulation, although her QTc was not so prolonged (0.47 seconds) in the presence of propranolol. An implantable cardioverter-defibrillator was then implanted.

Electrophysiological experiments

I_{Kr} and Kv11.1 currents were measured at 37°C using the standard whole-cell patch-clamp technique[19] with EPC-8 amplifier (HEKA, Lambrecht, Germany) and 2.5 to 3.5 MΩ glass electrodes. Series resistance (maximally approximately 8 MΩ) was not compensated, and leak subtraction was not performed. Liquid junction potential between normal Tyrode and intracellular solution (−10 mV) was corrected in the data.

I_{Kr} was elicited by 2-second depolarizations from holding potential of −50 mV (to inactivate sodium, transient outward potassium, and T-type calcium currents) followed by repolarizations to the holding potential to record tail currents. L-type calcium current was eliminated by addition of 0.4 μmol/l nisoldipine to the external solution. We were not able to detect HMR1556-sensitive current in HL-1 cardiomyocytes (I_{Ks}, data not shown), and I_{Ks} blocker was not used. Kv11.1 current in CHO cells was evoked from holding potential −70 mV by 2-second depolarizations and tails were recorded at repolarizing steps between −120 and −20 mV.

Composition of pipette solution was (in mmol/l): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP, 0.1 Li_2-GTP, 5 EGTA and 5 HEPES; pH adjusted to 7.2 with KOH. External solution, normal Tyrode, contained (in mmol/l): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose, and 5.0 HEPES; pH adjusted to 7.4 with NaOH. E-4031 (Wako, Japan), phenylephrine, 3-isobutyl-1-methylxantine (IBMX), and forskolin (Sigma, St. Louis, Missouri) were supplemented into external solution on the day of experiments diluting the necessary amount of stock solutions. Stock solutions (kept at −20°C) of E-4031 and phenylephrine were made in distilled water; IBMX and forskolin were stored in dimethyl sulfoxide. L-α-phosphatidyl-D-myo-inositol-4,5-bisphosphate (PIP_2, Calbiochem, San Diego, California) was added to the pipette solution to achieve concentration of 10 μmol/l and 5 ml aliquots were kept at −80°C maximally for 1 month.

Confocal microscopy

HL-1 cardiomyocytes constantly superfused with normal Tyrode solution at 37°C were examined in the recording chamber of a Zeiss LSM 510 confocal laser-scanning inverted microscope (Carl Zeiss, Oberkochen, Germany). A switch to a phenylephrine-containing (30 μmol/l) normal Tyrode solution tested the effect of α_{1A}AR stimulation on GFP-tagged pleckstrin homology domain of phospholipase C-δ_1 (PH-GFP) fluorescence. Confocal images were taken at 15- or 30-second time intervals, and fluorescence intensity was analyzed through the measurement of green plane intensity of the images in ImageJ software (Rasband, W. S.,

ImageJ, National Institutes of Health, Bethesda, Maryland; http://rsb.info.nih.gov/ij/, 1997–2008).

Statistical analysis

All averaged values are presented as mean ± SEM. Statistical comparisons were made by 2-tailed paired and unpaired Student *t*-test or 1-way ANOVA and differences were accepted as significant for $P < .05$.

Results

Stimulation of α_{1A}AR downregulated I_{Kr}

In HL-1 cardiomyocytes expressing human α_{1A}AR, I_{Kr} possessed typical properties as described for the rapid component of I_K.[20] Selective α_{1A}AR agonist phenylephrine, applied externally for 4 to 6 minutes at a concentration of 30 μmol/l, reduced quickly I_{Kr} amplitude, and the readministration of drug-free solution restored the current amplitude to 90% ± 6% (n = 8) of the control level (Figure 1A). Figure 1B shows typical I_{Kr} traces evoked by voltage commands between −40 and +50 mV before and during superfusion with 30 μmol/l phenylephrine, and in the presence of specific I_{Kr} inhibitor E-4031 (3 μmol/l). I_{Kr} block unmasked an existence of endogenous time-independent conductance present during depolarizing steps but not at holding potential. For accurate measurement of I_{Kr} amplitude, we used tail currents because after their complete inhibition by E-4031 no evident time-dependent conductance at −50 mV could be observed. Phenylephrine downregulated I_{Kr} in a voltage-dependent manner: inhibition of I_{Kr} tails varied from 0.68 ± 0.07 at −30 mV to 0.30 ± 0.01 at +50 mV (n = 9, $P < .05$, Figure 1C).

In addition to the reduction of current amplitude, α_{1A}AR stimulation modified I_{Kr} channel gating and facilitated the process of deactivation. The voltage at half-maximal activation ($V_{1/2}$) was shifted toward positive potentials, and fast and slow time constants of deactivation decreased in the presence of phenylephrine (Table 1, Figures 1D to 1F).

The α_{1A}AR modulation of I_{Kr}, evaluated as E-4031 sensitive current, was verified using a ventricular action potential clamp technique. Typical current traces (average of 5 consecutive recordings at steady state) are shown in Figure 2A. Similar to square depolarizations, α_{1A}AR-related decline of E-4031-sensitive current was voltage dependent: the range of measured current inhibition during action potential voltage clamp was between 0.35 at −50 mV and 0.20 at +30 mV (n = 7, $P < .05$, Figure 2B).

Fluctuation of PIP_2 content during α_{1A}AR activation

Variation of membrane PIP_2 concentration could be successfully shown by a method that takes advantage of high-affinity binding of pleckstrin homology domain of PLC-δ_1 (PH) to PIP_2.[21] Expression of GFP-tagged PH could be seen as predominantly cell-membrane-localized fluorescence under the microscope. The decrease of the membrane PIP_2 amount produces a decrease of membrane and an increase of cytosolic fluorescence because of redistribution of PH-GFP.

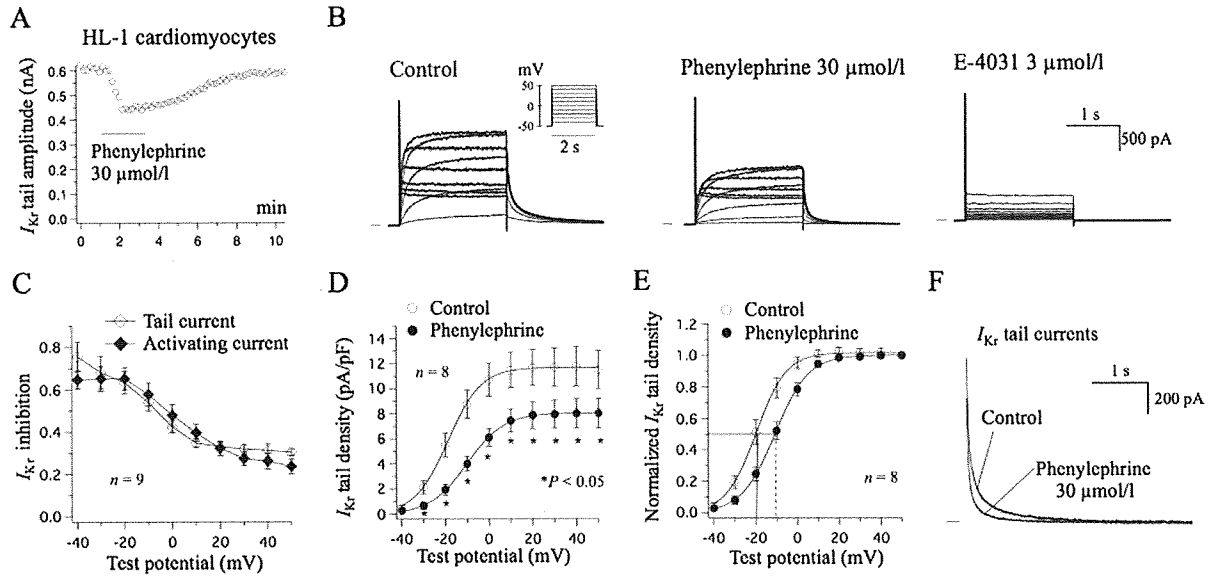

Figure 1 α_{1A}AR agonist phenylephrine downregulated I_{Kr} in HL-1 cardiomyocytes. **A:** Typical time course of I_{Kr} tail amplitudes when cells were exposed to 30 μmol/l phenylephrine: the current decreased quickly after α_{1A}AR stimulation. **B:** Line of control I_{Kr} currents, traces during α_{1A}AR stimulation, and conductance remaining after complete I_{Kr} inhibition by E-4031 (voltage-clamp protocol is in the inset). I_{Kr} suppression was more potent at negative potentials (**C**). **D:** Mean values of I_{Kr} tail densities before and during α_{1A}AR stimulation are plotted against the depolarizing voltages and fitted to the Boltzmann equation. The positive shift of I-V curve was shown when current densities were normalized to the maximal value (**E**). α_{1A}AR stimulation also accelerated I_{Kr} deactivation (Table 1). **F:** Typical tail currents recorded at −50 mV before and after α_{1A}AR stimulation.

Table 1 Activation and deactivation parameters of I_{Kr} in HL-1 cardiomyocytes and Kv11.1 currents in CHO cells

	Control	Phenylephrine	Control	Forskolin/IBMX
I_{Kr}				
$V_{1/2}$ (mV)	−18.7 ± 0.3	−9.9 ± 0.2* (8)†	−18.3 ± 1.3	−21.5 ± 1.1* (17)
k (mV)	+8.4 ± 0.1	+7.6 ± 0.2 (15)	+7.1 ± 0.3	+7.3 ± 0.4 (17)
τ_{fast} (ms)‡	60 ± 17	46 ± 19* (6)	47 ± 15	45 ± 16 (12)
τ_{slow} (ms)‡	586 ± 161	442 ± 212* (6)	451 ± 97	433 ± 107 (12)
Kv11.1 (WT)				
$V_{1/2}$ (mV)	−16.2 ± 0.6	−9.3 ± 0.3* (15)†	−16.4 ± 2.0	−20.9 ± 2.6* (10)
k (mV)	+7.5 ± 0.5	+8.7 ± 0.2 (15)	+8.2 ± 0.5	+8.1 ± 0.5 (10)
τ_{fast} (ms)‡	282 ± 17	205 ± 17* (8)	235 ± 24	224 ± 29 (8)
τ_{slow} (ms)‡	1,570 ± 104	875 ± 96* (8)	1,374 ± 119	1,293 ± 121 (8)
Kv11.1 (WT/Y43D)				
$V_{1/2}$ (mV)	−9.6 ± 0.5	−4.5 ± 0.3* (13)	−13.1 ± 2.1	−20.2 ± 1.6* (11)
k (mV)	+7.4 ± 0.4	+8.6 ± 0.2 (13)	+7.6 ± 0.3	+7.4 ± 0.2 (11)
τ_{fast} (ms)‡	129 ± 8	89 ± 7* (8)	166 ± 26	157 ± 18 (8)
τ_{slow} (ms)‡	642 ± 55	577 ± 48* (8)	810 ± 92	803 ± 100 (8)
Kv11.1 (WT/K595E)				
$V_{1/2}$ (mV)	−5.5 ± 0.6	0.09 ± 0.7* (9)	−3.3 ± 1.8	−8.6 ± 2.2* (11)
k (mV)	+7.5 ± 0.5	+8.6 ± 0.6 (9)	+6.6 ± 0.2	+6.5 ± 0.3 (11)
τ_{fast} (ms)‡	306 ± 32	225 ± 25* (8)	305 ± 37	301 ± 31 (9)
τ_{slow} (ms)‡	1,790 ± 162	1,184 ± 146* (8)	1,582 ± 111	1,577 ± 132 (9)

CHO = Chinese hamster ovary; IBMX = 3-isobutyl-1-methylxantine; WT = wild-type.
*P <.05 vs. control value.
†Figures in parentheses indicate the number of experiments.
‡Shown are the average values of deactivation at −50 mV for I_{Kr} and at −60 mV for Kv11.1 currents.

We used this technique to show the coupling of reconstituted human α_{1A}AR to G_q–PLC pathway. In HL-1 cardiomyocytes co-transfected with PH-GFP and α_{1A}AR cDNA (Figure 3A), phenylephrine caused rapid translocation of the fluorescence from the cell membrane to the cytoplasm (quantitatively analyzed in Figures 3B and 3C). On the contrary, in HL-1 cardiomyocytes expressing only PH-GFP, the membrane and cytosolic fluorescence were not altered by phenylephrine administration (not shown). The average ratio between green plane intensity in the images from cytosolic regions of interest measured in 7 HL-1 cardiomyocytes expressing α_{1A}AR and PH-GFP compared with the value from 4 cardiomyocytes transfected only with PH-GFP equaled 1.27 ± 0.06 and 0.99 ± 0.001, respectively (P <.05 between groups). The above results confirmed coupling between reconstituted human α_{1A}-AR and intracellular pathways.

In the system of HL-1 cardiomyocytes, the molecule responsible for I_{Kr} regulation through α_{1A}AR seemed to be PIP_2. When 10 μmol/l PIP_2 was loaded through the glass electrode (Figure 3D) I_{Kr} tail inhibition after α_{1A}AR activation was largely attenuated (e.g., reduction of mean tail amplitude after depolarization to +30 mV decreased from 31.1% to 11.5%, P <.05) and the phenylephrine-induced shift of I-V curve was only +4.2 mV (compared with +8.8 mV in the control state, P <.05). Deactivation time con-

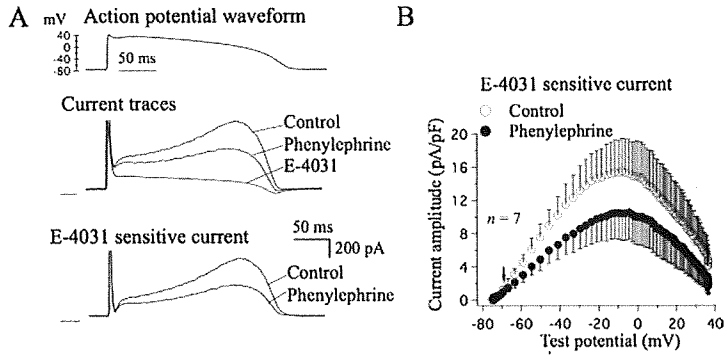

Figure 2 α_{1A}AR activation inhibited I_{Kr} during action potential voltage clamp. I_{Kr} was evoked by voltage-clamp command with ventricular action potential form (**A, top**) to confirm α_{1A}AR-related modulation during physiological variation of membrane potential. Reduction of recorded current by phenylephrine (30 μmol/l) and selective I_{Kr} inhibitor E-4031 (3 μmol/l) is shown (**A, middle**). The trace in the presence of E-4031 was subtracted from the remaining 2 to show E-4031-sensitive current (I_{Kr}) before and during α_{1A}AR stimulation (**A, bottom**). **B:** Average I-V relations of E-4031-sensitive current are presented. The corresponding points are statistically different at potentials more positive than −70 mV (arrow).

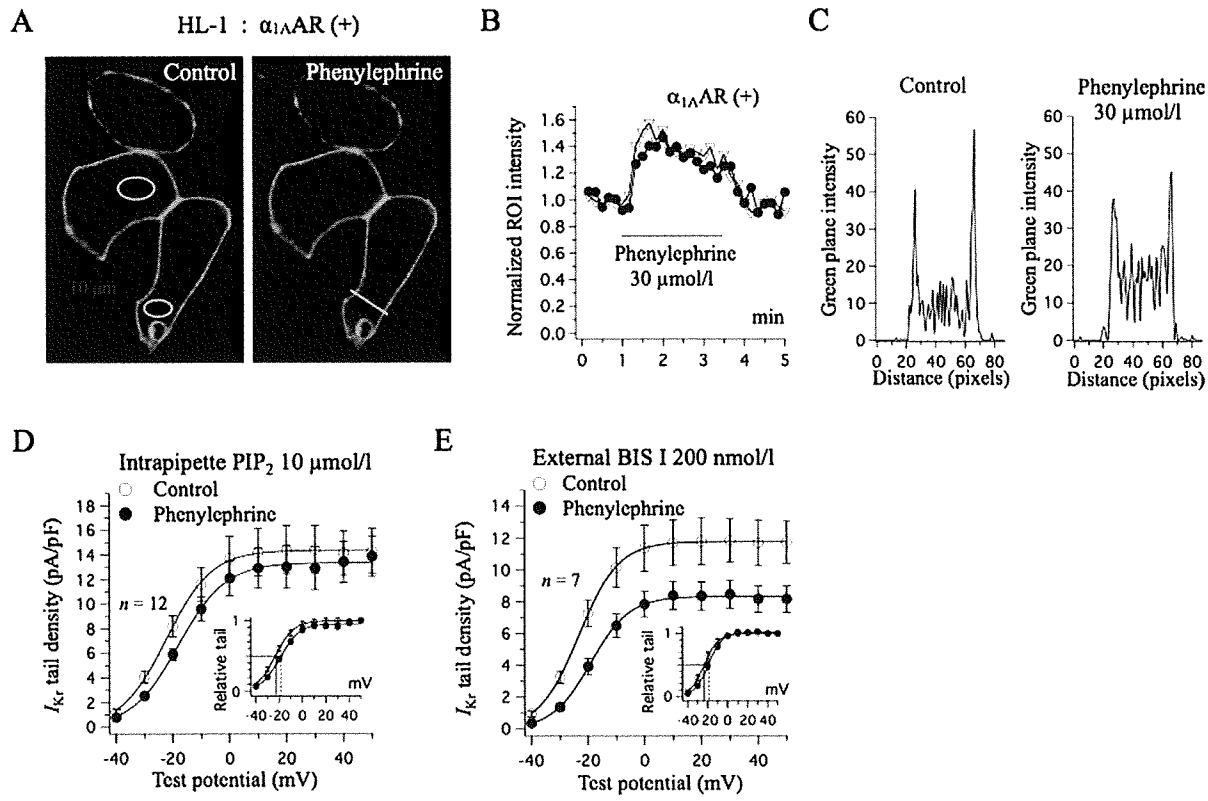

Figure 3 α_{1A}AR-induced depletion of membrane PIP_2 downregulated I_{Kr}. **A:** Confocal images from HL-1 cardiomyocytes expressing PH-GFP and α_{1A}AR. The fluorescence was located mainly in the cell periphery (membrane), and the signal from the cytosol intensified when external solution contained 30 µmol/l phenylephrine. This effect was quantified by exploring the green plane intensity of region of interests (ROI) and profile across the line (**B, C**), thus confirming functionality of reconstituted human α_{1A}AR. **D:** The mean I_{Kr} tail amplitudes (fitted to the Boltzmann equation) from 12 cardiomyocytes loaded with 10 µmol/l PIP_2 before and during α_{1A}AR stimulation. Inset illustrates the same relation when average tail amplitudes are normalized to the maximal value. Intrapipette PIP_2 significantly diminished phenylephrine-induced I_{Kr} reduction (see text). **E:** On the other hand, specific protein kinase C inhibitor BIS I (200 nmol/l) did not affect extensively I_{Kr} modulation: degree of tail inhibition, positive shift of activation, and facilitation of deactivation were similar to that in the control state.

stants were affected as in the absence of PIP_2 (not shown). On the contrary, the I_{Kr} response to phenylephrine remained identical in the presence of protein kinase C inhibitor bisindolylmaleimide I (BIS I) (Figure 3E): $V_{1/2}$ from the Boltzmann fit increased +5.3 mV ($P > .05$ vs. control value), average reduction of tail currents was comparable with control at all test voltages, and deactivation time constants were 53 ± 16 ms and 540 ± 191 ms in control vs. 35 ± 13 ms and 323 ± 169 ms after α_{1A}AR stimulation (n = 7, $P < .05$). BIS I (200 nmol/l) itself did not have a noticeable effect on control I_{Kr} amplitude.

cAMP-induced modulation of I_{Kr}

We stimulated $\beta_{1,2}$AR-coupled intracellular production of cAMP by exposing HL-1 cardiomyocytes simultaneously to adenylyl cyclase activator (forskolin, 5 µmol/l) and phosphodiesterase inhibitor (IBMX, 100 µmol/l). Figure 4A shows typical I_{Kr} traces in the absence and presence of forskolin/IBMX. Activation of transduction pathways coupled to $\beta_{1,2}$AR had a dual effect on I_{Kr}. Tail amplitudes increased by 43.3% to 7.1% after depolarizations between −50 and −30 mV (Figure 4B) but decreased by 4.1% to 5.5% after depolarization between +20 and +50 mV ($P < .05$). Combination forskolin/IBMX shifted I_{Kr} activation in a hyperpolarizing direction but did not affect considerably deactivation kinetics (Table 1). Thus in HL-1 cardiomyocytes, activation of $\beta_{1,2}$AR-coupled pathways tended to counterbalance the modulation of I_{Kr} by α_{1A}AR at negative potentials.

α_{1A}AR and $\beta_{1,2}$AR regulation of Kv11.1 channels

We investigated regulation of WT-Kv11.1 and 2 LQT2-associated mutant channels (see Methods section) reconstituted in CHO cells by α_{1A} and $\beta_{1,2}$AR in a way similar to that in HL-1 cardiomyocytes.

Figure 5A exemplifies currents recorded from CHO cells co-expressing WT-Kv11.1 and human α_{1A}AR in the control state and during exposure to 30 µmol/l phenylephrine. The α_{1A}AR agonist decreased I_{Kr} tail amplitude, shifted the midpoint of I-V relation rightward, and facilitated deactiva-

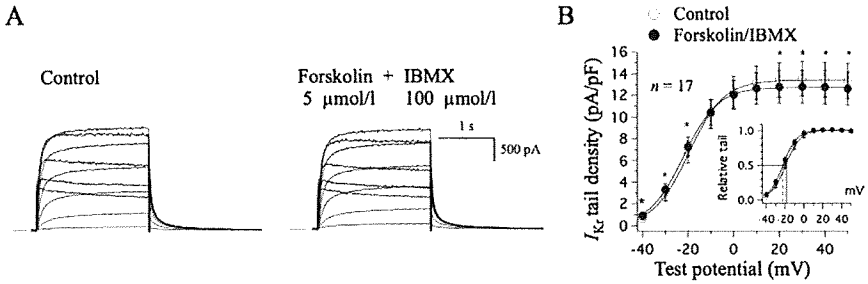

Figure 4 I_{Kr} modulation by $\beta_{1,2}$AR-coupled transduction. **A:** Typical I_{Kr} in HL-1 cardiomyocytes elicited by square voltage-clamp pulses (like in Figure 1B) in control state and during elevation of intracellular cAMP by 5 μmol/l forskolin and 100 μmol/l IBMX. **B:** I_{Kr} tail densities from 17 cells as a function of depolarizing voltage before and during forskolin/IBMX application. Smooth curves (fit to the Boltzmann equation) show −3.2 mV hyperpolarizing shift of I-V relation caused by the drugs (inset). Tail amplitudes were affected significantly after depolarizations between −40 mV and −20 mV (increased) and between +20 and +50 mV (reduced, $*P < .05$).

tion (Figures 7A and 7B). Activation of $\beta_{1,2}$AR-coupled signaling in CHO cells[18] with external forskolin/IBMX had dual effect on tail amplitude as in Figure 4B and shifted current activation to more negative potentials (Figure 5B, Table 1).

Expressed alone, mutant proteins Y43D-Kv11.1 and K595E-Kv11.1 did not produce functional channels (not shown). Co-expression with WT-Kv11.1 generated currents with loss-of-function properties. For WT/Y43D-Kv11.1, a positive shift (+6.6 mV, $P < .05$) of I-V relation and considerable acceleration of deactivation kinetics (Figures 6A, 7C, and 7D); for WT/K595E-Kv11.1, an even more prominent rightward shift of I-V relation (+10.7 mV, $P < .05$) and reduced I_{Kr} amplitude (between 82.5% at −30 mV and 49.3% at +50 mV, $P < .05$, Figure 6C). Nevertheless, stimulation of α_{1A}AR caused additional inhibition of the WT/Y43D-Kv11.1 and WT/K595E-Kv11.1 currents: $V_{1/2}$ was further increased with +5.1 and +5.6 mV, respectively, and time constants of deactivation had diminished values in the course of phenylephrine superfusion (Figures 6A, 6C, and 7C to 7F, Table 1). Forskolin/IBMX modulated both mutant currents similar to WT-Kv11.1: a negative shift of I-V relation and inhibition of tail amplitudes after positive depolarization for WT/Y43D-Kv11.1; a negative shift of the I-V curve for WT/K595E-Kv11.1 (Figures 6B and 6D, Table 1).

Taken together, both studied mutant currents retained regulation by α_{1A}AR and forskolin/IBMX comparable to that of WT-Kv11.1 channels.

Discussion

Acute adrenergic regulation of I_{Kr} and Kv11.1 channels

The main findings in this study can be summarized as follows. α_{1A}AR stimulation downregulated I_{Kr} and reconstituted WT-Kv11.1 channels by decreasing current amplitudes, creating a positive shift of I-V relations, and accelerating deactivation kinetics. The $\beta_{1,2}$AR-associated cAMP increase had dual action on I_{Kr} and Kv11.1: a negative shift of the I-V curves (augmenting the currents at negative potentials) and a decline of current amplitudes at positive

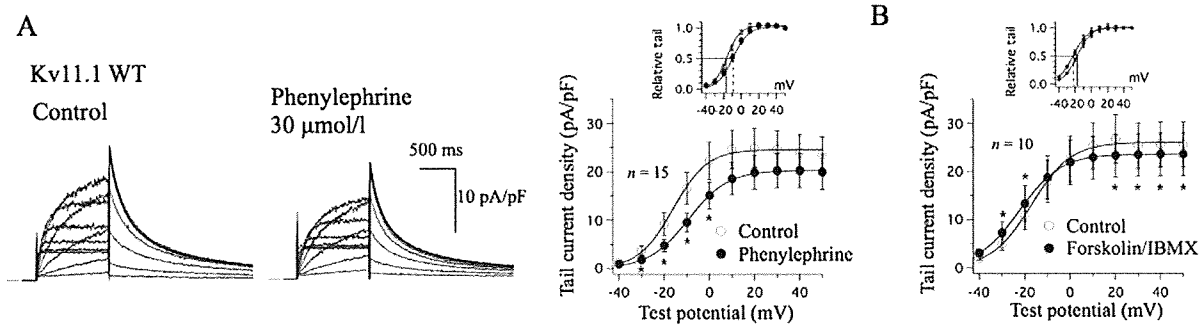

Figure 5 α_{1A}AR and $\beta_{1,2}$AR regulation of reconstituted WT-Kv11.1 channels. **A:** WT-Kv11.1 currents and average I-V relation of WT-Kv11.1 tails density (fitted to the Boltzmann equation) before and during exposure of CHO cells to 30 μmol/l phenylephrine are shown. α_{1A}AR stimulation reduced WT-Kv11.1 tail amplitudes significantly after depolarizations between −30 and 0 mV ($*P < .05$, maximal inhibition was 50.8% after −30 mV), shifted I-V curve in a rightward direction (+6.9 mV), and accelerated deactivation kinetics. Inset shows the mean amplitudes of WT-Kv11.1 tails normalized to the value after +40 mV step. **B:** The combination forskolin/IBMX shifted I-V curve to more negative potentials (−4.5 mV, $*P < .05$), significantly reduced tail amplitudes after depolarizations between +20 and +50 mV (8.0% to 13.6%), and increased tails after depolarizations to −30 and −20 mV (47.6% and 22.2%, respectively).

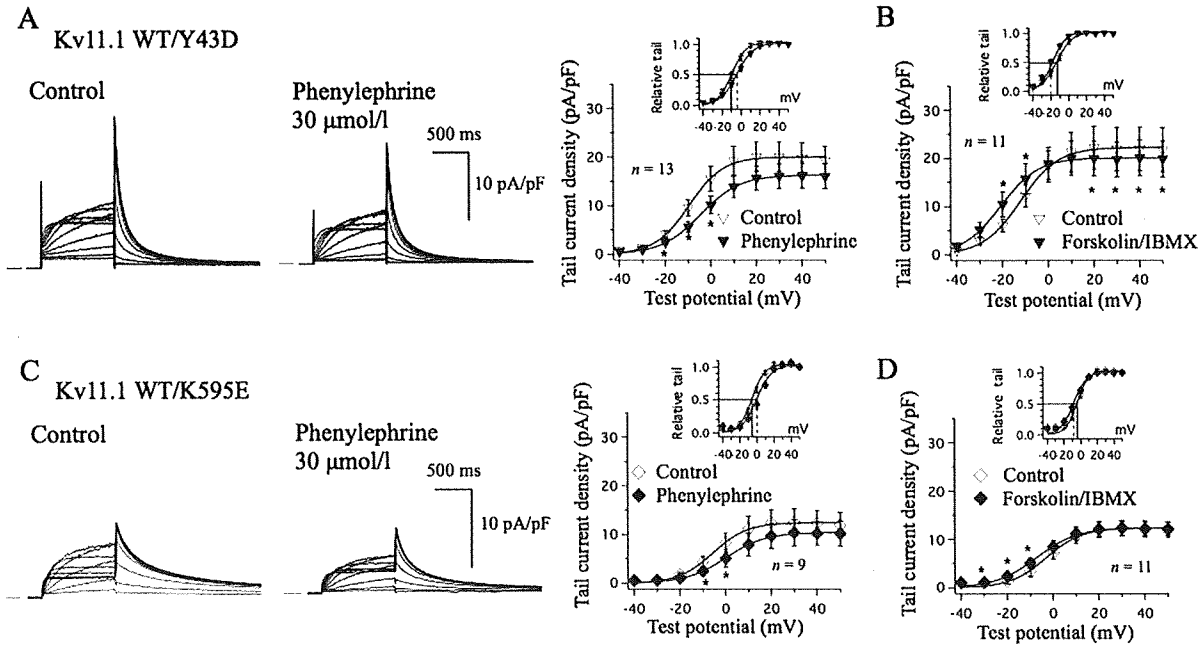

Figure 6 α_{1A}AR and $\beta_{1,2}$AR regulation of WT/Y43D- and WT/K595E-Kv11.1 channels. Phenylephrine and forskolin/IBMX effects on WT/Y43D-Kv11.1 (**A, B**) and WT/K595E-Kv11.1 (**C, D**) currents are presented identically to WT-Kv11.1 in Figure 5. These already deficient mutant channels were additionally downregulated by phenylephrine through inhibition of current amplitude, maximally after −10 mV depolarization (41.2% and 43.8% for WT/Y43D-Kv11.1 and WT/K595E-Kv11.1, respectively, $*P < .05$), positive shift of I-V relation and facilitation of deactivation. Forskolin/IBMX treatment changed the midpoint of I-V curves toward negative potentials for both mutants (Table 1). Forskolin/IBMX reduced the amplitude of WT/Y43D-Kv11.1 tail currents by 8.6% to 10.1% after steps between +20 and +50 mV and augmented tail amplitudes by 71.1% and 28.5% after depolarizations to −20 and −10 mV, respectively. **D:** WT/K595E-Kv11.1 tails increased by 99.0% to 52.6% after test voltages between −30 and −10 mV ($*P < .05$).

potentials. Moreover, 2 mutant channels found in LQT2 probands with loud noise-triggered syncope preserved the same pattern of acute adrenergic regulation.

α_{1A}AR-related modulation of I_{Kr} in ventricular myocytes[11] or Kv11.1 channels in expression systems[8,10] was shown. However, intracellular signaling linking α_{1A}AR and I_{Kr} channels is not sufficiently clarified because conflicting data have been published.[8,10] Our results are close to the reports for rabbit ventricular myocytes,[11] although we observed a positive shift of I-V relation and faster deactivation during α_{1A}AR stimulation. Stronger inhibition of I_{Kr} and Kv11.1 currents at negative potentials indicates that the significance of α_{1A}AR regulation is larger within the voltage range where the current is particularly important for ventricular repolarization.

$\beta_{1,2}$AR-coupled signaling produces complex changes in I_{Kr}: the net effect after cAMP application on the reconstituted Kv11.1 channel was downregulation, but co-expression of ancillary subunits (KCNE1 or KCNE2) attenuated current inhibition.[12] I_{Kr} in guinea pig ventricular myocytes was suppressed[14] or increased[15] by isoproterenol. In our data, stimulation of $\beta_{1,2}$AR-coupled signaling (Figures 4 to 6) induced together cAMP and protein kinase A-dependent effects on I_{Kr} and Kv11.1 currents. The negative shift of I-V relation would oppose the inhibition of I_{Kr} by α_{1A}AR activation.

Arrhythmogenic significance

For the first time (to our knowledge), we showed adrenergic regulation of 2 LQT-associated Kv11.1 channels (N-terminal Y43D and pore K595E mutations), co-expressed with WT-Kv11.1 in a mammalian cell line. The currents had a loss-of-function phenotype but nevertheless maintained responses to phenylephrine and forskolin/IBMX similarly to the WT-Kv11.1 channel. With these mutant channels, acute sympathetic stimulation via α_{1A}AR would act to additionally increase APD and thus promote arrhythmogenesis. Evidence for possible contribution of α_{1A}AR-mediated triggers for loud noise-induced syncope is the fact that in the Kv11.1-Y43D proband, symptoms had been successfully eliminated by carvedilol, but in the case of Kv11.1-K595E, propranolol was ineffective.

Arrhythmogenic potential of α_{1A}AR-coupled mechanisms has the following support: phenylephrine-induced ventricular tachyarrhythmias in a canine LQT model in vivo[22]; the α_1-adrenoceptor agonist methoxamine facilitated the proarrhythmic effect of almokalant (a class III antiarrhythmic drug) in rabbit[23]; phenylephrine and methoxamine prolonged the APD of canine Purkinje fibers[4] and other animals[5]; and phenylephrine increased the transmural dispersion of ventricular depolarization (TDR) in LQT2 patients.[24] Although physiological regulation of ion channels through α_{1A}AR is not restricted only to I_{Kr}, our data

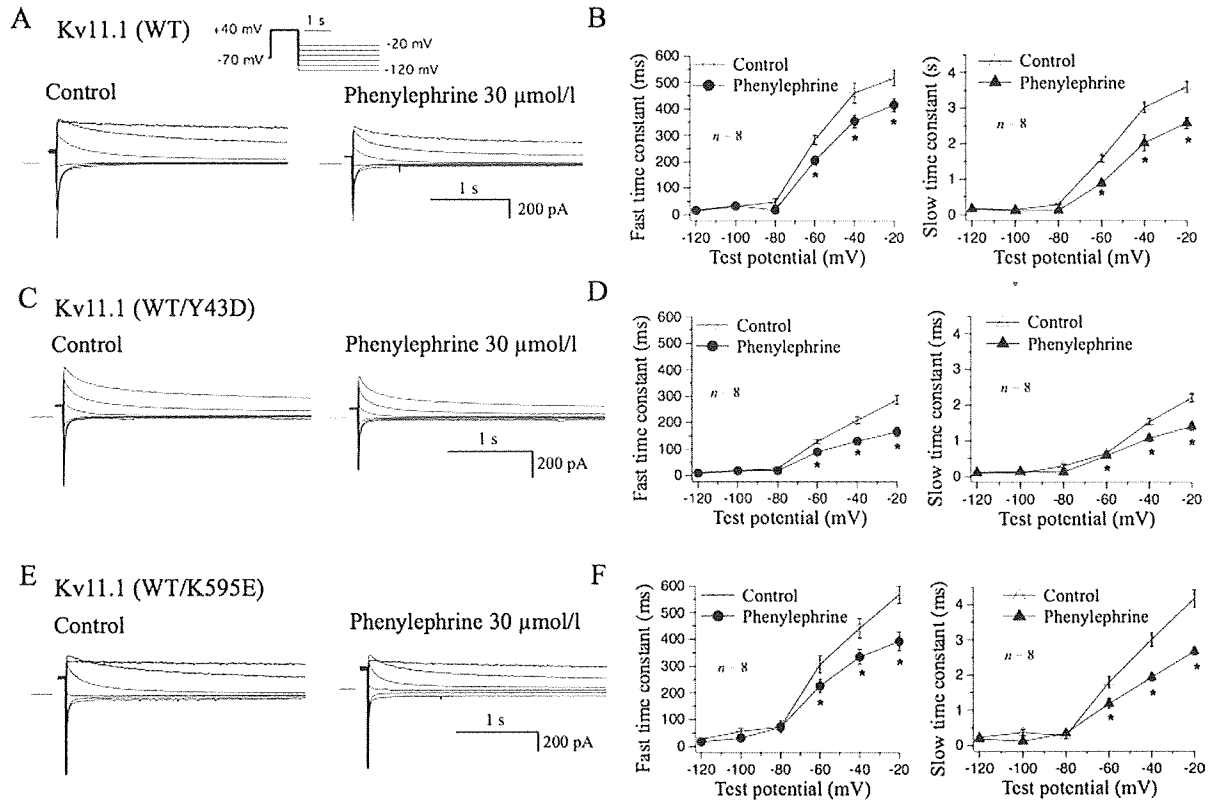

Figure 7 Deactivation kinetics of Kv11.1 currents. **A, C, E:** Representative Kv11.1 tail currents (WT, WT/Y43D, and WT/K595E, respectively) recorded at membrane potentials between −120 and −20 mV with a 20-mV increment after 2-second depolarizing step to +40 mV (**A, inset**) in control conditions and in the presence of 30 μmol/l phenylephrine. Similar to the results for I_{Kr} in HL-1 cardiomyocytes, deactivation of reconstituted WT and mutant Kv11.1 currents in CHO cells was facilitated by the α_{1A}AR agonist. **B, D, F:** The mean values of the fast and slow time constants of deactivation are plotted. The precise values of time constants at −60 mV are in Table 1 (see text).

suggest that it might be one of the currents involved in reported APD increase. In canine Purkinje fibers, the APD increase by phenylephrine was attributed mainly to I_K inhibition.[25]

Sympathetic stimulation is thought to initiate torsades de pointes in LQT by induction of triggered activity (early and delayed afterdepolarizations) and increase of TDR, which promotes propagation of premature electrical impulse.[26] In clinical studies, the effects of mainly $\beta_{1,2}$AR agonist epinephrine on QTc and duration of the interval between the peak and end of the T-wave on electrocardiogram ($T_{p\text{-}e}$, reflecting TDR) are known to be significantly longer in LQT1 than LQT2 at steady state.[28,29] On the other hand, in LQT2, an increase of QTc and $T_{p\text{-}e}$ by epinephrine is temporary and reversible at steady state (when comparisons with LQT1 in many studies were made[27,28]). Animal LQT models have shown the same dynamics in APD in the presence of epinephrine.[29] On the contrary, in LQT2 patients, phenylephrine was shown to prolong TDR considerably more than in LQT1.[24] Thus according to our results and the abovementioned reports, in LQT2 arrhythmia provoked shortly after sympathetic stress (loud noise), α_{1A}AR-mediated I_{Kr} reduction might be one potential triggering mechanism.

In brief, in the case of LQT2 and β-blockade, α_{1A}AR-mediated regulation of I_{Kr} could still cause sufficient derangements in ventricular repolarization to induce arrhythmia shortly after sympathetic stimulation. Higher resistance to β-blockers in LQT2 as well as the effectiveness of the α_1- and β-blocking agent labetalol[7] supports this statement. However, clinical investigations are needed to confirm this proposed mechanism.

Conclusion

This study investigated α_{1A}AR and $\beta_{1,2}$AR adrenergic regulation of 2 reconstituted Kv11.1 mutant channels found in loud noise-triggered symptomatic cases of congenital LQTS in comparison with wild-type Kv11.1 and native I_{Kr}. Although whole-cell currents from mutant Kv11.1 channels (co-expressed with wild-type Kv11.1) had a loss-of-function phenotype, they preserved negative regulation mediated through α_{1A}AR in a way similar to native I_{Kr} channel and wild-type Kv11.1.

The presented results implicate α_{1A}AR-coupled transduction in arrhythmogenesis in selected cases of congenital LQT2, specifically in patients with syncope after startled auditory stimuli, and suggest α_{1A}AR blockers as a beneficial treatment. It may worth clarifying α_{1A}AR regulation of long-QT-related Kv11.1 mutant channels, particularly in the cases of loud noise-induced arrhythmia or in patients resistant to β-blocking agents.

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Long-Term Prognosis of Probands With Brugada-Pattern ST-Elevation in Leads V_1–V_3

Shiro Kamakura, MD, PhD; Tohru Ohe, MD, PhD; Kiyoshi Nakazawa, MD, PhD; Yoshifusa Aizawa, MD, PhD; Akihiko Shimizu, MD, PhD; Minoru Horie, MD, PhD; Satoshi Ogawa, MD, PhD; Ken Okumura, MD, PhD; Kazufumi Tsuchihashi, MD, PhD; Kaoru Sugi, MD, PhD; Naomasa Makita, MD, PhD; Nobuhisa Hagiwara, MD, PhD; Hiroshi Inoue, MD, PhD; Hirotsugu Atarashi, MD, PhD; Naohiko Aihara, MD; Wataru Shimizu, MD, PhD; Takashi Kurita, MD, PhD; Kazuhiro Suyama, MD, PhD; Takashi Noda, MD, PhD; Kazuhiro Satomi, MD, PhD; Hideo Okamura, MD; Hitonobu Tomoike, MD, PhD; for the Brugada Syndrome Investigators in Japan

Background—The prognosis of patients with saddleback or noncoved type (non–type 1) ST-elevation in Brugada syndrome is unknown. The purpose of this study was to clarify the long-term prognosis of probands with non–type 1 ECG and those with coved (type 1) Brugada-pattern ECG.

Methods and Results—A total of 330 (123 symptomatic, 207 asymptomatic) probands with a coved or saddleback ST-elevation ≥1 mm in leads V_1–V_3 were divided into 2 ECG groups—type 1 (245 probands) and non–type 1 (85 probands)—and were prospectively followed for 48.7±15.0 months. The absence of type 1 ECG was confirmed by drug provocation test and multiple recordings. The ratio of individuals with a family history of sudden cardiac death (14%) was lower than previous studies. Clinical profiles and outcomes were not notably different between the 2 groups (annual arrhythmic event rate of probands with ventricular fibrillation; type 1: 10.2%, non–type 1: 10.6%, probands with syncope; type 1: 0.6%, non–type 1: 1.2%, and asymptomatic probands; type 1: 0.5%, non–type 1: 0%). Family history of sudden cardiac death at age <45 years and coexistence of inferolateral early repolarization with Brugada-pattern ECG were independent predictors of fatal arrhythmic events (hazard ratio, 3.28; 95% confidence interval, 1.42 to 7.60; P=0.005; hazard ratio, 2.66; 95% confidence interval, 1.06 to 6.71; P=0.03, respectively, by multivariate analysis), although spontaneous type 1 ECG and ventricular fibrillation inducibility by electrophysiological study were not reliable parameters.

Conclusions—The long-term prognosis of probands in non–type 1 group was similar to that of type 1 group. Family history of sudden cardiac death and the presence of early repolarization were predictors of poor outcome in this study, which included only probands with Brugada-pattern ST-elevation. (***Circ Arrhythmia Electrophysiol.* 2009;2:495-503.)**

Key Words: death, sudden ■ prognosis ■ follow-up studies ■ electrocardiography ■ Brugada syndrome

Brugada syndrome is a hereditary arrhythmogenic disease characterized by ST-elevation in the right precordial lead of standard ECGs and an increased risk of sudden cardiac death (SCD).[1] The prognosis for this condition and the management approaches have been reported in several multicenter studies of patients with the coved type 1 ECG. However, no prospective data have been reported in patients with saddleback type or noncoved Brugada-pattern ST-elevation before, because they were excluded from previous

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studies as atypical Brugada patients showing a benign clinical course. Besides, the data from previous studies are all conflicting with regard to the prognosis of the typical Bru-

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From the Division of Cardiology (S.K., N.A., W.S., T.K., K.S., T.N., K.S., H.O., H.T.), National Cardiovascular Center, Suita, Japan; the Department of Cardiovascular Medicine (T.O.), Okayama University Graduate School of Medicine, Okayama, Japan; the Department of Cardiology (K.N.), St Marianna University, Kawasaki, Japan; the Division of Cardiology (Y.A.), Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; the Division of Cardiology (A.S.), Yamaguchi University Graduate School of Medicine, Ube, Japan; the Department of Cardiovascular Medicine (M.H.), Shiga University of Medical Science, Otsu, Japan; the Department of Cardiopulmonary Medicine (S.O.), Keio University, Tokyo, Japan; the Second Department of Internal Medicine (K.O.), Hirosaki University School of Medicine, Hirosaki, Japan; the Second Department of Internal Medicine (K.T.), Sapporo Medical University School of Medicine, Sapporo, Japan; the Division of Cardiovascular Medicine (K.S.), Toho University Medical Center Ohashi Hospital, Tokyo, Japan; the Department of Cardiovascular Medicine (N.M.), Hokkaido University Graduate School of Medicine, Sapporo, Japan; the Department of Cardiology (N.H.), Tokyo Women's Medical University; the Second Department of Internal Medicine (H.I.), Toyama University, Toyama, Japan; and the Department of Internal Medicine, Nippon Medical School, Tama-Nagayama Hospital, Tokyo, Japan.

Correspondence to Shiro Kamakura, MD, PhD, Division of Cardiology, Department of Internal Medicine, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565, Japan. E-mail kamakura@hsp.ncvc.go.jp

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gada syndrome.[2-5] This may be caused by cohort studies that included a significant number of family members other than probands, in which the prognosis of pedigree members can be affected by the disease severity of probands. Furthermore, a selection bias can be present if the data are analyzed retrospectively. Therefore, we aimed to investigate the long-term prognosis of probands with noncoved type ST-elevation in leads V_1–V_3, prospectively, and compared it with that of probands with the type 1 ST-elevation.

Methods

Patient Population

A total of 330 individuals with spontaneous ST-elevation were registered consecutively in this study, namely, "a multicenter study for risk stratification and management in patients with Brugada syndrome." The study was conducted at 26 institutions across Japan beginning in July 2001. These individuals were prospectively followed up for more than 12 months to the end of March 2007. Subjects were enrolled in this study if they met the following inclusion criteria: (1) proband, (2) J-point (QRS-ST junction) amplitude of ≥0.1 mV (1 mm) with either coved or saddle back type ST-segment elevation in at least 2 of the 3 precordial leads (V_1–V_3) on resting standard 12-lead ECG, (3) normal findings on physical examination, and (4) no abnormality in either right or left ventricular morphology and/or function demonstrated by chest radiography and echocardiography. Patients with vasospastic angina and those with vasovagal syncope were excluded from this study. Patients were not administered antiarrhythmic drugs and did not have electrolyte abnormalities at the time of baseline ECG recording and other examinations.

Classification of Groups

We divided the 330 patients with Brugada-pattern ECG into 3 groups according to their symptoms: The ventricular fibrillation (VF) group consisted of 56 probands with aborted sudden death and/or documented VF, the syncope group consisted of 67 probands with syncope without documented arrhythmias that was not typical for vasovagal syncope, and the asymptomatic group consisted of 207 asymptomatic individuals whose ECGs were mainly detected by individual annual medical checkup or health screening in their place of employment.

We also divided these patients into 2 groups according to ECG morphology: The type 1 group consisted of 245 probands with a spontaneous type 1 ECG or those who developed type 1 ECG with a drug provocation test. The non–type 1 group consisted of the remaining 85 probands who never showed type 1 ST-elevation even with the drug provocation test (Figure 1) and during the follow-up on standard 12-lead ECGs.

Clinical Data, ECG, and Electrophysiological Testing

Clinical data including age at the enrollment, sex, family history of SCD, and the presence of atrial fibrillation were collected for all patients. The standard ECGs were recorded more than 5 times during the follow-up period in all patients. ECG recording on higher intercostals spaces (third and/or second) in leads V_1–V_3[6] was encouraged in patients who had cardiac events during the follow-up period.

A type 1 ECG was defined as a prominent coved ST-segment elevation displaying J-point wave amplitude or ST-segment elevation ≥2 mm or 0.2 mV.[7,8] ECG patterns with a prominent coved ST-elevation ≥2 mm followed by a positive or flat T wave were also included in type 1 group (Figure 2A through C). A non–type 1 ECG was defined as one of the following: type 2 ECG,[7] type 3 ECG,[7] and ECG displaying coved or saddleback ST-elevation with J-wave amplitude ≥1 mm and <2 mm (Figures 1 and 2D through 2G).

The presence of early repolarization in the inferolateral leads[9] was evaluated by baseline 12-lead ECGs at the time of enrollment to elucidate ECG findings associated with Brugada syndrome. Early repolarization was defined as an elevation of the J point in at least 2 leads. The amplitude of the J wave or J-point elevation had to be at least 1 mm above the baseline level, either as QRS slurring or notching in the inferior lead (II, III, and aVF), lateral (I, aVL, and V_4–V_6) lead, or both.[9]

ECGs were evaluated by 3 independent investigators (S.K., N.A., and W.S.) who were unaware of the patients' other clinical information. The ECG type or morphology was established by the evaluation in which at least 2 of the 3 observers were in agreement.

Sodium channel blocker pilsicainide (1 mg/kg body weight at a rate of 5 to 10 mg/min), disopyramide (1.5 mg/kg, 10 mg/min), flecainide (2 mg/kg, 10 mg/min), or procainamide (10 mg/kg, 100 mg/min) was administered intravenously in 270 (82%) patients (233, 15, 14, and 8, respectively) to test the conversion to typical coved ST-elevation.[8,10,11]

Baseline electrophysiological studies (EPS) were performed in 232 (70%) patients. A maximum of 3 ventricular extrastimuli were delivered from 2 right ventricular (RV) sites (RV apex and RV outflow tract) unless VF or polymorphic ventricular tachycardia (VT) (lasting ≥10 beats) that terminated spontaneously within 30 seconds, causing syncope, or requiring intervention to be terminated was elicited at a previous step. Premature beats were started in late diastole; coupling intervals were then reduced in 10-ms decrements until refractoriness was reached. Stimulation was performed at twice the diastolic threshold. Patients with inducible ventricular arrhythmias lasting less than 10 beats were classified as noninducible. The indices including age, sex distribution, a family history of SCD at

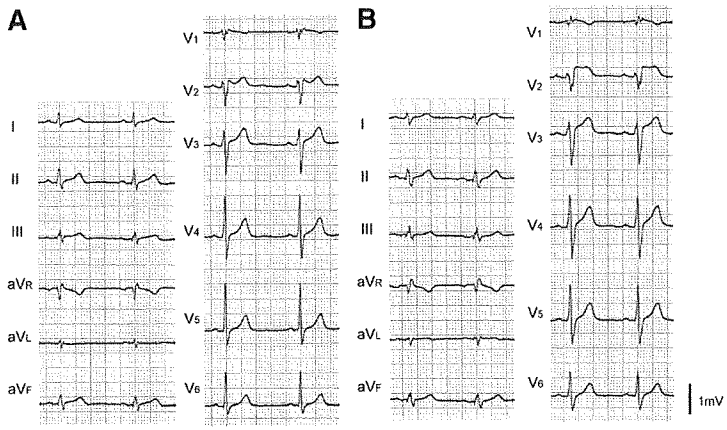

Figure 1. Presentation of 12-lead ECGs of a patient with non–type 1 ST-elevation. A, Baseline 12-lead ECG; B, 12-lead ECG after provocation by intravenous administration of 50 mg pilsicainide in the same patient. Saddleback-type ST-elevation in leads V_1 and V_2 was enhanced after pilsicainide but was not changed to type 1 ST-elevation. This 46-year-old male patient with a history of syncope but with no family history of SCD had inducible VF by electrophysiological study. He had spontaneous VF 11 months after enrollment.

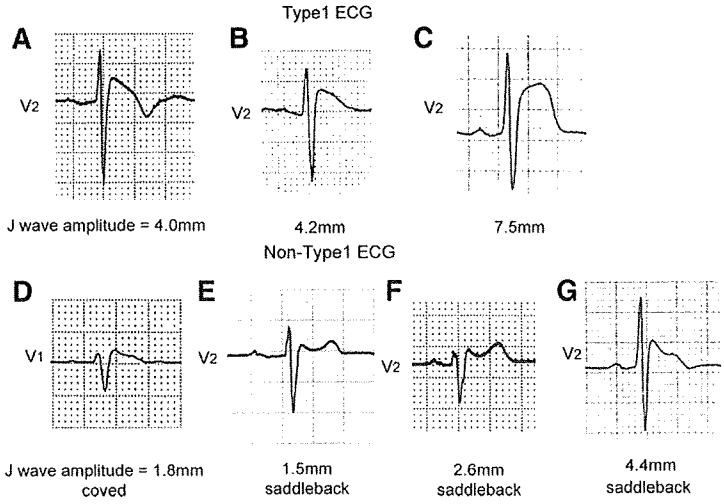

Figure 2. Presentation of type 1 and non–type 1 ECG. Coved-type ST-elevation with a J-wave amplitude ≥2 mm followed by a negative T wave (A) or a positive/flat T wave (B), and a coved ST-elevation followed by a smaller J wave than T wave (C) were defined as type 1 ECG. Coved (D) or saddleback-type ST-elevation (E) with a J-wave amplitude <2 mm, a saddleback ST-elevation with a J-wave amplitude ≥2 mm (F), and a saddleback ST-elevation displaying bigger J wave than T wave (G) were defined as non–type 1 ECG.

less than 45 years of age, and VF/polymorphic VT inducibility were compared with those reported in previously published studies[2,3,5] (Table 1). In addition to these parameters, the presence of atrial fibrillation, cardiac events at night, and inferolateral early repolarization were compared between type 1 and non–type 1 groups.

Patient treatment was based on clinical judgment of the participating hospital. Twenty-eight (8%) probands received antiarrhythmic drugs (quinidine sulfate ≤400 mg, bepridil ≤200 mg, disopyramide ≤300 mg, aprindine ≤30 mg, and amiodarone ≤200 mg/d) for prevention of atrial fibrillation or VF. Calcium antagonists were administered in 18 (5%) probands for hypertension. Quinidine and bepridil were administered only after a documentation of VF during follow-up. Among the 330 patients, 125 (38%) received an implantable cardioverter-defibrillator (ICD). During follow-up, patients were considered to have an arrhythmic event if sudden death occurred or VF was documented.

Statistical Analysis

Data are presented as mean±standard deviation. The Fisher exact test or the χ^2 test was used for categorical variables. One-way ANOVA was used for comparisons of continuous variables among the different groups. Survival curves were plotted by the Kaplan-Meier method and analyzed by the log-rank test. Cox proportional hazards models were used to analyze factors associated with the time to the first arrhythmic event during follow-up in all probands as well as in type 1, non–type 1, VF, and non-VF (syncope and asymptomatic) groups. Variables were included in the multivariate analysis with the use of a forward stepwise procedure with a criteria of $P<0.05$ for inclusion and $P>0.15$ for removal from the model. A probability value of $P<0.05$ was considered statistically significant.

This study was performed under the ethical code approved by the Health, Labor, and Welfare Ministry of Japan. Written informed consent was obtained from all individuals.

Results

Clinical Profiles of All Probands

The mean age of the 330 probands was 51.4±14.8 years (median, 53 years; range, 4 to 86 years). The majority (315; 95%) of probands were male. A low percentage (14%) of patients had a family history of SCD occurring before the age of 45 years. The induction rate of VF/polymorphic VT by EPS was higher (77/109: 72%, $P<0.005$) in symptomatic than asymptomatic probands (61/123: 50%) (Table 1).

Comparison of Clinical Characteristics Between Type 1 and Non–Type 1 Groups

Type 1 ECG was found in 245 probands (VF group: 45, 18%; syncope group: 46, 19%; and asymptomatic group: 154, 63%). Of these 245 probands, 173 (71%) showed type 1 ECG spontaneously and the remaining 72 (29%) showed characteristic type 1 morphology after class Ic or Ia antiarrhythmic drug administration. In 85 probands of the non–type 1 group (VF group: 11, 13%; syncope group: 21, 25%; and asymptomatic group: 53, 62%), non–type 1 ECG remained during the drug provocation test (type 2: 61,

Table 1. Comparison of Patient Characteristics Among 3 Large Registries

	Brugada et al[2]		Eckardt et al[5]		Kamakura et al	
	Sympt	Asympt	Sympt	Asympt	Sympt (VF, S)	Asympt
No.	144	190	89	123	123 (56, 67)	207
Age, y	41±16*	40±16	46±14	44±14	50.4±16.6	51.9±13.6
Men, %	83	71	76	68	96	95
FH of SCD, %	34	72	21	33	19 (25, 13)	11
VF/VT inducibility, %	73	33	63	39	71 (65, 75)	50

Values in parentheses are for the patients with aborted sudden death and an episode of syncope. Sympt indicates symptomatic; Asympt, asymptomatic; S, syncope; FH of SCD, prevalence of patients with a family history of sudden cardiac death at <45 years old; and VF/VT inducibility, induction rate of VF or polymorphic ventricular tachycardia by EPS.

*Age of patients with VF.

Table 2. Comparison of Clinical Profiles Between Probands With Type 1 ECG and Those With Non–Type 1 ECG

	Type 1 (n=245)			Non-Type 1 (n=85)			
	VF	Syncope	Asympt	VF	Syncope	Asympt	*P* Value
No.	45	46	154	11	21	53	0.33
Age, y	48.2±17.8	52.5±15.6	52.3±13.1	48.0±18.1	51.9±15.8	50.7±15.2	0.99
Men, n (%)	44 (98)	44 (96)	146 (95)	11 (100)	19 (90)	51 (96)	0.90
FH of SCD, n (%)	11 (24)	8 (17)	17 (11)	3 (27)	1 (5)	5 (9)	0.06
Event at night, n (%)	37/45 (82)	15/45 (33)		5/9 (56)	7/18 (39)		0.06
Inferolateral ER, n (%)	8 (18)	3 (7)	15 (10)	2 (18)	1 (5)	4 (8)	0.85
Prevalence of AF, n (%)	19 (42)	7 (15)	21 (14)	4 (36)	3 (14)	8 (15)	0.87
VF/VT inducibility, n (%)	27/41 (66)	31/40 (78)	52/91 (57)	7/11 (64)	12/17 (71)	9/32 (28)	0.04

n (%) indicates the number and the ratio of patients with each parameter; event at night, event developed at night (8 PM to 8 AM); inferolateral ER, inferolateral early repolarization; AF, atrial fibrillation; VF/VT inducibility, induction rate of VF or polymorphic ventricular tachycardia by EPS.

72%; coved with J-point amplitude <2 mm: 24, 28%) and the follow-up period. Most of the clinical parameters except for VF/VT inducibility, namely, age, sex distribution, the prevalence of atrial fibrillation, the presence of a family history of SCD, cardiac events at night (8 PM to 8 AM), and early repolarization, were of similar occurrence between type 1 and non–type 1 groups (Table 2). Only 8% (7/85) of probands in the non–type 1 group and 11% (26/245) of those in the type 1 group were associated with early repolarization in the inferolateral leads.

Follow-Up and Predictors of Outcome

The mean follow-up period for the entire study population was 48.7±14.9 months. Follow-up time was similar among VF (51.9±15.0 months), syncope (48.5±14.0 months), and asymptomatic (47.7±15.0 months) groups and between type 1 (48.6±15.2 months) and non–type 1 (48.9±14.2 months) groups. Twenty-four patients had fatal arrhythmic events during follow-up. The frequency of events in the type 1 group—15 of 45 (33%) in patients with VF, 1 of 46 (2%) in syncope patients, and 3 of 154 (2%) in asymptomatic patients— was similar to that in the non–type 1 group (4/11: 36%, 1/21: 5%, and 0/53: 0%, respectively, $P=0.22$; Figure 3). In 5 patients who had events in the non–type 1 group, 2 had shown a type 1 ST-elevation only in the higher (second or third) intercostal spaces—1 in a follow-up ECG and 1 after drug provocation test. The observed frequency of arrhythmic events was significantly highly in patients with early repolarization in the inferolateral leads (7/33; 21% versus 17/297; 6%, $P<0.005$), although there was no difference in risk between the 2 groups (type 1: 6/26; 23%, non–type 1: 1/7; 14%, $P=0.67$). One asymptomatic patient with type 1 ECG died suddenly 3 months after enrollment. Six patients died of nonarrhythmic causes; 3 died of cancer, 1 because of rupture of abdominal aortic aneurysm, 1 because of pneumonia, and cause of death for 1 patient was unknown. Seven percent of all patients who entered the study dropped out, the most frequent reason for drop-out was inability of follow-up due to patient's change of address.

Figure 4 shows the Kaplan–Meier analysis of arrhythmic events in probands with type 1 and non–type 1 ECG. Probands in the VF group had significantly worse prognosis than those in the syncope and asymptomatic groups. The

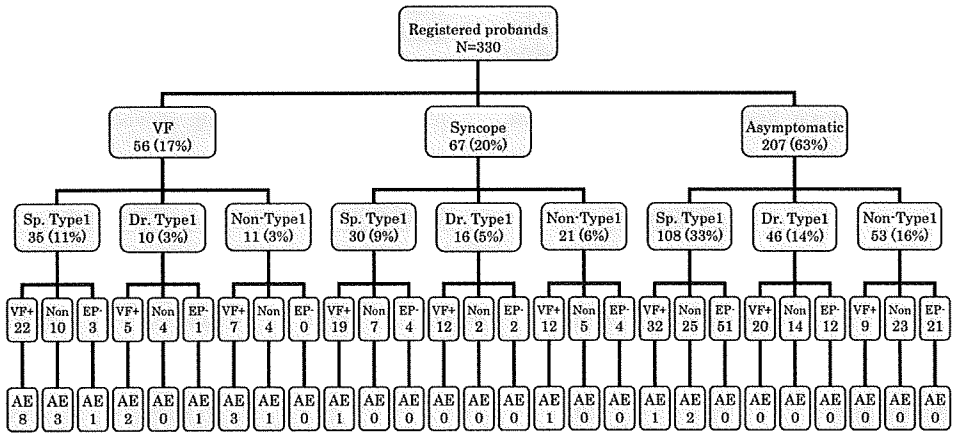

Figure 3. Flow chart of proband groups categorized according to symptom, ECG morphology, and VF/VT inducibility by electrophysiological study. Sp. Type 1 indicates spontaneous type 1 group; Dr. Type 1, drug-induced type 1 group; VF+, a group with inducible VF/VT; Non, a group with noninducible VF/VT; EP−, a group in which electrophysiological study was not performed; AE, fatal arrhythmic event during follow-up. The number indicates the number of probands in each category.

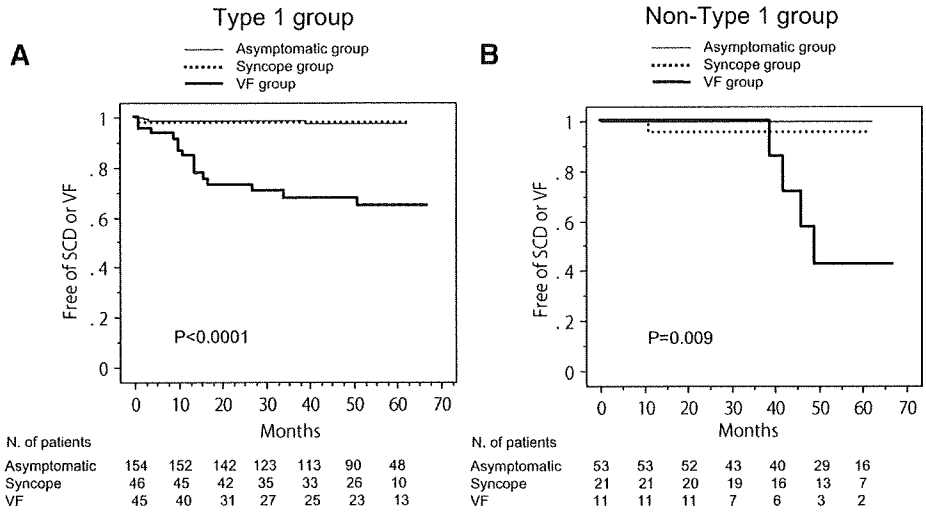

Figure 4. Kaplan–Meier analysis of arrhythmic events (SCD or documented VF) during follow-up depending on the clinical presentation (VF/aborted sudden death, syncope, or asymptomatic) in probands with type 1 ECG (A) and those with non–type 1 ECG (B). $P<0.0001$ represents overall comparison, and $P=0.009$ is for comparison between the VF group and the syncope group. There was no statistically significant difference ($P=0.95$) in the events-free survival of VF probands comparing type 1 and non–type 1 groups.

annual rate of arrhythmic events in probands with type 1 ECG was 10.2% in the VF group, 0.6% in the syncope group, and 0.5% in the asymptomatic group (Figure 4A). The cumulative rate of arrhythmic events in probands with non–type 1 ECG was similar to those with type 1 ECG. The annual arrhythmic event rate was 10.6%, 1.2%, and 0%, respectively (Figure 4B).

By univariate analysis, a family history of SCD was a predictor for arrhythmic events in the type 1 group (hazard ratio [HR], 5.1; 95% CI, 2.0 to 12.8; $P=0.0004$) and the non–type 1 group (HR, 12.3; 95% CI, 2.0 to 74.8; $P=0.006$). Coexistence of posterolateral early repolarization with precordial Brugada-pattern ECG was another predictor in the type 1 group (HR, 4.2; 95% CI, 1.6 to 11.2; $P=0.003$); however, other parameters were not reliable. Figure 5 shows the Kaplan–Meier curves of arrhythmic events in the type 1 group during follow-up, depending on the presence of a family history of SCD (Figure 5A), inferolateral early repolarization (Figure 5B), a spontaneous type 1 ST-elevation (Figure 5C), and inducibility of ventricular arrhythmias by EPS (Figure 5D). Multivariate analysis in all probands identified that the former 2 parameters were independent risk factors for arrhythmic events (a family history of SCD: HR, 3.28; 95% CI, 1.42 to 7.60; $P=0.005$; early repolarization: HR, 2.66; 95% CI, 1.06 to 6.71; $P=0.03$, Table 3) as well as a family history of SCD in analysis of probands without VF (syncope and asymptomatic groups) (HR, 12.5; 95% CI, 2.0 to 75.0; $P=0.005$).

Discussion

Main Findings

We present one of the largest series of consecutive patients with Brugada-pattern ECG. Importantly, in the present study only probands were included. Also, this study has the longest follow-up ever reported. The main finding is that probands who have a non–type 1 ECG, even after challenged with a sodium channel blocker, do not necessarily have a better prognosis than patients with spontaneous or drug-induced type 1 ECG. Patients presenting with aborted cardiac arrest had a grim prognosis and those presenting with syncope or no symptoms had an excellent prognosis irrespective of their ECG pattern (that is, type 1 versus non–type 1). Also, a family history of sudden death at age <45 years and coexistence of early repolarization in the inferolateral leads were predictors of poor outcome. In contrast, VF/VT inducibility during EPS was not a predictor of outcome.

Comparison With Previous Studies

In this study, the follow-up time was uniform among the 3 groups. The mean follow-up time for the asymptomatic individuals was the longest (47.7±15.0 months) compared with the studies by Brugada et al[2] (27±29 months), Priori et al[3] (34±44 months), and Eckardt et al[5] (33.7±52.2 months). The percentage of female patients (5%) and patients with a family history of SCD (14%) was significantly smaller than 2 of these previous reports (5% versus 24% to 28%[2,3,5]; $P<0.001$, and 14% versus 28% to 54%[2,3,5]; $P<0.001$), although the percentage (14%) of a family history of SCD was similar to that of probands (20%) that Priori et al[3] had reported. The values observed in the present study may reflect the true profile of the probands of Brugada syndrome in contrast to previous studies in which a significant number of family members were also enrolled.

Prognosis of Probands Presenting With Syncope and Without Symptoms

The prognosis of probands in the syncope and asymptomatic groups was very good, and the annual rate of arrhythmic events was ≤1.2%. In the syncope group, this rate is far less than reported in previous studies,[2–5] although the

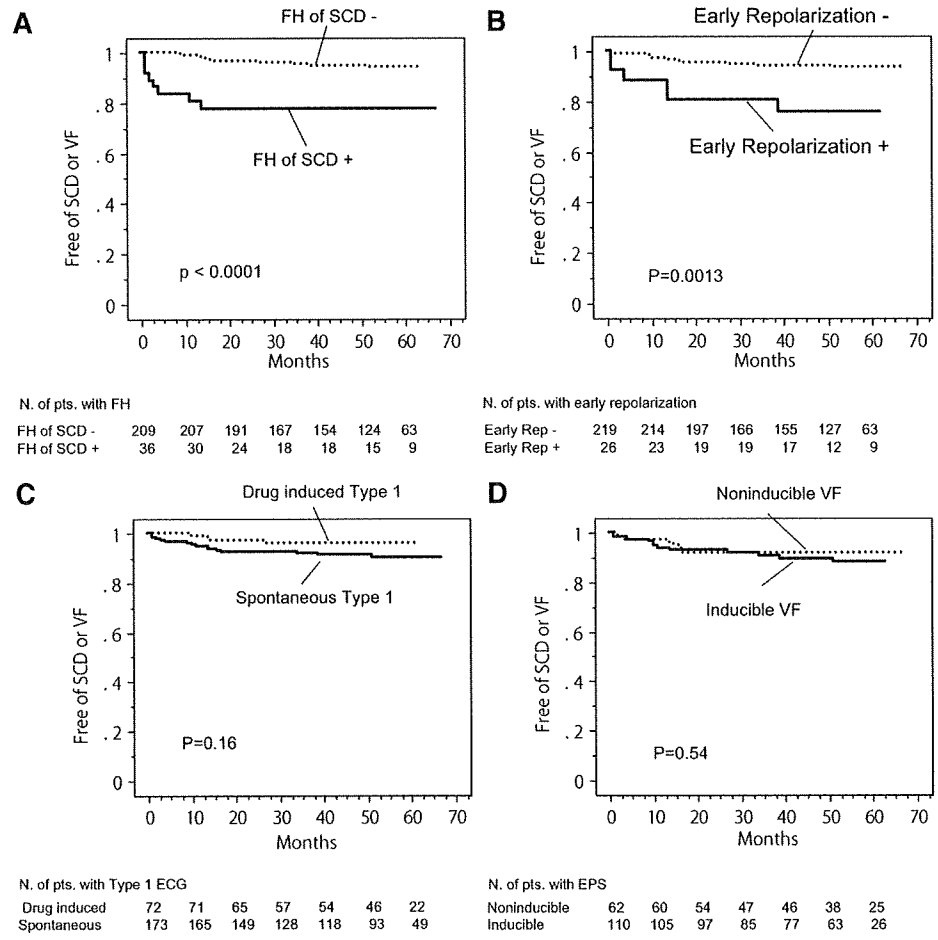

Figure 5. Kaplan–Meier analysis of fatal arrhythmic events during follow-up depending on a family history (FH) of SCD (FH of SCD− versus FH of SCD +) (A), inferolateral early repolarization (early repolarization− versus early repolarization+) (B), a spontaneous type 1 ST-elevation (drug-induced type 1 versus spontaneous type 1) (C), and inducibility of ventricular arrhythmias by EPS (noninducible VF versus inducible VF) (D).

rate in the asymptomatic group is similar to that in the Eckardt registry[5] and the rate of around 10% for the VF group is comparable to the rate reported in the Brugada registries.[2,8] The reason that the patients in the syncope group showed excellent prognosis is not entirely clear but may be related to the method of registry. Poor prognosis in prior studies is possibly related to the retrospective design of the studies consisting of probands and family members,[2,3,5] in which only severe syncope directly linked to VF tends to be categorized later as a syncope, despite difficulty to determine the cause of syncope at the onset. Even so, we cannot exclude the possibility that some patients with vasovagal syncope were inevitably included in the syncope group because not a few patients have undefined syncope and >30% of Brugada patients are reported to have both vasovagal syncope and the syncope due to ventricular arrhythmia.[12] Another reason for the good prognosis is the difference of genetic background. Brugada syndrome is known to be common in Asian people, which possibly relates to the higher prevalence of polymorphism of haplotype B, associated with the cardiac sodium channel.[13,14] The average prognosis of Asian patients with Brugada syndrome may be better than that of the white population, because individuals without a critical genetic defect are easily detected as a Brugada patient in a routine medical checkup. Further genetic studies are required to clarify the racial difference of outcome. Nevertheless, the patients in this study with an aborted sudden death showed worse prognosis than European people in the study by Eckardt et al[5] and had a similar outcome to those who underwent ICD implantation.[15]

Prognosis of Probands With Non–Type 1 ECG

The outcome of probands with non–type 1 ECG was similar to those with type 1 ECG and the rate of arrhythmic events in the VF group was considerably higher. Some of these patients had shown a coved (type 1) ST-elevation only in the higher (second or third) intercostal spaces during the drug provocation test or follow-up. Miyamoto et al[16] reported that men with a spontaneous type 1 ECG

Table 3. Probability of Sudden Death or VF During Follow-Up Depending on Clinical and Electrophysiological Variables in All Probands (Type 1 and Non–Type 1 Groups)

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Prior VF	21.46	8.00–57.53	<0.0001	17.48	6.22–49.11	<0.0001
FH of SCD	6.35	2.84–14.19	<0.0001	3.28	1.42–7.60	0.005
Inferolateral ER	4.14	1.71–10.00	0.001	2.66	1.06–6.71	0.03
AF	2.15	0.92–5.03	0.07	0.87	0.36–2.09	0.75
Syncope	0.35	0.08–1.09	0.15			
Sp. type1	2.31	0.67–7.94	0.18			
VF induc. (apex/OT)	1.81	0.72–4.70	0.20			
VF induc. (apex)	1.58	0.60–4.11	0.34			
Male		NA				

FH indicates family history; inferolateral ER, inferolateral early repolarization; AF, atrial fibrillation; Sp. type 1, spontaneous type 1 ST-elevation on 12-lead ECG at baseline; VF induc. (apex/OT), VF induction by programmed pacing at the RV apex or RV outflow tract; and VF induc. (apex), VF induction by programmed pacing at the RV apex.

recorded only at the higher leads V_1 and V_2 showed a prognosis similar to that of men with a type 1 ECG when using standard leads. In the past, patients with non–type 1 ST-elevation in standard ECG had been excluded from studies as a benign entity of Brugada syndrome. However, if patients had a history of aborted sudden death or agonizing nocturnal dyspnea, non–type 1 Brugada-pattern ECG should not be disregarded. Careful follow-up including ECG recording at the higher intercostals spaces and the implantation of ICD is probably required in such a patient to prevent SCD.

Clinical Features of Probands With Non–Type 1 ECG

The clinical profiles of probands were very similar between the non–type 1 group and the type 1 group (Table 2). Inferolateral early repolarization occurred equally in small percentage of patients in both groups (8% and 11%, respectively), which is comparable to the prevalence (12%) of early repolarization that Letsas et al[17] reported in patients with Brugada syndrome. This means that the patient characteristics of the non–type 1 group are much closer to Brugada syndrome than early repolarization syndrome reported by Haïssaguerre et al,[9] in which the VF occurrence rate during sleeping was low (19%) and VF inducibility by EPS was only 34%. Moreover, they reported that several aspects including the relapsing VF and the efficacy of isoproterenol and quinidine,[9,18] which were observed in some patients with early repolarization, were exactly like those of typical Brugada syndrome. Haïssaguerre et al[9] excluded patients with Brugada syndrome, defined as right bundle-branch block and ST-segment elevation >0.2mV in leads V_1–V_3, at the enrollment. However, considering that they possibly included patients with non–type 1 ECG as non-Brugada pattern in their study, some patients with prior VF and early repolarization might have represented non–type 1 Brugada patients of high risk.

Predictors of Outcome

It was reported that male sex, a previous episode of syncope, a spontaneous type 1 ECG, and inducibility of ventricular arrhythmias by EPS are predictors for poor outcome.[2–4] Brugada et al demonstrated that inducibility of ventricular arrhythmias was a reliable marker in patients with and without VF/SCD,[2,4] although Priori et al[3] did not find any significant difference in the analysis of all patients. A spontaneous type 1 ECG was also indicated as a reliable marker of poor prognosis by Brugada et al[4] in the analysis of patients without VF/SCD and by Eckardt et al[5] in all patients.[5] However, we could not find any reliability in these markers (Figures 3 and 5). Inducibility of ventricular arrhythmias was not a significant predictor even if it was evaluated by programmed pacing only from the RV apex (type 1 group: HR, 1.9 [95% CI, 0.7 to 5.2], $P=0.18$; all probands: HR, 1.5 [95% CI, 0.6 to 4.1], $P=0.34$, by univariate analysis).

In contrast, a family history of SCD occurring at age of <45 years is an independent risk factor of a poor prognosis in probands of any groups irrespective of their ECG type (type 1 or non–type 1) or symptoms (with VF or without VF). This was probably caused by a smaller proportion of probands with a family history of SCD as compared with previous studies[2–5] A family history was not found to be a marker in studies that enrolled many patients with SCD or a family history of Brugada syndrome. These results indicate that we should evaluate risks for arrhythmic events cautiously in studies with a significant number of family members.

Early repolarization pattern in the inferolateral leads was another indicator of poor prognosis, although Letsas et al[17] did not find any association with arrhythmic events in the data collected from 3 European centers, which also included ≈30% of patients with a family history of SCD. The reason for the poor outcome in probands with early repolarization in this study is not clear. However, it is conceivable that the combination of precordial Brugada-pattern ST-elevation with inferolateral early repolarization may represent electric heterogeneity in extensive regions of ventricles, which can result in lethal ventricular arrhythmias.

Study Limitations

In this study, premature ventricular electric stimulation was given until refractoriness was reached. The minimal

coupling interval of extrastimuli was not constant between participating hospitals and was sometimes shortened to <200 ms to induce ventricular arrhythmias.

We did not show the results of genetic analysis in this report, although more than half of the patients underwent genetic screening. Detailed results will be presented in a future report. So far, no positive relationship between genetic findings and patient outcomes has been found.[3,19]

We did not record ECGs at the higher intercostals spaces systematically except for probands with cardiac events, because the importance of "high-recording" became apparent in the course of this study.[6] Therefore, some patients of the non–type 1 group may have shown type 1 ST-elevation at the higher precordial positions.

Conclusions

This study described the long-term prognosis of probands with noncoved (non–type 1) Brugada-pattern ECG compared with type 1 ECG. The annual incidence of fatal arrhythmic events was similar between the 2 groups, which reached 10.6% in probands with non–type 1 ECG and a prior episode of VF. A family history of SCD occurring at age of <45 years and the presence of early repolarization were indicators of poor outcome although VF inducibility and a spontaneous type 1 ST-elevation were not reliable indicators in this prospective study including only probands.

Appendix

The following investigators and institutions participated in this study: A. Hukui, Yamagata University, Yamagata; M. Hiraoka, Tokyo Dental and Medical University, Tokyo; S. Takata, Kanazawa University, Kanazawa; H. Sakurada, Hiroo Metropolitan Hospital, Tokyo; Y. Eki, Ibaragi-higashi National Hospital, Tokai; Y. Sasaki, Nagano National Hospital, Ueda; Y. Tomita, Nagoya Medical Center, Nagoya; U. Shintani, Mie-chuo Medical Center, Tsu; T. Hashizume, Minami-Wakayama Medical Center, Tanabe; Y. Fujimoto, Okayama Medical Center, Okayama; W. Matsuura, Higashihiroshima Medical Center, Higashihiroshima; K. Sakabe, Zentuuji National Hospital, Zentuuji; and I. Matsuoka, Kagoshima Medical Center, Kagoshima, Japan.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The prognosis of patients with saddleback or noncoved type (non–type 1) ST-elevation in Brugada syndrome is unknown. We compared the long-term prognosis of 85 probands with non–type 1 ECG with 245 probands with coved (type 1) Brugada-pattern ECG prospectively. The absence of type 1 ECG was confirmed by drug provocation test and multiple recordings. Clinical profiles and outcomes did not differ between the non–type 1 and type 1 groups. The annual rate of fatal arrhythmic events was very low in asymptomatic probands and those with syncope but was higher in probands with ventricular fibrillation. A family history of sudden cardiac death at age <45 years and the presence of inferolateral early repolarization were indicators of poor prognosis, although ventricular fibrillation inducibility and a spontaneous type 1 ST-elevation were not reliable parameters in this prospective study including only probands.

Clinical Characteristics and Genetic Background of Congenital Long-QT Syndrome Diagnosed in Fetal, Neonatal, and Infantile Life

A Nationwide Questionnaire Survey in Japan

Hitoshi Horigome, MD; Masami Nagashima, MD; Naokata Sumitomo, MD; Masao Yoshinaga, MD; Hiroya Ushinohama, MD; Mari Iwamoto, MD; Junko Shiono, MD; Koh Ichihashi, MD; Satoshi Hasegawa, MD; Tadahiro Yoshikawa, MD; Tamotsu Matsunaga, MD; Hiroko Goto, MD; Kenji Waki, MD; Masaki Arima, MD; Hisashi Takasugi, MD; Yasuhiko Tanaka, MD; Nobuo Tauchi, MD; Masanobu Ikoma, MD; Noboru Inamura, MD; Hideto Takahashi, PhD; Wataru Shimizu, MD; Minoru Horie, MD

Background—Data on the clinical presentation and genotype-phenotype correlation of patients with congenital long-QT syndrome (LQTS) diagnosed at perinatal through infantile period are limited. A nationwide survey was conducted to characterize how LQTS detected during those periods is different from that in childhood or adolescence.

Methods and Results—Using questionnaires, 58 cases were registered from 33 institutions. Diagnosis (or suspicion) of LQTS was made during fetal life (n=18), the neonatal period (n=31, 18 of them at 0 to 2 days of life), and beyond the neonatal period (n=9). Clinical presentation of LQTS included sinus bradycardia (n=37), ventricular tachycardia/torsades de pointes (n=27), atrioventricular block (n=23), family history of LQTS (n=21), sudden cardiac death/aborted cardiac arrest (n=14), convulsion (n=5), syncope (n=5), and others. Genetic testing was available in 41 (71%) cases, and the genotype was confirmed in 29 (71%) cases, consisting of LQT1 (n=11), LQT2 (n=11), LQT3 (n=6), and LQT8 (n=1). Ventricular tachycardia/torsades de pointes and atrioventricular block were almost exclusively observed in patients with LQT2, LQT3, and LQT8, as well as in those with no known mutation. In LQT1 patients, clues to diagnosis were mostly sinus bradycardia or family history of LQTS. Sudden cardiac death/aborted cardiac arrest (n=14) was noted in 4 cases with no known mutations as well as in 4 genotyped cases, although the remaining 6 did not undergo genotyping. Their subsequent clinical course after aborted cardiac arrest was favorable with administration of β-blockers and mexiletine and with pacemaker implantation/implantable cardioverter-defibrillator.

Conclusions—Patients with LQTS who showed life-threatening arrhythmias at perinatal periods were mostly those with LQT2, LQT3, or no known mutations. Independent of the genotype, aggressive intervention resulted in effective suppression of arrhythmias, with only 7 deaths recorded. (***Circ Arrhythm Electrophysiol.* 2010;3:10-17.**)

Key Words: arrhythmia ■ long-QT syndrome ■ genes ■ death (sudden)

Congenital long-QT syndrome (LQTS) is an inherited disorder characterized by polymorphic ventricular tachycardia (VT), or torsades de pointes (TdP), syncope, and sudden cardiac death.[1] LQTS is often diagnosed in children from school age to young adulthood[2] and sometimes during fetal, neonatal, and infantile life.[3–5] Previous case reports

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From the Department of Child Health (H.H.), Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan; the Department of Cardiology (M.N.), Aichi Children's Health and Medical Center, Ohbu, Japan; the Department of Pediatrics and Child Health (N.S.), Nihon University School of Medicine, Tokyo, Japan; the Department of Pediatrics (M.Y.), National Hospital Organization Kagoshima Medical Center, Kagoshima, Japan; the Department of Cardiology (H.U.), Fukuoka Children's Hospital and Medical Center for Infectious Diseases, Fukuoka, Japan; the Department of Pediatric Cardiology (M. Iwamoto), Yokohama City University School of Medicine, Yokohama, Japan; the Department of Pediatrics (J.S.), Ibaraki Children's Hospital, Mito, Japan; the Department of Pediatrics (K.I.), Jichi Medical University, Shimotsuke, Japan; the Department of Pediatrics (S.H.), Niigata University Graduate School of Medical and Dental Science, Niigata, Japan; the Department of Pediatrics (T.Y.), Sakakibara Heart Institute, Fuchu, Japan; the Department of Pediatric Cardiology (T.M.), International Medical Center, Saitama Medical University, Hidaka, Japan; the Department of Pediatrics (H.G.), Gifu Prefectural General Medical Center, Gifu, Japan; the Department of Pediatrics (K.W.), Kurashiki Central Hospital, Kurashiki, Japan; the Department of Pediatrics (M.A.), Sent Marianna University School of Medicine, Kawasaki, Japan; the Department of Pediatrics (H. Takasugi), Kochi Medical School, Kochi, Japan; the Department of Cardiology (Y.T.), Shizuoka Children's Hospital, Shizuoka, Japan; the Department of Pediatric Cardiology (N.T.), Ogaki Municipal Hospital, Ogaki, Japan; the Department of Pediatrics (M. Ikoma), Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan; the Department of Pediatric Cardiology (N.I.), Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; the Department of Epidemiology and Biostatistics (H. Takahashi), School of Medicine, University of Tsukuba, Tsukuba, Japan; the Division of Cardiology (W.S.), Department of Internal Medicine, National Cardiovascular Center, Suita, Japan; and the Department of Cardiology and Respiratory Medicine (M.H.), Shiga University of Medical Science, Otsu, Japan.

Correspondence to Hitoshi Horigome, MD, PhD, Department of Child Health, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan. E-mail hhorigom@md.tsukuba.ac.jp

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Table 1. Questionnaire Items

1. Patient: Serial No. in each institution, initials, birth year, and month, sex
2. Age at diagnosis or suspicion (including gestational age for a fetus)
3. Clinical symptoms: Fetal arrhythmias, fetal heart failure, syncope, convulsion, heart failure, aborted cardiac arrest, others
4. ECG findings and arrhythmias (heart rate, QTc on ECG at presentation, sinus bradycardia, VT/TdP, atrioventricular block, other arrhythmias)
5. Family history of LQTS or other arrhythmias or sudden cardiac death (which member, and their outcome?)
6. Genotype
7. Treatment (acute therapy and maintenance therapy)
   pharmacotherapy (which drug, dose, age at initiation, and duration)
   device therapy (pacemaker implantation/implantable cardioverter-defibrillator) and age at application
8. Duration of follow-up
9. Outcome (alive or death, and neurological sequels of cardiac arrest)

suggest that the latter cases are at higher risk of development of life-threatening arrhythmias necessitating emergency treatment[3–5] and show higher mortality rates than the former age groups.[3,5–11] For example, recent progress in molecular biology has clarified that LQTS partly contributes to sudden infant death syndrome (SIDS).[12,13] Unfortunately, prenatal diagnosis of LQTS has been extremely difficult to confirm except for a limited number of cases for which prenatal gene screening[14] or fetal magnetocardiography (fMCG)[15–17] was applied.

Clinical Perspective on p 17

Thus, the clinical presentation, the genotype-phenotype correlation, and the outcome of patients with fetal, neonatal, or infantile presentation of LQTS remain to be elucidated. The purposes of this study were first, to report the findings of a nationwide survey conducted to define the clinical characteristics and the genotype-phenotype correlation, and second, to report the outcome of patients with LQTS diagnosed before birth and in the first year of life.

Methods

Population

The study population included fetuses, neonates, and infants (<1 year of age) diagnosed with LQTS based on ECG findings including prolonged QTc >0.46 seconds (using Bazett formula), with or without VT/TdP, who had no structural heart disease, family history of LQTS, or had undergone genetic testing. Those with normal QTc duration and no gene mutation known to cause LQTS were excluded. Patient data were collected using questionnaires. The form was sent to those councilors of the Japanese Society of Pediatric Cardiology and Cardiac Surgery who responded to a preliminary survey that they had 1 or more cases of LQTS diagnosed during fetal, neonatal, and infantile life. The items obtained from the responders are presented in Table 1.

The study protocol was approved by the Ethics Committee of the University Hospital of Tsukuba, and informed consent was obtained from each patient (or parents, if the patient was younger than 15 years of age) by a coordinator in charge in each institution before the patient's data were registered.

Genetic Analysis and Genotype-Phenotype Correlation

Genetic analyses were performed in 4 established laboratories in Japan. DNA was isolated from blood samples in each patient. Screening for mutations of at least 3 major genes causing LQTS (*KCNQ1, KCNH2, SCN5A*) was performed using polymerase chain reaction (PCR)/single-strand conformation polymorphism or denatured high-performance liquid chromatography analysis. For aberrant PCR products, DNA sequencing was conducted with a DNA sequencer (ABI 3700 and ABI 3130xl, Applied Biosystems, Foster City, Calif). For those subjects in whom genotype was confirmed and those who underwent genetic analysis but found to have no mutation, genotype-phenotype correlations (or mutation-negative phenotype correlations) with the aforementioned items (Table 1) were investigated.

Statistical Analysis

All statistical calculations were conducted using the R software. Quantitative variables (heart rate [HR] and QTc) are presented as mean±SD and categorized variables (presence of FH, sinus bradycardia, VT/TdP, and atrioventricular block [AVB]) as proportions (percentages). One-way ANOVA was applied for comparisons of continuous variables, followed by pairwise comparisons with Bonferroni adjustment of probability values among 4 groups (LQT1, LQT2, LQT3, and mutation-negative groups). The equality of proportions for categorical variables among the 4 groups was examined by the χ^2 test (global test). When there was a significant difference in proportions, we performed pairwise comparisons between pairs of proportions with correction for multiple testing using Bonferroni inequality of probability values. Tests were 2-sided, and a probability value <0.05 was considered significant.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Population

A total of 58 cases (all Japanese; males 30, females 28) were registered from 33 institutions. Forty-one were born during the last 10 years (between 1999 and 2008), 14 between 1989 and 1998, 1 in 1986, and 2 in 1984. LQTS was diagnosed or suspected during fetal life at 18 to 40 weeks of gestation in 18 individuals, during neonatal life at 0 to 28 days in 31, and in infancy (<1 year) at 1 to 9 months in 9.

Clinical Features

For 18 fetuses with LQTS, clinical presentation (or clues to diagnosis or suspicion of LQTS) included bradycardia (15 cases), AVB (8 cases), VT/TdP (7 cases), and family history of LQTS (6 cases), including 1 family with a previous intrauterine death (items overlapped in some cases). Two fetuses were confirmed to be LQTS by fMCG, with QTc values of 570 and 680 on fMCG, and 590 and 700 on ECG soon after birth, respectively (these 2 cases have been reported previously).[16,17] No fetal death was noted in this group.

For 31 neonates with LQTS, the most frequent feature was sinus bradycardia (17 cases), followed by VT/TdP (15 cases), positive family history of LQTS (15 cases), including 1 with previous intrauterine death and 1 with infantile death, AVB (10 cases), syncope (5 cases), convulsion (5 cases), and others (items overlapped in some cases). Among the 31 neonatal cases, 18 (70%) were diagnosed within 2 days of life, and 8 of them had some significant fetal presentation (4 bradycardia or bradyarrhythmias, 4 tachyarrhythmias, and 1 hydrops), retrospectively.

As described above, the number of patients with LQTS diagnosed during infancy beyond the neonatal period was only 9. The clinical presentation of these patients included sinus bradycardia (5 cases), sudden cardiac death (SCD)/