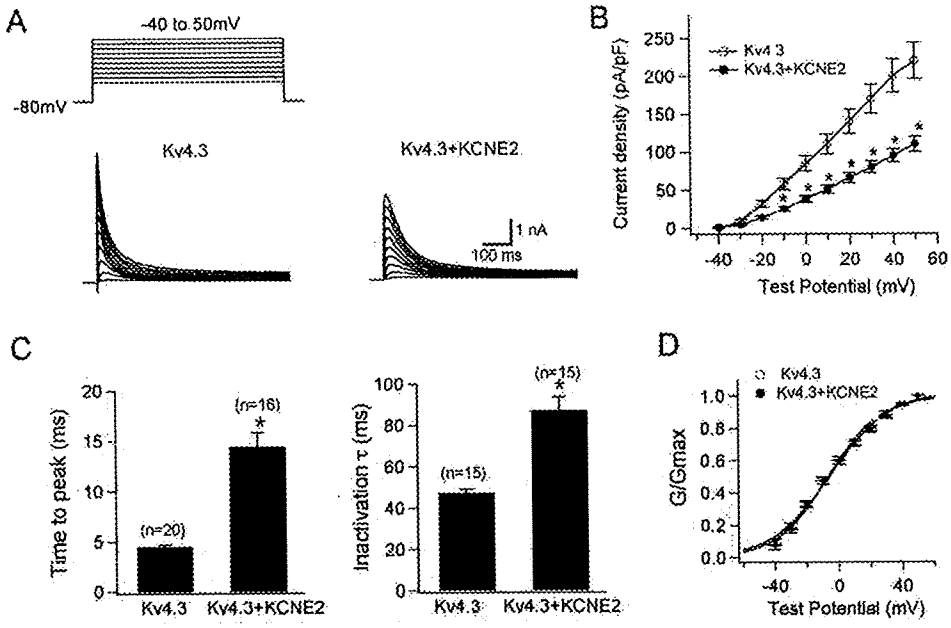

Figure 1 *KCNE2* co-expression with Kv4.3 produces smaller I_{to}-like currents with slower activation/inactivation kinetics. **A:** Representative current traces recorded from Chinese hamster ovary (CHO) cells expressing Kv4.3 **(left)** and Kv4.3 + *KCNE2* **(right).** As shown in the **inset** in panel A, depolarizing step pulses of 1-second duration were introduced from a holding potential of −80 mV to potentials ranging from −40 to +50 mV in 10-mV increments. **B:** Current–voltage relationship curve showing peak current densities in the absence and presence of co-transfected *KCNE2* (*P <.05 vs Kv4.3). **C:** Bar graphs showing the kinetic properties of reconstituted channel currents: time to peak of activation course **(left)** and inactivation time constants **(right)** measured using test potential to +20 mV (*P <.05 vs Kv4.3). Numbers in parentheses indicate numbers of experiments. **D:** Normalized conductance–voltage relationship for peak outward current of Kv4.3 and Kv4.3 + *KCNE2* channels.

Abbott et al reported that three *KCNE2* variants (Q9E, M54T, I57T) caused a loss of function in I_{Kr} and thereby were associated with the congenital or drug-induced long QT syndrome.[6,7] However, the reported QTc values in two index patients with M54T and I57T variants, both located in the transmembrane segment of MiRP1, were only mildly prolonged (390–500 ms and 470 ms).[6] We recently identified the same missense *KCNE2* variant, I57T, in which isoleucine was replaced by threonine at codon 57, in three unrelated probands showing a Brugada type 1 ECG. These findings are difficult to explain on the basis of a loss of function in I_{Kr}, thus leading us to explore other mechanisms.

Recent studies have demonstrated that interaction between α and β subunits (*KCNE*s) of voltage-gated K^+ channel is more promiscuous; for example, MiRP1 has been shown to interact with Kv7.1,[8–10] HCN1,[11] Kv2.1,[12] and Kv4.2.[13] These studies suggest that MiRP1 may also co-associate with Kv4.3 and contribute to the function of transient outward current (I_{to}) channels.[14] Indeed, a recent study reported that I_{to} is diminished in *kcne2* (−/−) mice.[15]

In the human heart, I_{to} currents are of critical importance in regulating myocardial electrical properties during the very early phase of the action potential and are thought to be central to the pathogenesis of Brugada-type ECG manifestations.[16] Antzelevitch et al demonstrated that a gain of function in I_{to} secondary to a mutation in *KCNE3* contributes to a Brugada phenotype by interacting with Kv4.3 and thereby promoting arrhythmogenicity.[14]

We hypothesized that mutations in *KCNE2* may have similar actions and characterize the functional consequences of interaction of wild-type (WT) and two mutant (I57T, M54T) MiRP1 with Kv4.3[17,18] using heterologous co-expression of these α and β subunits in Chinese hamster ovary (CHO) cells.

Methods

Heterologous expression of hKv4.3 and β subunits in CHO cells

Full-length cDNA fragment of *KCNE2* in pCR3.1 vector[10] was subcloned into pIRES-CD8 vector. This expression vector is useful in cell selection for later electrophysiologic study (see below). Two *KCNE2* mutants (M54T, I57T) were constructed using a Quick Change II XL site-directed mutagenesis kit according to the manufacturer's instructions (Stratagene, La Jolla, CA, USA) and subcloned to the same vector. Two *KCNE2* mutants were fully sequenced (ABI3100x, Applied Biosystems, Foster City, CA, USA) to ensure fidelity. Full-length cDNA encoding the short isoform of human Kv4.3 subcloned into the pIRES-GFP (Clontech, Palo Alto, CA, USA) expression vector was kindly provided by Dr. G.F. Tomaselli (Johns Hopkins University). Full-length cDNA encoding Kv channel-interacting protein (*KCNIP2*) subcloned into the PCMV-IRS expression vector was a kind gift from Dr. G.-N. Tseng (Virginia Commonwealth University). *KCND3* was transiently transfected into CHO cells together with *KCNE2* (or M54T or I57T) cDNA at equimolar ratio (*KCND3* 1.5 μg,

Table 1 Effects of *KCNE2* on Kv4.3 and Kv4.3 + KChIP2b

Parameter	Kv4.3	Kv4.3 *KCNE2*	Kv4.3 KChIP2b	Kv4.3 KChIP2b *KCNE2*
Current density at +20 mV (pA/pF)	142.0 ± 16.0 (n = 12)	66.0 ± 6.6* (n = 12)	191.5 ± 33.8 (n = 15)	77.8 ± 5.9† (n = 20)
Steady-state activation ($V_{0.5}$ in mV)	−6.5 ± 2.1 (n = 9)	−5.5 ± 1.7 (n = 11)	−7.5 ± 1.7 (n = 8)	−7.4 ± 1.4 (n = 8)
Steady-state inactivation ($V_{0.5}$ in mV)	−46.0 ± 1.3 (n = 10)	−40.8 ± 1.7* (n = 8)	−49.8 ± 1.4 (n = 7)	−44.5 ± 1.9† (n = 7)
τ of inactivation at +20 mV (τ_{inact} in ms)	47.3 ± 2.0 (n = 15)	87.2 ± 6.2* (n = 15)	47.5 ± 2.2 (n = 15)	66.6 ± 3.5† (n = 15)
Time to peak at +50 mV (TtP in ms)	4.5 ± 0.2 (n = 20)	14.4 ± 1.4* (n = 16)	4.1 ± 0.2 (n = 15)	6.1 ± 0.5† (n = 21)
τ of recovery from inactivation (ms)	419.6 ± 18.8 (n = 6)	485.6 ± 74.8 (n = 6)	89.2 ± 5.3 (n = 6)	60.2 ± 6.9† (n = 6)

*Significantly different from Kv4.3.
†Significantly different from Kv4.3 + KChIP2b.

KCNE2 1.5 μg) using Lipofectamine (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. In one set of experiments, we also co-transfected equimolar levels of KChIP2b (*KCND3* 1.5 μg, *KCNE2* 1.5 μg, *KCNIP2* 1.5 μg). The transfected cells were then cultured in Ham's F-12 medium (Nakalai Tesque, Inc., Kyoto, Japan) supplemented with 10% fetal bovine serum (JRH Biosciences, Inc., Lenexa, KS, USA) and antibiotics (100 international units per milliliter penicillin and 100 μg/mL streptomycin) in a humidified incubator gassed with 5% CO_2 and 95% air at 37°C. The cultures were passaged every 4 to 5 days using a brief trypsin-EDTA treatment. The trypsin-EDTA treated cells were seeded onto glass coverslips in a Petri dish for later patch-clamp experiments.

Electrophysiologic recordings and data analysis

After 48 hours of transfection, a coverslip with cells was transferred to a 0.5-mL bath chamber at 25°C on an inverted microscope stage and perfused at 1 to 2 mL/min with extracellular solution containing the following (in mM): 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 7.4 with NaOH. Cells that emitted green fluorescence were chosen for patch-clamp experiments. If co-expressed with *KCNE2* (or its mutants), the cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450, Dynal, Norway) for 15 minutes. In these cases, cells that emitted green fluorescence and had attached beads were chosen for electrophysiologic recording. Whole-cell membrane currents were recorded with an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany), and data were low-pass filtered at 1 kHz, acquired at 5 kHz through an LIH-1600 analog-to-digital converter (HEKA), and stored on hard disk using PulseFit software (HEKA). Patch pipettes were fabricated from borosilicate glass capillaries (Narishige, Tokyo, Japan) using a horizontal microelectrode puller (P-97, Sutter Instruments, Novato, CA, USA) and the pipette tips fire-polished using a microforge. Patch pipettes had a resistance of 2.5 to 5.0 MΩ when filled with the following pipette solution (in mM): 70 potassium aspartate, 50 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 Na_2-ATP (Sigma, Japan, Tokyo), 0.1 Li_2-GTP (Roche Diagnostics GmbH, Mannheim, Germany), 5 EGTA, and 5 HEPES (pH 7.2).

Cell membrane capacitance (C_m) was calculated from 5 mV-hyperpolarizing and depolarizing steps (20 ms) applied from a holding potential of −80 mV according to Equation 1[19]:

$$C_m = \tau_c I_0 / \Delta V_m (1 - I_\infty / I_0), \quad (1)$$

where τ_c = time constant of capacitance current relaxation, I_0 = initial peak current amplitude, ΔV_m = amplitude of voltage step, and I_∞ = steady-state current value. Whole-cell currents were elicited by a family of depolarizing voltage steps from a holding potential of −80 mV. The difference between the peak current amplitude and the current at the end of a test pulse (1-second duration) was referred to as the transient outward current. To control for cell size variability, currents were expressed as densities (pA/pF).

Steady-state activation curves were obtained by plotting the normalized conductance as a function of peak outward potentials. Steady-state inactivation curves were generated by a standard two-pulse protocol with a conditioning pulse of 500-ms duration and obtained by plotting the normalized current as a function of the test potential. Steady-state inactivation/activation kinetics were fitted to the following Boltzmann equation (Eq. 2):

$$Y(V) = 1/(1 + \exp[(V_{1/2} - V)/k]), \quad (2)$$

where Y = normalized conductance or current, $V_{1/2}$ = potential for half-maximal inactivation or activation, respectively, and k = slope factor.

Data relative to inactivation time constants, time to peak, and mean current levels were obtained by using current data recorded at +50 mV or +20 mV. Recovery from inactivation was assessed by a standard paired-pulse protocol: a 400-ms test pulse to +50 mV (P1) followed by a variable

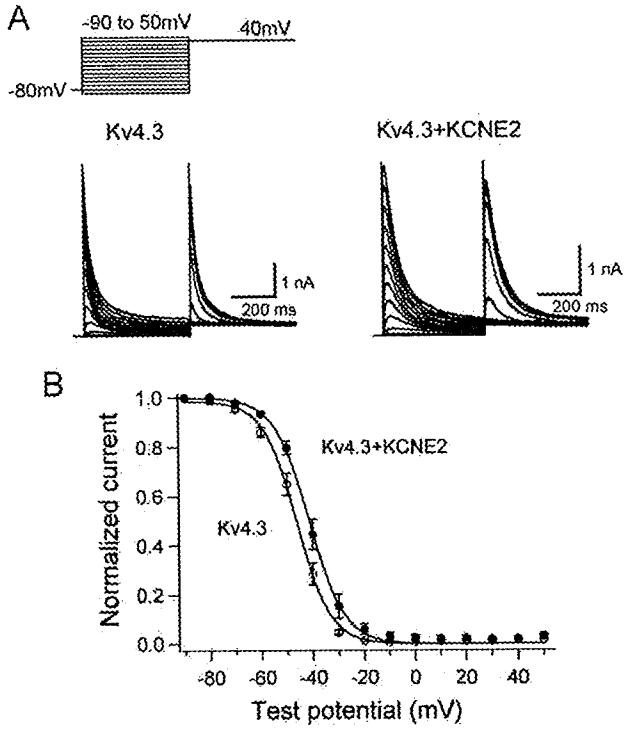

Figure 2 *KCNE2* co-expression with Kv4.3 causes a positive shift of voltage dependence of steady-state inactivation. **A:** Representative Kv4.3 and Kv4.3 + *KCNE2* current traces induced by 500-ms pulses (P1) from −90 to +50 mV applied from the holding potential −80 mV in 10-mV steps followed by a second pulse (P2) to +40 mV. **B:** Steady-state inactivation curves for Kv4.3 *(open circles)* and Kv4.3 + *KCNE2 (closed circles)* channels.

recovery interval at −80 mV and then a second test pulse to +50 mV (P2). Both the inactivation time constants and the time constant for recovery from inactivation were determined by fitting the data to a single exponential (Eq. 3):

$$I(t) \text{ (or P2/P1)} = A + B_{exp}(-t/\tau), \quad (3)$$

where I(t) = current amplitude at time t, A and B = constants, and τ = inactivation time constant or time constant for recovery from inactivation. For measurement of recovery from inactivation, the plot of P2/P1 instead of I(t) was used.

All data were given as mean ± SEM. Statistical comparisons between two groups were analyzed using Student's unpaired t-test. Comparisons among multiple groups were analyzed using analysis of variance followed by Dunnett test. $P < .05$ was considered significant.

Results

Effects of *KCNE2* on Kv4.3 currents and its gating kinetics

WT *KCNE2* initially was co-expressed with *KCND3*, the gene encoding Kv4.3, the α subunit of the I_{to} channel,[17,18] in CHO cells. Figure 1A shows representative whole-cell current traces recorded from cells transfected with *KCND3* and co-transfected with (right) or without (left) *KCNE2*. Cells expressing Kv4.3 channels alone showed rapidly activating and inactivating currents. Co-expression of *KCNE2* significantly reduced peak current densities as summarized in the current–voltage relationship curve shown in Figure 1B and slowed both activation and inactivation kinetics (Table 1). Figure 1C (left) shows mean time intervals from the onset of the pulse to maximum current (time to peak), whereas the right panel shows time constants of inactivation (at +20 mV) obtained using Equation 3. Thus, co-transfection of *KCNE2* significantly increased both the time to peak and the time constant.

In contrast, *KCNE2* did not affect the voltage dependence of steady-state activation as assessed by plotting the normalized conductance as a function of test potential (Figure 1D). Fitting to the Boltzmann equation (Eq. 2) yielded half-maximal activation potentials of −6.5 ± 2.1 mV for Kv4.3 alone (open circles) and −5.5 ± 1.7 mV for Kv4.3 + *KCNE2* channels (filled circles, P = NS; Table 1). These findings are consistent with those previously reported for studies using *Xenopus* oocytes, CHO cells, and HEK293 cells.[20,21]

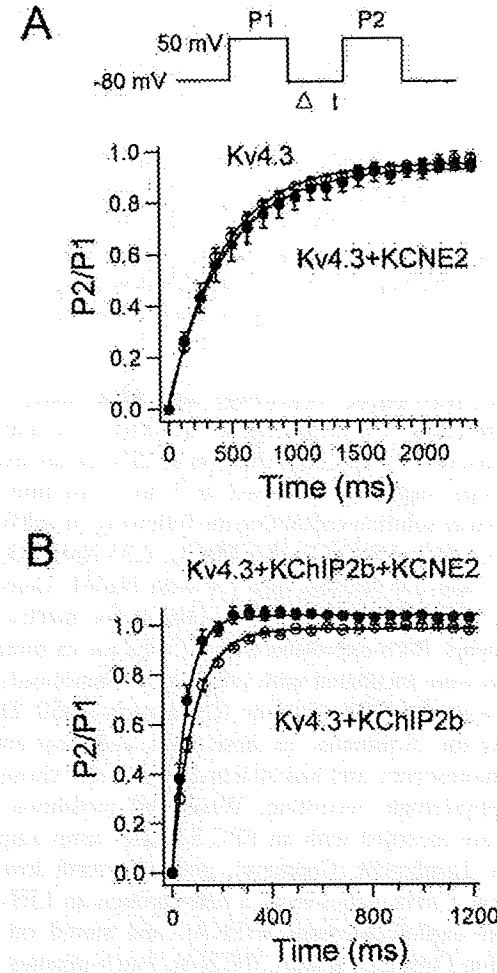

Figure 3 Effects of *KCNE2* co-expression on recovery from inactivation of Kv4.3 **(A)** and Kv4.3 + KChIP2b **(B)** currents. Recovery from inactivation was assessed by a two-pulse protocol **(A, inset)**: a 400-ms test pulse to +50 mV (P1) followed by a variable interval at −80 mV, then by a second test pulse to +50 mV (P2). Data were fit to a single exponential.

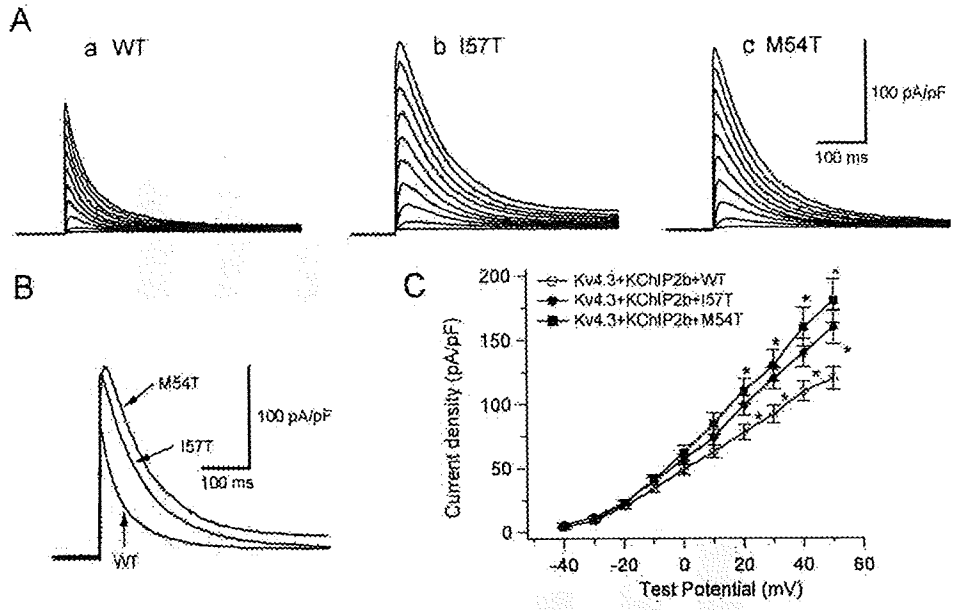

Figure 4 Two *KCNE2* transmembrane variants, I57T and M54T, increase the reconstituted Kv4.3 + KChIP2b channel current and slow its inactivation. **A**: Three sets of current traces elicited by depolarizing pulses for 500 ms from a holding potential of −80 mV to potentials ranging between −40 and +50 mV in 10-mV increments (same protocol as in experiments of Figure 1A). **B**: Superimposition of three original current traces recorded upon depolarization showing variant-related increase in peak outward current density. **C**: Current–voltage relationship curve showing average peak outward current densities (*P <.05 vs Kv4.3 + KChIP2b + WT). WT = wild type.

KCNE2 co-expression also caused a positive shift (approximately +5 mV) of voltage dependence of steady-state inactivation. Steady-state inactivation was assessed using a double-step pulse method (Figure 2A, inset). Peak outward currents recorded at various levels of prepulse (Figure 2A) were normalized by that measured after a 500-ms prepulse at −90 mV and are plotted as a function of prepulse test potentials (Figure 2B). Half-inactivation potentials of steady-state inactivation, determined by fitting data to the Boltzmann equation (Eq. 2), were −46.0 ± 1.3 mV for Kv4.3 (open circles) and −40.8 ± 1.7 mV for Kv4.3 + *KCNE2* (filled circles, P <.01), consistent with the observation of Tseng's group.[13]

A double-pulse protocol (Figure 3A, inset) was used to test the effect of *KCNE2* co-expression on the time course for recovery from inactivation. Figure 3A shows the time course of recovery of Kv4.3 alone (open circles) and Kv4.3 + *KCNE2* (filled circles). Mean time constants for recovery from inactivation were not significantly different, indicating that co-transfection of *KCNE2* did not affect the time course of recovery from inactivation.

Effects of *KCNE2* on Kv4.3 + KChIP2b current and its gating kinetics

For human native cardiac I_{to}, KChIP2 has been shown to serve as a principal β subunit.[22–25] Accordingly, in another series of experiments, we examined the effect of WT and mutant *KCNE2* on Kv4.3 + KChIP2b current. Consistent with previous reports, in the presence of KChIP2, Kv4.3 currents showed a significantly faster recovery from inactivation (Figure 3B and Table 1).[26,27] Co-expression of WT *KCNE2* produced similar changes on Kv4.3 + KChIP2b current as on Kv4.3 current (Table 1). Kv4.3 + KChIP2b current recovery from inactivation was further accelerated: average time constant was 89.2 ± 6.5 ms for Kv4.3 + KChIP2b alone (open circles) and 60.2 ± 8.4 ms for Kv4.3 + KChIP2b + *KCNE2* (filled circles, P <.05). In 16 of 21 cells transfected with *KCNE2*, we observed an "overshoot" phenomenon, which is commonly seen during recording of native I_{to} in human ventricular myocytes.[28]

KCNE2 variants increase Kv4.3 + KChIP2b current and alter its gating kinetics

The I57T variant was first identified in an asymptomatic middle-aged woman with very mild QT prolongation.[6] In addition to this variant, the authors reported another *KCNE2* variant of the transmembrane segment (M54T) that was associated with ventricular fibrillation during exercise in a middle-aged woman. This patient appeared to show a wide range of QTc interval (390–500 ms). Therefore, we tested the functional effects of these two transmembrane *KCNE2* variants on Kv4.3 + KChIP2b currents.

The three panels of Figure 4A show three sets of current traces elicited by depolarizing pulses from a holding potential of −80 mV in cells co-transfected with WT (a), I57T (b), or M54T (c) *KCNE2*. Neither variant caused a significant shift of half-maximal activation voltage: −7.4 ± 1.4 mV (n = 8) for co-expression of WT *KCNE2*, −6.1 ± 1.5 mV (n = 8) for I57T, and −6.6 ± 1.6 mV (n = 8) for M54T. Both variants significantly increased I_{to} density: 125.0 ± 10.6 pA/pF in WT *KCNE2* (n = 21), 178.1 ± 12.1 pA/pF with I57T (n = 9), and 184.3 ± 27.9 pA/pF with M54T (n = 9, Figure 4C).

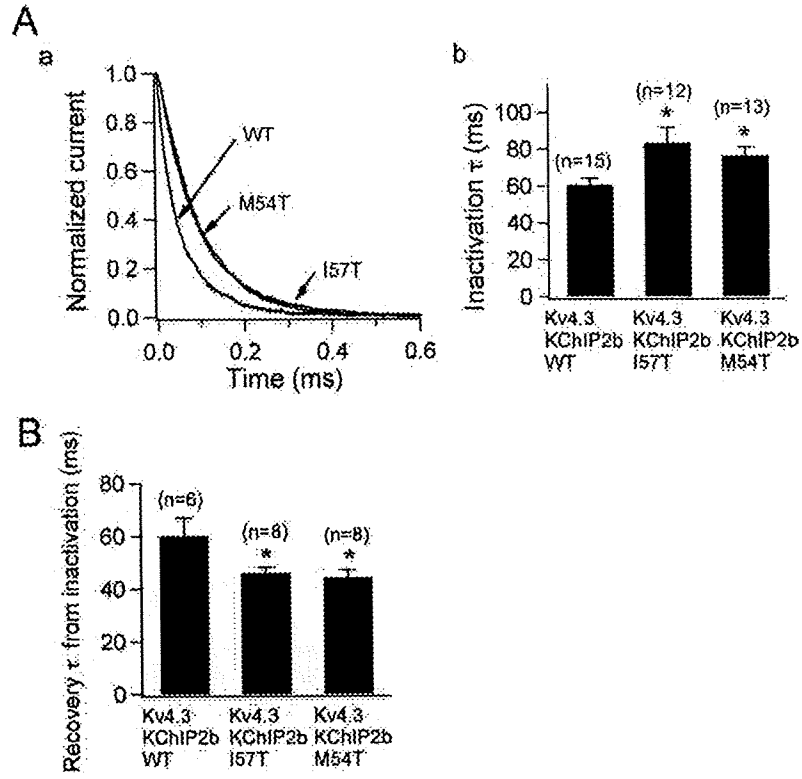

Figure 5 Two *KCNE2* variants slow inactivation kinetics and accelerate recovery from inactivation. **A, a:** Three current traces obtained from Chinese hamster ovary (CHO) cells transfected with wild-type (WT), I57T, and M54T *KCNE2* variant co-expressed with Kv4.3 and KChIP2b. Traces, which are normalized and superimposed, show that the variants slow inactivation. **A, b:** Time constants of decay at +20 mV for WT and variant *KCNE2* (*$P < .05$ vs Kv4.3 + KChIP2b + WT). Numbers in parentheses indicate numbers of observations. **B:** Time constants of recovery from inactivation recorded using a double-pulse protocol (*$P < .05$ vs Kv4.3 + KChIP2b + WT). Numbers in parentheses indicate numbers of observations.

Figure 5A shows the three traces depicted in Figure 4B normalized to their peak current level. This representation shows that the time course of inactivation of the two variant currents is slowed. The current decay was fitted by Equation 3 and the time constants (at +20 mV) summarized in Figure 5A, panel b. Finally, Figure 5B shows that the time constants of recovery of the two mutant channels from inactivation were significantly reduced. Thus, compared to WT *KCNE2*, recovery of reconstituted Kv4.3 + KChIP2b channels from inactivation was significantly accelerated with both I57T and M54T mutants.

Discussion

Kv4.3/KChIP2/MiRP1 complex can recapitulate the native I_{to}

In the present study, co-expression of WT *KCNE2* produced changes in kinetic properties (Figures 1–3 and Table 1) that led to close recapitulation of native cardiac I_{to}.[28,29] Notably, in addition to causing a positive shift of steady-state inactivation (Figure 2), *KCNE2* co-expression hastened the recovery of Kv4.3 + KChIP2b channels from inactivation (Figure 3). These modifications rendered Kv4.3 + KChIP2b channels more similar to native cardiac I_{to}, suggesting that *KCNE2* may be an important component of the native I_{to} channel complex. In contrast to a previous observation in HEK293 cells,[21] *KCNE2* co-expression decreased the current density of Kv4.3 and Kv4.3 + KChIP2b channel current in the present study, which seems to be a more reasonable result as the native I_{to} density reportedly was smaller in isolated human heart.[28] *KCNE2* co-expression has also been shown to reduce the density of Kv7.1[8,9] and HERG[6,7] channels.

Similar to the result of Deschenes and Tomaselli,[21] we failed to observe an overshoot during recovery from inactivation when *KCNE2* was co-expressed with Kv4.3 (Figure 3A), which is in contrast to the report of another group.[13] However, co-expression of *KCNE2* with Kv4.3 + KChIP2 channels produced an overshoot (Figure 3B), consistent with the report of Wettwer's group.[25] Wettwer et al also found that other *KCNE* subunits either were ineffective or induced only a small overshoot in CHO cells. Therefore, both MiRP1 and KChIP2 subunits are sufficient and necessary to recapitulate native I_{to} in the heart. Considering that the overshoot phenomenon has been described only in human ventricular I_{to} channels of the epicardial but not endocardial region,[28] these results may further implicate participation of MiRP1 and KChIP2 in the I_{to} channel complex in epicardium.

KCNE2 variants may alter the arrhythmogenic substrate by modulating I_{to}

Heterologous expression in CHO cells was conducted to examine the functional effects of I57T and M54T variants on Kv4.3 + KChIP2 channels. Both I57T and M54T

KCNE2 variants significantly (1) increased peak transient outward current density (Figure 4), (2) slowed the decay of the reconstituted I_{to} (Figure 5A), and (3) accelerated its recovery from inactivation (Figure 5B). Both variants thus caused an important gain of function in human I_{to}. These sequence changes may play a role in modulating I_{to} and thereby predispose to some inherited fatal rhythm disorders.

Functional effects on I_{to} induced by I57T and M54T resemble each other, increasing I_{to} density and accelerating its recovery from inactivation. The gain of function in I_{to} opposes the fast inward Na^+ currents during phase 0 of the action potential, leading to all or none repolarization at the end of phase 1 and loss of the epicardial action potential dome, thus promoting phase 2 reentry and fatal ventricular arrhythmias.[30]

Another *KCNE2* variant (M54T) associated with fatal arrhythmias was first identified in a woman who had a history of ventricular fibrillation and varied QT intervals.[6] It is possible that her arrhythmia was also related to a gain of function in I_{to} secondary to this variation in *KCNE2*. Interestingly, the I57T variant has been reported to produce a loss of function of HERG or Kv7.1 channels, thereby predisposing to long QT syndrome,[6,8] indicating that the same *KCNE2* variant could cause two different cardiac rhythm disorders, similar to long QT syndrome and Brugada syndrome caused by *SCN5A* mutations.[31,32]

References

1. Kass RS, Freeman LC. Potassium channels in the heart: cellular, molecular, and clinical implications. Trends Cardiovasc Med 1993;3:149–159.
2. MacKinnon R. Determination of the subunit stoichiometry of a voltage-activated potassium channel. Nature 1991;350:232–235.
3. Abbott GW, Goldstein SA. A superfamily of small potassium channel subunits: form and function of the MinK-related peptides (MiRPs). Q Rev Biophys 1998;31:357–398.
4. Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and IsK (minK) proteins associate to form the I_{Ks} cardiac potassium current. Nature 1996;384:78–80.
5. Sanguinetti MC, Curran ME, Zou AR, et al. Coassembly of KvLQT1 and minK (I_{Ks}) proteins to form cardiac I_{Ks} potassium channel. Nature 1996; 384:80–83.
6. Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. Cell 1999;97:175–187.
7. Sesti F, Abbott GW, Wei J, et al. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc Natl Acad Sci U S A 2000;97: 10613–10618.
8. Tinel N, Diochot S, Borsotto M, Lazdunski M, Barhanin J. *KCNE2* confers background current characteristics to the cardiac KCNQ1 potassium channel. EMBO J 2000;19:6326–6330.
9. Wu DM, Jiang M, Zhang M, Liu XS, Korolkova YV, Tseng GN. *KCNE2* is colocalized with KCNQ1 and *KCNE1* in cardiac myocytes and may function as a negative modulator of $I(_{Ks})$ current amplitude in the heart. Heart Rhythm 2006;3:1469–1480.
10. Toyoda F, Ueyama H, Ding WG, Matsuura H. Modulation of functional properties of KCNQ1 channel by association of KCNE1 and KCNE2. Biochem Biophys Res Commun 2006;344:814–820.
11. Yu H, Wu J, Potapova I, et al. MinK-related peptide 1: a beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. Circ Res 2001;88:E84–E87.
12. McCrossan ZA, Roepke TK, Lewis A, Panaghie G, Abbott GW. Regulation of the Kv2.1 potassium channel by MinK and MiRP1. J Membr Biol 2009;228:1–14.
13. Zhang M, Jiang M, Tseng GN. MinK-related peptide 1 associates with Kv4.2 and modulates its gating function: potential role as beta subunit of cardiac transient outward channel? Circ Res 2001;88:1012–1019.
14. Delpon E, Cordeiro JM, Nunez L, et al. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. Circ Arrhythm Electrophysiol 2008;1:209–218.
15. Roepke TK, Kontogeorgis A, Ovanez C, et al. Targeted deletion of *KCNE2* impairs ventricular repolarization via disruption of $I_{K,slow1}$ and $I_{to,f}$. FASEB J 2008;22:3648–3660.
16. Calloe K, Cordeiro JM, Di Diego JM, et al. A transient outward potassium current activator recapitulates the electrocardiographic manifestations of Brugada syndrome. Cardiovasc Res 2009;81:686–694.
17. Dixon JE, Shi W, Wang HS, et al. Role of the Kv4.3 K^+ channel in ventricular muscle. A molecular correlate for the transient outward current. Circ Res 1996;79:659–668.
18. Kääb S, Dixon J, Duc J, et al. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. Circulation 1998;98:1383–1393.
19. Benitah JP, Gomez AM, Bailly P, et al. Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts. J Physiol 1993;469:111–138.
20. Singleton CB, Valenzuela SM, Walker BD, et al. Blockade by N-3 polyunsaturated fatty acid of the Kv4.3 current stably expressed in Chinese hamster ovary cells. Br J Pharmacol 1999;127:941–948.
21. Deschênes I, Tomaselli GF. Modulation of Kv4.3 current by accessory subunits. FEBS Lett 2002;528:183–188.
22. Wang S, Bondarenko VE, Qu Y, Morales MJ, Rasmusson RL, Strauss HC. Activation properties of Kv4.3 channels: time, voltage and $[K^+]_o$ dependence. J Physiol 2004;557:705–717.
23. An WF, Bowlby MR, Betty M, et al. Modulation of A-type potassium channels by a family of calcium sensors. Nature 2000;403:553–556.
24. Decher N, Uyguner O, Scherer CR, et al. hKChIP2b is a functional modifier of hKv4.3 potassium channels: cloning and expression of a short hKChIP2b splice variant. Cardiovasc Res 2001;52:255–264.
25. Radicke S, Cotella D, Graf EM, et al. Functional modulation of the transient outward current I_{to} by *KCNE* beta-subunits and regional distribution in human non-failing and failing hearts. Cardiovasc Res 2006;1:695–703.
26. Deschênes I, DiSilvestre D, Juang GJ, Wu RC, An WF, Tomaselli GF. Regulation of Kv4.3 current by KChIP2b splice variants: a component of native cardiac I_{to}? Circulation 2002;106:423–429.
27. Radicke R, Vaquero M, Caballero R, et al. Effects of MiRP1 and DPP6 β-subunits on the blockade induced by flecainide of Kv4.3/KChIP2 channels. Br J Pharmacol 2008;154:774–786.
28. Wettwer E, Amos GJ, Posival H, Ravens U. Transient outward current in human ventricular myocytes of subepicardial and subendocardial origin. Circ Res 1994; 75:473–482.
29. Patel SP, Campbell DL. Transient outward potassium current, "I_{to}," phenotypes in the mammalian left ventricle: underlying molecular, cellular and biophysical mechanisms. J Physiol 2005;569:7–39.
30. Antzelevitch C. Brugada syndrome. Pacing Clin Electrophysiol 2006;29:1130–1159.
31. Bezzina C, Veldkamp MW, van den Berg MP, et al. A single Na^+ channel mutation causing both long-QT and Brugada syndromes. Circ Res 1999;85: 1206–1213.
32. Van den Berg MP, Wilde AA, Viersma TJW, et al. Possible bradycardic mode of death and successful pacemaker treatment in a large family with features of long QT syndrome type 3 and Brugada syndrome. J Cardiovasc Electrophysiol 2001;12:630–636.

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IMAGES IN CARDIOLOGY

Aortopulmonary Artery Dissection

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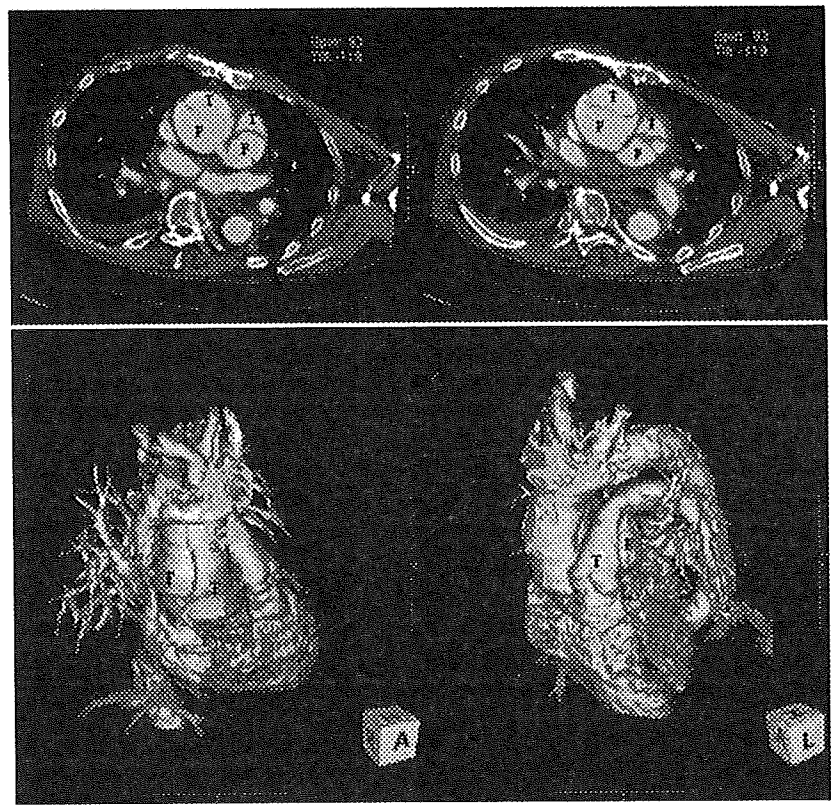

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A 71-year-old man was brought to the hospital by ambulance and admitted because of intermittent back pain. Physical examination revealed continuous heart murmur. Chest X-ray showed cardiomegaly and pulmonary congestion. The patient received continuous hydration for renal dysfunction and severe metabolic acidosis (base excess of −16.5 mmol/l and pH of 7.24) and underwent enhanced chest computed tomography. The chest computed tomography showed not only ascending aortic dissection of Stanford type A but also pulmonary artery dissection with an aortopulmonary window (**red arrow** = aortopulmonary window, T = true lumen, F = false lumen). An aortopulmonary shunt could not only increase pulmonary circulation and cause untreatable congestive pulmonary edema but also cause severe metabolic acidosis. Aortopulmonary artery dissection is a very rare disease but is fatal, requiring the surgical repair as rapidly as possible.

Endothelin-1 as a predictor of atrial fibrillation recurrence after pulmonary vein isolation

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BACKGROUND A considerable rate of atrial fibrillation (AF) recurrence is one of the major limitations of pulmonary vein isolation (PVI). Although endothelin-1 (ET-1) is involved in atrial remodeling, it is unknown whether plasma ET-1 level before PVI can be used as a predictive factor for AF recurrence.

OBJECTIVE The goal of this study was to clarify whether the plasma ET-1 level, before PVI, can be used as a predictive factor for AF recurrence after PVI.

METHODS Fifty-one patients without structural heart disease who underwent PVI for symptomatic and drug-refractory paroxysmal/persistent AF were included in the study. Neurohumoral factors were measured, and transthoracic echocardiography was performed before and 6 months after each PVI. Mean left atrial (LA) pressure and arterial blood pressure (BP) were evaluated just before PVI. AF recurrence was detected by 12-lead electrocardiogram (ECG), Holter ECG, and event ECG monitor recordings, 3 to 6 months after PVI.

RESULTS Among plasma levels of ET-1, atrial and brain natriuretic peptides, renin, angiotensin II, and aldosterone before PVI, only ET-1 was significantly higher in the recurrence group compared with the nonrecurrence group (2.15 ± 0.51 vs. 1.65 ± 0.35 pg/ml, $P < .001$). Both mean LA pressure and diastolic BP in the recurrence group were significantly higher than in the nonrecurrence group (mean LA pressure, 10 ± 3 vs. 8 ± 3 mm Hg, $P < .01$; diastolic BP, 82 ± 11 vs. 71 ± 12 mm Hg, $P < .01$). The plasma ET-1 level and mean LA pressure were correlative. Multiple logistic regression analyses showed that higher levels of plasma ET-1 and diastolic BP were significant prognostic predictors of AF recurrence 3 to 6 months after PVI ($P < .01$ and $P < .05$, respectively).

CONCLUSION Our findings suggest that the plasma ET-1 level before PVI could be a crucial predictor of AF recurrence 3 to 6 months after PVI.

KEYWORDS Atrial fibrillation; Pulmonary vein isolation; Endothelin-1; Recurrence; Predictive factor; Neurohumoral factor; Remodeling

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Introduction

Atrial fibrillation (AF) is the most common type of arrhythmia seen in clinical practice, and its prevalence increases with age. Because pulmonary veins (PVs) are important sources of ectopic beats for the initiation of paroxysmal AF (PAF),[1] PV isolation (PVI) by catheter ablation can cure PAF. Circumferential PVI was reported to be effective for PAF and chronic AF.[2] However, there is a considerable rate of AF recurrence (10% to 40%).[3,4] Verma et al[4] showed that left atrial (LA) scarring is a strong predictor of AF recurrence; however, it requires an invasive technique to quantify the extent of LA fibrosis.

Endothelin-1 (ET-1), an endothelium-derived vasoconstrictor peptide,[5] participates in the pathophysiology of AF via membrane ion channels and atrial remodeling. In fact, ET-1 shortens the action potential duration (APD) via both the inhibition of the L-type calcium current and activation of the muscarinic potassium current.[6] ET-1 is also reported to be proarrhythmic through the modulation of the intracellular calcium dynamics of atrial myocytes.[7-9] Furthermore, the biological effects of ET-1 include the activation of neurohumoral factors, modulation of the renin-angiotensin-aldosterone system, augmentation of myocardial inotropic function, and stimulation of cardiac hypertrophy.[10] All of these effects, derived from ET-1, facilitate electrical or structural remodeling during AF,[11] and cause AF chronicity.[12]

It remains, however, unclear whether the plasma ET-1 level, before PVI, can be used as a predictive factor for AF recurrence after PVI. Therefore, we evaluated the plasma levels of several neurohumoral factors including the ET-1 before PVI and investigated the PVI outcome in AF patients.

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Methods

Patient characteristics

This prospective study included 51 consecutive patients who underwent circumferential PVI for symptomatic and

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drug-refractory PAF and/or persistent AF between October 2005 and February 2008. According to the international consensus,[13] PAF, persistent AF, and permanent AF were defined as follows: PAF = self-terminating ≤7 days, persistent AF = lasting >7 days and requiring pharmacologic therapy or electrical cardioversion for termination, and permanent AF = failing to terminate with electrical cardioversion or relapsing within 24 hours after successful cardioversion. Patients with structural heart disease, pulmonary disease, or a history of catheter ablation of AF were not enrolled in this study. All patients gave their written informed consent, and the Ethical Committee of Shiga University of Medical Science approved the study protocol.

Study protocol

Class I antiarrhythmic drugs were ceased at least 3 days before PVI. The patients were allowed to continue other drugs; class III antiarrhythmic drugs and beta-blockers. Oral anticoagulation (warfarin) with a target international normalized ratio of 2.0 to 2.6 was required for at least 1 month before PVI. Transthoracic echocardiography was performed before and 6 months after PVI. The LA diameter was measured in the parasternal long-axis view. The LA medial-lateral and superior-inferior lengths were measured in the apical 4-chamber view. The LA volume index (LAVI) (ml/m^2) was calculated using Equation 1.[14]

$$LAVI = \pi/6(LA_{S1} \cdot LA_{S2} \cdot LA_L)/BSA \times 1000 \quad (1)$$

where LA_{S1} (mm) indicates the M-mode LA diameter of the short axis in the parasternal view, LA_{S2} (mm) and LA_L (mm) indicate measurements of the short and long axes, in the apical 4-chamber view at ventricular end-systole, and BSA (m^2) indicates body surface area. Valvular abnormality, left ventricular (LV) wall motion analysis, and LV ejection fraction (LVEF) were also assessed.

Because neurohumoral factors are considered to be predictive of AF recurrence after cardioversion,[15-17] for the blood sampling and hormone assays, we measured the plasma levels of ET-1, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), renin, angiotensin II, and aldosterone before and 6 months after the completed PVI procedure. The blood samples were obtained from the antecubital vein in the supine position after a resting period of 20 min before PVI and 6 months after PVI. At the timing of the blood sampling before PVI, we measured the mean LA pressure transseptally and blood pressure (BP) in the femoral artery. The details of the hormone assay can be found elsewhere.[18-21]

PVI procedure

The LA catheterization was performed with a 2-puncture, 3-sheath technique. Intravenous heparin was administered to maintain an activated clotting time of >250 sec after the atrial transseptal procedure. In all cases, circumferential PVI targeting the PV antrum potentials of all PVs was performed using a circular mapping catheter (Lasso, Johnson & Johnson K.K. Medical Company, Tokyo, Japan). The Lasso catheter was positioned at the ostium of each PV for recording the PV potentials. The ipsilateral left and right PVs were encircled with a lesion line by circumferential PV ablation. Radiofrequency energy was delivered with a target temperature of 55°C and maximum power output of 30 to 40 W for 30 to 60 sec (CABLE-IT, Japan Lifeline Co., Tokyo, Japan) using an 8-mm-tip catheter (Ablaze Fantasista, Japan Lifeline Co., Tokyo, Japan). The endpoint of the PVI procedure was the bidirectional block between the PV antrum and left atrium,[22] with elimination of all recordable high-frequency potentials confirmed by the Lasso catheter. Cavotricuspid isthmus ablation was also performed in all patients.

Follow-up

All patients were examined at 1-month intervals for 6 months after PVI. During the follow-up period, neither digoxin nor verapamil was administered in any of the patients. During the same period, although the preventive administration of class I antiarrhythmic drugs was not allowed, the patients were permitted to treat themselves with single administrations of class I antiarrhythmic drugs after any AF recurrence. No class III antiarrhythmic drugs were added after PVI. Warfarin with a target international normalised ratio of 2.0 to 2.6 was continued during the follow-up period.

Assessment of the AF attacks

All of the patients were contacted and asked if they had had any AF attacks before and after PVI (every visit). A 12-lead electrocardiogram (ECG) was conducted at every visit. Holter ECG recordings were performed before and 6 months after PVI. All patients were also given an event ECG monitor (HCG-801/901, Omron Health Care Co., Kyoto, Japan) at 2 weeks before, 3 months after, and 6 months after PVI. Then the patients were asked to record their rhythm at least twice per day and whenever they had any symptoms. The existence of AF was defined when 12-lead ECG, Holter ECG, and event ECG monitor recordings repeatedly detected AF continuing for >20 sec. AF recurrence was defined as: (1) AF repeatedly detected by the event ECG monitor recording for ≥7 days, or (2) symptomatic AF detected by the conventional ECG recording and/or event ECG monitor recording more than several times on an outpatient basis 3 to 6 months after the successful completion of PVI.

Statistical analysis

Continuous variables of 2 groups were compared using an unpaired Student *t* test, and categorical variables (yes/no) as binary dependent variables of 2 groups were compared using a chi-square test. All continuous variables are expressed as the mean values and standard deviation (SD). Pairwise associations were examined by a Pearson correlation coefficient test when the data were on a continuous scale. We drew a receiver-operator characteristic (ROC) curve and a calculated area under the curve (AUC) to

investigate the clinical value of the ET-1 and factors that showed a statistical difference between the 2 groups with nonrecurrence and recurrence of AF. The ROC curve was performed to test the best cutoff for the prediction of AF recurrence. Comparisons between the 2 groups were assessed by univariate and multivariate logistic regression analyses with continuous or categorical variables. The variables associated with the relevant outcomes by the univariate analysis ($P < .05$) were included in the multivariate logistic regression analyses. Adjusted odds ratios and 95% confidence interval (CI) were determined for the variables that were associated with each outcome. Statistical differences with $P < .05$ were considered significant. All data were analyzed using SPSS software (Version 11, SPSS Japan, Inc., Tokyo, Japan).

Results

AF recurrence and clinical characteristics

All PVs in each of the patients in this study were electrically isolated. The AF nonrecurrence rate after the PVI was 71% when we used the conventional definition of AF recurrence using 12-lead ECG and Holter ECG. However, AF episodes not detected by the conventional ECG recordings in 9 patients (18%) were detected by event ECG monitors. Thus, the nonrecurrence rate in this study was 53% using both event ECG monitors and the conventional ECG recordings. Although atrial tachycardia/flutter (AT/AFL) was observed in 5 patients, the nonrecurrence rate was not altered because the AT/AFL was always accompanied by AF recurrences.

Table 1 Clinical characteristics before PVI in the nonrecurrence and recurrence of AF groups

	Nonrecurrence (n = 27)	Recurrence (n = 24)
Age (years)	60 ± 11	56 ± 10
Sex (male/female)	23/4	22/2
AF duration (months)	50 ± 64	70 ± 83
PAF/persistent AF (n)	23/4	16/8
Delivered RF energy (J)		
PVI	78,948 ± 20,856	87,339 ± 28,979
CT isthmus block line	25,262 ± 13,638	25,304 ± 13,281
Mean LA pressure (mm Hg)	8 ± 3	10 ± 3*
BP obtained from the femoral artery		
Systole (mm Hg)	138 ± 31	142 ± 19
Diastole (mm Hg)	71 ± 12	82 ± 11*
Heart rate (beats/min)	68 ± 13	77 ± 19
Hypertension [n (%)]	11 (41)	9 (38)
Hyperlipidemia [n (%)]	11 (41)	11 (46)
Medication [n (%)]		
Antiarrhythmic class I	20 (74)	22 (92)
Antiarrhythmic class III	4 (15)	5 (21)
Digoxin	12 (44)	8 (33)
Beta-blocker	14 (52)	7 (29)
Verapamil	8 (30)	9 (38)
Other CCB	6 (22)	3 (13)
ACE-I	2 (7)	4 (17)
ARB	5 (19)	6 (25)
Statin	4 (15)	2 (8)

ACE-I = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BP = blood pressure; CCB = calcium-channel blocker; CT = cavotricuspid; LA = left atrial; PAF = paroxysmal atrial fibrillation; PVI = pulmonary vein isolation; RF = radiofrequency.
*$P < .01$.

Table 2 Transthoracic echocardiographic parameters before and 6 months after PVI

	Nonrecurrence (n = 27)	Recurrence (n = 24)	P value
Before PVI			
LA diameter; M-mode (mm)	40 ± 4	41 ± 5	.22
LAVI (ml/m^2)	26 ± 8	26 ± 8	.86
LVEF (%)	66 ± 9	62 ± 7	.16
Six months after PVI			
LA diameter; M-mode (mm)	37 ± 4‡	39 ± 6†	.32
LAVI (ml/m^2)	20 ± 8†	22 ± 6*	.59
LVEF (%)	64 ± 7	63 ± 10	.57

LA = left atrial; LAVI = left atrial volume index; LVEF = left ventricular ejection fraction; PVI = pulmonary vein isolation.
*$P < .05$ vs. before PVI. †$P < .01$ vs. before PVI. ‡$P < .001$ vs. before PVI.

The clinical characteristics before PVI in the AF nonrecurrence and recurrence groups are listed in Table 1. A comparison of the delivered radiofrequency energy between the 2 groups showed no significant difference. There were no statistical differences between the 2 groups in terms of the number of patients with hypertension or hyperlipidemia. Both the mean LA pressure and diastolic BP just before PVI were significantly higher in the recurrence group than in the nonrecurrence group (mean LA pressure, 10 ± 3 vs. 8 ± 3 mm Hg, $P < .01$; diastolic BP, 82 ± 11 vs. 71 ± 12 mm Hg, $P < .01$). In contrast, no significant difference was found in the other characteristics: age, sex, AF duration, presence of persistent AF, systolic BP, and heart rate. There was no significant difference in the frequency of the administration of antiarrhythmic drugs (class I/III), digoxin, beta-blockers, calcium channel blockers, angiotensin-converting-enzyme inhibitors, angiotensin II receptor blockers, or statins.

In addition, as shown in Table 2, there were no statistical differences in the echocardiographic parameters (LA diameter, LAVI, and LVEF) before PVI. When comparing the echocardiographic parameters before and 6 months after PVI, we found that both the LA diameter and LAVI had significantly decreased and the LVEF had not become significantly altered by the PVI regardless of AF recurrence.

Plasma levels of ET-1 and other neurohumoral factors

Table 3 shows the plasma neurohumoral factor levels before and 6 months after PVI. Before PVI, among the ET-1, ANP, BNP, renin, angiotensin II, and aldosterone levels, only the ET-1 level in the recurrence group was significantly higher than that in the nonrecurrence group (2.15 ± 0.51 vs. 1.65 ± 0.35 pg/ml, $P < .001$). In contrast, at 6 months after PVI, although there was no significant difference in the ET-1 level

Table 3 Plasma neurohumoral factor levels before and 6 months after PVI

	Nonrecurrence (n = 27)	Recurrence (n = 24)	P value
Before PVI			
ET-1 (pg/ml)	1.65 ± 0.35	2.15 ± 0.51	<.001
ANP (pg/ml)	41.6 ± 30.6	55.6 ± 43.3	.19
BNP (pg/ml)	46.1 ± 45.3	54.2 ± 43.3	.52
Renin (pg/ml)	16.3 ± 32.0	10.4 ± 9.3	.39
Angiotensin II (pg/ml)	12.2 ± 16.6	11.0 ± 5.7	.76
Aldosterone (pg/ml)	67.8 ± 44.4	77.4 ± 40.2	.42
Six months after PVI			
ET-1 (pg/ml)	1.76 ± 0.46	1.85 ± 0.46*	.44
ANP (pg/ml)	18.0 ± 8.7†	26.1 ± 25.6*	.17
BNP (pg/ml)	23.7 ± 18.2*	59.4 ± 88.7	<.05
Renin (pg/ml)	32.0 ± 68.8	16.9 ± 28.3	.35
Angiotensin II (pg/ml)	25.2 ± 43.6	18.3 ± 21.3	.52
Aldosterone (pg/ml)	75.8 ± 41.0	69.5 ± 36.0	.62

ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; ET-1 = endothelin-1; PVI = pulmonary vein isolation.
*$P < .05$ vs. before PVI. †$P < .01$ vs. before PVI.

Table 4 Univariate and multivariate predictors of AF recurrence

Variables	Univariate chi-square	P value	Multivariate chi-square odds ratio (95% CI)	P value
Age (years)	1.32	.25		
Sex (male = 1)	0.50	.48		
AF duration (months)	0.89	.35		
ET-1 (>1.68 pg/ml = 1)	9.88	<.01	8.71 .06 (.01–0.38)	<.01
Mean LA pressure (mm Hg)	6.73	<.01		
Diastolic BP (mm Hg)	7.57	<.01	6.10 1.13 (1.03–1.25)	<.05
LA diameter; M-mode (mm)	1.49	.22		
LVEF (%)	1.97	.16		

AF = atrial fibrillation; BP = blood pressure; CI = confidence interval; ET-1 = endothelin-1; LA = left atrial; LVEF = left ventricular ejection fraction.

between the 2 groups, the BNP level in the recurrence group was significantly higher than that in the nonrecurrence group (59.6 ± 90.6 vs. 23.6 ± 18.2 pg/ml, $P < .05$). Although the ET-1 level in the recurrence group was significantly decreased by the PVI ($P < .05$), the ET-1 level in the nonrecurrence group was not statistically altered. In addition, the plasma ANP level in both groups and the plasma BNP level in the nonrecurrence group were significantly decreased by the PVI.

ROC curves and the best cutoff values

Figure 1 shows the ROC curves of the plasma ET-1 level, mean LA pressure, and diastolic BP. The AUC for the ET-1 (Figure 1A) was 0.81 with a 95% CI of 0.69 to 0.93, and a cutoff value of 1.68 pg/ml practically and reasonably separated the patients with or without AF recurrence 3 to 6 months after the PVI (sensitivity 92%, specificity 56%, positive predictive value 67%, and negative predictive value 87%). Because we think that a sufficiently low rate of AF recurrence should be required in AF patients with an ET-1 level lower than the cutoff point, we chose the ET-1 cutoff value as the point satisfying a sufficiently high negative predictive value. On the other hand, we obtained AUC values of 0.74 with a 95% CI of 0.60 to 0.89 for the mean LA pressure (Figure 1B) and 0.75 with a 95% CI of 0.62 to 0.88 for the diastolic BP (Figure 1C). A statistically significant correlation was observed between the plasma ET-1 level and mean LA pressure before PVI ($r = 0.438$, $P < .01$).

Univariate and multivariate predictors of AF recurrence

As shown in Table 4, 8 clinical, neurohumoral, and hemodynamic variables (age, sex, AF duration, plasma ET-1 level, mean LA pressure, diastolic BP, LA diameter, and LVEF) were analyzed using univariate and multivariate logistic regression analyses. In the univariate logistic regression analysis, higher levels of the plasma ET-1, mean LA pressure, and diastolic BP were associated with AF

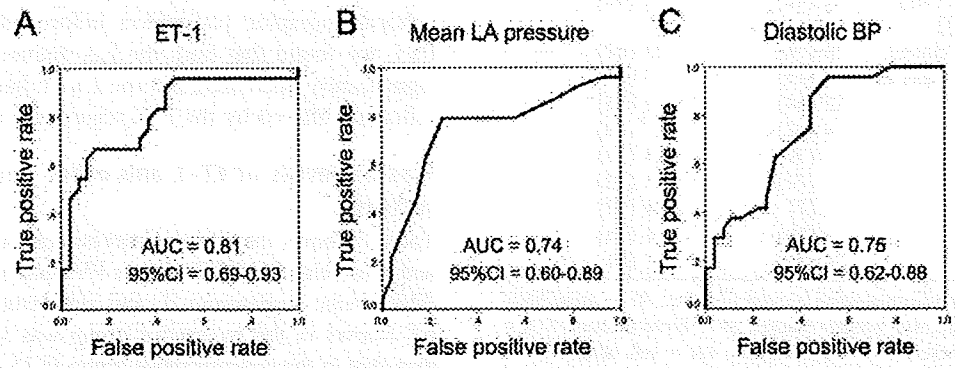

Figure 1 Receiver-operator characteristic curves of the endothelin-1 **(A)**, mean left atrial pressure **(B)**, and diastolic blood pressure **(C)** used to separate the patients with or without atrial fibrillation recurrence 3 to 6 months after pulmonary vein isolation. AUC = area under the curve; 95% CI = 95% confidence interval.

recurrence 3 to 6 months after PVI. In the multivariate logistic regression analysis, higher levels of the plasma ET-1 and diastolic BP were significant predictors of AF recurrence 3 to 6 months after PVI ($P < .01$ and $P < .05$, respectively).

Discussion

The major findings of the present study were: (1) a higher plasma ET-1 level before PVI was a strong predictor of AF recurrence 3 to 6 months after PVI; (2) compared with the previously proposed predictive factors for AF recurrence after PVI, the plasma ET-1 level before PVI was the strongest predictive factor; (3) the plasma ET-1 level was correlated with the mean LA pressure.

The predictive factors of AF recurrence in previous studies

Previous studies showed that AF recurrence after PVI depends on various factors, such as LA diameter,[3] LVEF,[23] and LA scarring.[4] In the present study, because patients with structural heart disease were excluded, there were no significant differences in the echocardiographic parameters before PVI between the AF nonrecurrence and recurrence groups (Table 2).

Katritsis et al[24] suggested a relationship between AF recurrence and hypertension. On the other hand, in this study we found that although the diastolic BP before PVI was one of the predictive factors, the plasma ET-1 level before PVI was the strongest (Table 4).

Yamada et al[25] reported that a significant reduction in the plasma BNP level 3 months after PVI is a marker for nonrecurrence of AF; however, the BNP level before PVI was not useful for the prediction of AF recurrence. Similar to their data,[25] our present data showed a significant reduction in the BNP level 6 months after PVI in the nonrecurrence group and no difference in the BNP levels before PVI between the AF nonrecurrence and recurrence groups (Table 3).

ET-1 and electrical remodeling

Shortening of the atrial effective refractory period or APD has been indicated as an important factor for the maintenance of AF in recent experimental and clinical studies.[12,26,27] Ono et al[6] showed that ET-1 regulated the L-type calcium current and muscarinic potassium current, and therefore the ET-1 shortened the APD in mammalian atrial myocytes. Other studies[8,9] showed that ET-1 altered the calcium transient via activation of inositol 1,4,5-triphosphate, and thus the ET-1 shortened the atrial APD. In contrast, Redpath et al[28] suggested that ET-1 has antiarrhythmic effects through its antiadrenergic action on human atria.

As mentioned above, it is speculated that ET-1 has both proarrhythmic and antiarrhythmic effects on atrial myocytes. However, we considered that the electrical remodeling does not have such a strong effect on the AF recurrence 3 to 6 months after PVI because the electrical remodeling is reversible within 1 week of normal sinus rhythm.[12]

ET-1 and structural remodeling

Fujisaki et al[29] reported that the angiotensin II-induced ET-1 gene expression in cardiac fibroblasts may serve as autocrine/paracrine growth factors for the cardiac fibroblasts themselves. van Wamel et al[30] elucidated the role of ET-1 as an autocrine/paracrine mediator of stretch-induced cardiomyocyte hypertrophy. According to their report,[30] ET-1 is released by stretched cardiac cells, such as cardiomyocytes, cardiac fibroblasts, endothelial cells, and vascular smooth muscle cells. Interestingly, although angiotensin II is one of the major factors in cardiac fibrogenesis,[31] they[30] further found that the stretched cardiac fibroblasts can produce ET-1 even in the absence of angiotensin II.

Our results showed that the plasma ET-1 level correlated well with the mean LA pressure when the blood samples were obtained just before PVI. This indicates that the elevation in the mean LA pressure causes atrial stretch and therefore releases ET-1, initiating atrial structural remodeling before PVI. Because the pre-existing LA structural remodeling (scarring) is a strong independent predictor of AF recurrence after PVI,[4] we think that the ET-1 level can serve as a strong predictor of AF recurrence after PVI. In addition, we speculate that atrial structural remodeling is already in progress to some extent even in PAF patients. This idea is supported by our additional results showing that a higher ET-1 level before PVI resulted in a higher probability of AF recurrence in PAF patients (even excluding persistent AF patients) 3 to 6 months after PVI ($P < .001$).

Clinical implications

The measurement of the plasma ET-1 level is a noninvasive and an easy procedure, and therefore we can measure the plasma ET-1 level even in the outpatient clinic. Furthermore, because the plasma ET-1 level is an effective and certain predictive factor for the recurrence of AF 3 to 6 months after PVI as shown in this study, it would be beneficial to AF patients who plan to undergo PVI. Because ET-1 is involved in cardiac fibroblast proliferation in atria, a higher ET-1 level might reflect the progression of atrial structural remodeling. Actually, as found in this study, a higher rate of AF recurrence 3 to 6 months after PVI resulted from a plasma ET-1 level of >1.68 pg/ml before PVI (Table 4).

Some clinicians[32,33] have used a stepwise ablation approach, which includes PVI, LA roof linear ablation, mitral isthmus ablation, and complex fractionated potential ablation. We infer from our results that the stepwise ablation approach should be recommended for the higher ET-1 group regardless of the AF duration or AF type.

Study limitations

Because we used patient activation-type event ECG monitors instead of automatic continuous ECG monitoring systems, recurrences of both asymptomatic AF and AF during sleep might have been missed.

The cardiac rhythm when the blood samples were obtained was normal sinus rhythm in most cases, whereas in some cases the blood sampling was performed under AF because of its resistance to conventional treatment. However, we found in this study that the plasma ET-1 level before PVI under AF was not significantly higher than that during normal sinus rhythm (1.99 ± 0.52 vs. 1.82 ± 0.48 pg/ml, $P = .23$). In addition, we found that the existence of AF at the time of blood sampling in the recurrence group was not significantly higher than that in the nonrecurrence group ($P = .13$).

In this study, the reasons why both the LA diameter and LAVI were significantly decreased by PVI regardless of AF recurrence (Table 2) and why the plasma ET-1 level was significantly decreased by PVI even in the AF recurrence group (Table 3) are unexplained. A possible explanation for the reduction in the ET-1 level is that the PVI procedure mechanically modifies LA condition and negates the effect of LA stretch on the ET-1 elevation. Further studies are needed to elucidate these issues.

Conclusion

The plasma ET-1 level before PVI is a crucial predictor of AF recurrence 3 to 6 months after PVI. Although this study is hypothesis generating and the clinical utility of the plasma ET-1 level needs to be prospectively validated, focusing on the ET-1 level in AF patients may open a new avenue for the treatment of AF.

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References

1. Haïssaguerre M, Jaïs P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659–666.
2. Pappone C, Rosanio S, Oreto G, et al. Circumferential radiofrequency ablation of pulmonary vein ostia: a new anatomic approach for curing atrial fibrillation. Circulation 2000;102:2619–2628.
3. Oral H, Scharf C, Chugh A, et al. Catheter ablation for paroxysmal atrial fibrillation: segmental pulmonary vein ostial ablation versus left atrial ablation. Circulation 2003;108:2355–2360.
4. Verma A, Wazni OM, Marrouche NF, et al. Pre-existent left atrial scarring in patients undergoing pulmonary vein antrum isolation: an independent predictor of procedural failure. J Am Coll Cardiol 2005;45:285–292.
5. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–415.
6. Ono K, Tsujimoto G, Sakamoto A, et al. Endothelin-A receptor mediates cardiac inhibition by regulating calcium and potassium currents. Nature 1994;370:301–304.
7. Duru F, Barton M, Lüscher TF, et al. Endothelin and cardiac arrhythmias: do endothelin antagonists have a therapeutic potential as antiarrhythmic drugs? Cardiovasc Res 2001;49:272–280.
8. Mackenzie L, Bootman MD, Laine M, et al. The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. J Physiol 2002;541:395–409.
9. Li X, Zima AV, Sheikh F, et al. Endothelin-1-induced arrhythmogenic Ca^{2+} signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate (IP3)-receptor type 2-deficient mice. Circ Res 2005;96:1274–1281.
10. Lüscher TF, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. Circulation 2000; 102:2434–2440.
11. Burrell KM, Molenaar P, Dawson PJ, et al. Contractile and arrhythmic effects of endothelin receptor agonists in human heart in vitro: blockade with SB 209670. J Pharmacol Exp Ther 2000;292:449–459.
12. Wijffels MC, Kirchhof CJ, Dorland R, et al. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. Circulation 1995; 92:1954–1968.
13. Lévy S, Camm AJ, Saksena S, et al. International consensus on nomenclature and classification of atrial fibrillation: a collaborative project of the Working Group on Arrhythmias and the Working Group of Cardiac Pacing of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. J Cardiovasc Electrophysiol 2003;14:443–445.
14. Murray JA, Kennedy JW, Figley MM, et al. Quantitative angiocardiography: II. The normal left atrial volume in man. Circulation 1968;37:800–804.
15. Mabuchi N, Tsutamoto T, Maeda K, et al. Plasma cardiac natriuretic peptides as biochemical markers of recurrence of atrial fibrillation in patients with mild congestive heart failure. Jpn Circ J 2000;64:765–777.
16. Wozakowska-Kaplon B, Opolski G. Concomitant recovery of atrial mechanical and endocrine function after cardioversion in patients with persistent atrial fibrillation. J Am Coll Cardiol 2003;41:1716–1720.
17. Goette A, Hoffmanns P, Enayati W, et al. Effect of successful electrical cardioversion on serum aldosterone in patients with persistent atrial fibrillation. Am J Cardiol 2001;88:906–909.
18. Tsutamoto T, Wada A, Maeda Y, et al. Relation between endothelin-1 spillover in the lungs and pulmonary vascular resistance in patients with chronic heart failure. J Am Coll Cardiol 1994;23:1427–1433.
19. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997;96:509–516.
20. Wada A, Tsutamoto T, Matsuda Y, et al. Cardiorenal and neurohumoral effects of endogenous atrial natriuretic peptide in dogs with severe congestive heart failure using a specific antagonist for guanylate cyclase-coupled receptors. Circulation 1994;89:2232–2240.
21. Tsutamoto T, Wada A, Maeda K, et al. Digitalis increases brain natriuretic peptide in patients with severe congestive heart failure. Am Heart J 1997;134: 910–916.
22. Gerstenfeld EP, Dixit S, Callans D, et al. Utility of exit block for identifying electrical isolation of the pulmonary veins. J Cardiovasc Electrophysiol 2002; 13:971–979.
23. Chen MS, Marrouche NF, Khaykin Y, et al. Pulmonary vein isolation for the treatment of atrial fibrillation in patients with impaired systolic function. J Am Coll Cardiol 2004;43:1004–1009.
24. Katritsis DG, Toumpoulis IK, Giazitzoglou E, et al. Latent arterial hypertension in apparently lone atrial fibrillation. J Interv Card Electrophysiol 2005;13:203–207.
25. Yamada T, Murakami Y, Okada T, et al. Plasma brain natriuretic peptide level after radiofrequency catheter ablation of paroxysmal, persistent, and permanent atrial fibrillation. Europace 2007;9:770–774.
26. Yue L, Feng J, Gaspo R, et al. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. Circ Res 1997;81:512–525.
27. Van Wagoner DR, Pond AL, Lamorgese M, et al. Atrial L-type Ca2+ currents and human atrial fibrillation. Circ Res 1999;85:428–436.
28. Redpath CJ, Rankin AC, Kane KA, et al. Anti-adrenergic effects of endothelin on human atrial action potentials are potentially anti-arrhythmic. J Mol Cell Cardiol 2006;40:717–724.
29. Fujisaki H, Ito H, Hirata Y, et al. Natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking endothelin-1 gene expression. J Clin Invest 1995;96:1059–1065.
30. van Wamel AJ, Ruwhof C, van der Valk-Kokshoom LE, et al. The role of angiotensin II, endothelin-1 and transforming growth factor-beta as autocrine/paracrine mediators of stretch-induced cardiomyocyte hypertrophy. Mol Cell Biochem 2001;218:113–124.
31. Li D, Shinagawa K, Pang L, et al. Effects of angiotensin-converting enzyme inhibition on the development of the atrial fibrillation substrate in dogs with ventricular tachypacing-induced congestive heart failure. Circulation 2001;104: 2608–2614.
32. Hocini M, Jaïs P, Sanders P, et al. Techniques, evaluation, and consequences of linear block at the left atrial roof in paroxysmal atrial fibrillation: a prospective randomized study. Circulation 2005;112:3688–3696.
33. O'Neill MD, Jaïs P, Takahashi Y, et al. The stepwise ablation approach for chronic atrial fibrillation—evidence for a cumulative effect. J Interv Card Electrophysiol 2006;16:153–167.

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

A Nationwide Questionnaire Survey in Japan

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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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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)/

aborted cardiac arrest (ACA) (5 cases), AVB (5 cases), VT/TdP (5 cases), and other miscellaneous abnormalities.

The ECG on diagnosis, or immediately after birth for fetal cases, showed that the HR and QTc interval (corrected using Bazett formula) ranged from 50 to 160 (102±28) bpm, and from 360 to 774 (563±70) ms, respectively.

Genotype-Phenotype Correlation

Among 41 patients who underwent genetic testing, mutations were identified in 29 (71%) cases; including *KCNQ1* gene mutations (LQT1) in 11, *KCNH2* mutations (LQT2) in 11, *SCN5A* mutations (LQT3) in 6, and *CACNA1C* (LQT8) in 1. Twelve patients also underwent genotyping, but no mutation was found. Table 2 lists the demographic and clinical features of these subjects (references 16, 17, and 23 reported the same cases 2, 12, and 27 in Table 2) and of those with no known mutations.

The remaining 17 subjects (6 fetuses, 8 neonates, 3 infants) did not undergo genetic analysis due to lack of such analysis at that time, death soon after birth, or refusal by parents. Five had SCD/ACA (Table 3), including a 1-day-old neonate who had AVB and died at 57 days of age in 1984. This case was later assumed to be LQT8, based on characteristic phenotypes such as syndactyly. AVB and VT/TdP were observed in 7 and 5 cases, respectively, in this group.

Although HR and QTc values were not different among LQT1, LQT2, LQT3, and mutation-negative groups, the incidence of VT/TdP was higher in LQT2 and LQT3 compared with LQT1 (Table 4). The incidence of AVB tended to be higher in LQT3 compared with LQT1 but statistically insignificant. On the other hand, the presence of family history of LQTS was more frequent in LQT1 than the mutation-negative group. The incidence of sinus bradycardia was comparable among the 4 groups (Table 4).

Table 3 lists cases with SCD/ACA; only 4 genetically confirmed cases were included, and 4 were mutation-negative, although the remaining 6 cases did not undergo genotyping. These individuals showed bradycardia (97±31 bpm; 10/14 showed HR <110 bpm) and markedly prolonged QTc (617±81 ms).

Treatment

With regard to the treatment of fetal VT/TdP, antiarrhythmic agents were administered transplacentally in 4 of 18 fetal cases (propranolol in 3 cases, lidocaine in 1, mexiletine in 1, flecainide in 1, and magnesium in 1), using the method described in detail in our previous report.[17] None of the 4 cases was genetically confirmed prenatally. When 2 or 3 of the following findings of sinus bradycardia, VT, and AVB were observed in a structurally normal heart, LQTS was strongly suggested, and β-blockers, sodium channel blockers (lidocaine, mexiletine), and magnesium (Mg) were selected as typical antiarrhythmic agents, instead of amiodarone or sotalol, which may prolong the QT interval. These drugs were used in combination until VT/TdP was controlled and proved effective in all 4 cases. However, preterm delivery was conducted in 2 cases both at 33 weeks of gestation due to recurrent VT/TdP and depression of fetal physical activity in one and to fetal hydrops and distress in the other. In the remaining 14 cases, pharmacotherapy was initiated after confirmation of the type of arrhythmias after birth. However, no fetal death was noted.

For 15 neonatal cases who presented with VT/TdP (including those who did not undergo genotyping), acute pharmacotherapy consisted of 2 or more of the following drugs: β-blockers, mexiletine, lidocaine, Mg, phenytoin, and others, except for 2 cases who were treated with phenytoin alone and 1 with mexiletine alone. Most of these cases were judged to respond the combination therapy. In 5 neonates in whom LQT3 was strongly suggested based on a typical ECG finding called late-appearing T wave, mexiletine was first administered but proved insufficient, and β-blockers were also added in all 5.

For those with LQTS presenting in infancy, 6 cases received acute pharmacotherapy (2 or all of propranolol, mexiletine, and Mg). No additional agent was administered. Thus, in all age groups, the acute therapy for VT/TdP consisted of a single drug to which 1 or more drugs was then added until the arrhythmia was controlled, independent of the genotype. Actually, the genotype was not identified during the acute phase in most cases. Furthermore, genotyping was not conducted in those 17 cases who presented before 1999.

Maintenance therapy consisted mainly of β-blockers (or no therapy) for LQT1 and mostly of mexiletine/β-blockers for LQT2 and LQT3 (Table 2). β-Blockers were added in 8 LQT2 cases after confirmation of the genotype. In all 6 LQT3 cases, mexiletine was maintained (combined with β-blockers) from acute through chronic phase after determination of the genotype.

Nine patients underwent pacemaker implantation (PMI), 5 with ventricular pacing mode (VVI) and 1 with atrial pacing mode (AAI), from age 1 day to 8 years due to severe bradycardia caused by AVB, inducing VT/TdP. In 6 cases, VT was completely suppressed after PMI. Only 2 patients had an implantable cardioverter-defibrillator (ICD) at ages 4 (LQT3) and 25 months (mutation negative), respectively, due to recurrent VT/TdP with satisfactory results.

Outcome

During the follow-up period of 8 days to 23.5 years (median, 4.25 years), 7 SCD and 7 ACA were registered (age at SCD or ACA range, 8 days to 10 years; median, 10.5 months); 6 did not have genetic testing, whereas 4 showed no mutation. Only 4 were genetically confirmed (Table 3). One case was later suspected to be LQT8, based on the phenotype including syndactyly. Among the 14 SCD/ACA cases, 12 had been under pharmacotherapy, 5 with both β-blockers and sodium channel blockers, and 2 had had PM or ICD. Four cases developed significant neurological deficits after cardiorespiratory resuscitation.

Discussion

The noteworthy finding of the present study was that 49 of 58 cases (84%) were diagnosed at the fetal or neonatal period, although this survey covered the entire infantile period. Remarkably, two thirds of the neonatal cases were diagnosed within 2 days of life; this period should be recognized as the most vulnerable period. The number of cases diagnosed after the neonatal period was only 9. Considering that the average age at appearance of symptoms in LQT2 and LQT3 is after

Table 2. Clinicogenetic Details

Case	LQT Type	Mutation	Age at Diagnosis/Sex	Clinical Presentation	FH	HR, bpm	QTc, ms
1	LQT1	Thr587Met	Fetus/M	FH, brady	+	109	561
2	LQT1	Ala341Val	Fetus/M	Brady	+	110	590
3	LQT1	Ala341Val	Neonate/M	FH	+	110	520
4	LQT1	Ile313Lys	Neonate/M	FH	+	102	589
5	LQT1	Ile313Lys	Neonate/M	FH	+	115	554
6	LQT1	276delSer	Neonate/M	Prolonged QT	+	115	570
7	LQT1	Asp611Tyr	Neonate/M	Brady	+	80	550
8	LQT1	Asp611Tyr	Neonate/F	FH	+	ND	ND
9	LQT1	Thr458Met	Neonate/M	FH	+	126	530
10	LQT1	Gly643Ser	Infant/M	ACA	−	109	554
11	LQT1	Gly269Ser	Infant/F	Cyanosis	−	113	586
					82%	109±12	560±24
12	LQT2	Gly628Ser	Fetus/M	VT/TdP, AVB	−	50	631
13	LQT2	del(7)(q32qter)	Fetus/F	TdP	−	111	492
14	LQT2	Ser243+112X	Fetus/F	FH	+	160	360
15	LQT2	Gly628Ala	Fetus/F	Syncope, VT/TdP, AVB	+	78	570
16	LQT2	Thr613Met	Fetus/M	VT/TdP, AVB	−	60	578
17	LQT2	Ala561Val	Neonate/M	Cyanosis, VT/TdP	−	86	520
18	LQT2	Gly628Ser	Neonate/M	TdP, brady	−	111	550
19	LQT2	Thr613Met	Neonate/M	convulsion, VT	−	140	599
20	LQT2	Gly572Ser	Neonate/F	TdP, AVB	−	91	520
21	LQT2	Ala614Val	Neonate/F	Syncope, VT	+	98	500
22	LQT2	Asn633Ser	Infant/F	VT/TdP, AVB	−	60	600
					27%	95±34	538±74
23	LQT3	Ala1186Thr	Fetus/M	AVB	+	78	679
24	LQT3	Asn1774Asp	Fetus/M	convulsion, VT/TdP, AVB	−	115	670
25	LQT3	Val176Met	Neonate/F	TdP, AVB	+	63	600
26	LQT3	Asn406Lys	Neonate/M	Syncope, TdP	+	129	598
27	LQT3	Arg1623Gln	Neonate/F	Heart failure	−	79	483
28	LQT3	Leu1772Val	Infant/M	ACA	−	138	520
					50%	100±31	592±79
29	LQT8	Gly406Arg	Neonate/M	AVB	−	141	581
30	Unidentified	−	Fetus/F	Brady	+	80	554
31	Unidentified	−	Fetus/M	Brady	−	100	510
32	Unidentified	−	Fetus/M	VT	−	85	590
33	Unidentified	−	Fetus/M	AVB	−	80	600
34	Unidentified	−	Neonate/F	Syncope	−	100	647
35	Unidentified	−	Neonate/F	Arrhythmia	−	126	586
36	Unidentified	−	Neonate/F	ACA	−	111	638
37	Unidentified	−	Neonate/M	Brady	−	93	550
38	Unidentified	−	Neonate/F	FH	+	120	520
39	Unidentified	−	Infant/F	ACA	−	160	470
40	Unidentified	−	Infant/F	ACA	−	100	774
41	Unidentified	−	Infant/F	PAC with block	−	60	460
					17%	104±32	575±86

(Continued)

Cases 2, 12, and 27 are reported in references 16, 17, and 23, respectively. ACA indicates aborted cardiac arrest; AVB, atrioventricular block; BB, β-blocker; brady, bradycardia; FH, family history; HR, heart rate; ICD, implantable cardioverter-defibrillator; Isp, isoproterenol; Lido, lidocaine; Mexil, mexiletine; Mg, magnesium; Nifed, nifedipine; PAC, premature atrial contraction; Pheny, phenytoin; PM, pacemaker; SCD, sudden cardiac death.

Table 2. Continued

Sinus Brady	VT/TdP	AVB	Acute Therapy	Maintenance Therapy	PMI/ICD	Follow-Up	Outcome
+	–	+	–	–	–	0 mo	Alive
+	–	–	–	BB	–	9 y	Alive
+	–	–	–	BB	–	4 y, 1 mo	Alive
+	–	–	–	BB	–	11 y, 10 mo	Alive
+	–	–	–	BB	–	10 mo	Alive
+	–	–	–	–	–	11 mo	Alive
+	–	–	–	–	–	7 y, 3 mo	Alive
+	–	–	–	–	–	5 y, 8 mo	Alive
–	–	–	–	–	–	4 y, 5 mo	Alive
+	–	–	Lido, Mexil	Mexil	–	9 y, 1 mo	Alive
+	–	–	–	–	–	7 y, 8 mo	Alive
73%	0%	9%				Median 68 mo	
+	+	+	Lido, Mg, BB, Mexil, Pacing	BB, Mexil	PM	3 y	Alive
+	+	–	–	BB	–	1 y	Alive
–	–	–	–	BB	–	2 y, 2 mo	Alive
+	+	+	Lido, Mg, BB, Mexil, pacing	BB, Mexil	PM	8 y, 1 mo	Alive
+	+	+	Mg, Mexil	BB, Mexil	–	8 mo	Alive
+	+	–	Lido, Mg, Mexil	BB, Mexil	–	11 y, 4 mo	Alive
+	+	+	Mexil	BB, Mexil	–	7 mo	Alive
–	+	–	Mg, BB	BB	–	8 y	Alive
+	+	+	Pheny	BB, Mexil	–	18 y, 5 mo	Alive
+	+	–	Pheny, DC	Pheny, BB	–	23 y, 6 mo	Alive
+	+	+	–	BB, Mexil	PM	15 y, 4 mo	Alive
82%	91%	55%				Median 96 mo	
+	+	+	Mexil	Mexil	PM ICD	3 y, 4 mo	Alive
+	+	+	BB, Mexil, Mg	BB, Mexil, Flecainide	PM	11 y, 4 mo	Alive
+	+	+	Lido, Mg, BB, Mexil	BB, Mexil	–	1 y, 3 mo	Alive
+	+	–	Lido, BB	BB, Mexil	–	11 mo	Alive
+	+	+	BB, Mexil, Lido	BB, Mexil	PM	8 y	Alive
–	+	+	Mg, BB, Mexil	BB, Mexil	–	3 y, 2 mo	Alive
83%	100%	83%				Median 39 mo	
–	+	+	BB, Mexil, Nifed	BB, Mexil, Nifed	–	3 y, 2 mo	Alive
+	–	+	–	BB, Mexil	–	2 y, 5 mo	Alive
+	–	–	–	BB	–	6 y, 5 mo	Alive
+	+	–	Lido, Mg	Mexil	–	5 y, 5 mo	Alive
+	–	+	BB, Mexil, Mg	BB, Mexil	–	4 mo	Alive
+	–	–	Lido, Mg, Isp	Mexil	–	4 y, 3 mo	Died
+	+	–	BB, Mg	BB	–	9 y, 5 m	Alive
–	+	–	Lido, BB, pheny, Mexil	Mexil	–	11 y, 9 mo	Alive
+	–	–	–	–	–	9 y, 6 mo	Alive
–	–	–	–	–	–	6 mo	Alive
–	+	–	BB, Mexil	BB, Mexil	ICD	7 y, 2 mo	Alive
+	+	+	Mexil	Mexil	–	4 y3 mo	Alive
+	–	–	BB, Mexil	BB, Mexil	–	7 y, 5 mo	Alive
75%	42%	25%				Median 71 mo	

Table 3. Clinicogenetic Details of Cases With Sudden Cardiac Death or Aborted Cardiac Arrest

Case	Case No. in Table 2	Genotyping	Age at Diagnosis	Age at SCD or ACA	HR, bpm	QTc, ms	Maintenance Therapy Until SCD/ACA	Acute Therapy for SCD/ACA Event
1	23	LQT3 (Ala1186Thr)	Fetus (28 wk)	1 y, 10 mo (aborted)	78	679	Mexil	Mexil, DC
2	...	No gene test	Fetus (31 wk)	8 d	60	570	...	Lido, Isp, Pacing, DC
3	...	No gene test	Fetus (36 wk)	57 d	90	600	BB, Mexil	DC
4	29	LQT8 (Gly406Arg)	Neonate (0 d)	1 y, 5 mo (aborted)	141	581	BB, Nifed	Mexil, Mg
5	...	Negative result	Neonate (0 d)	4 y	100	647	Mexil	DC
6	...	Negative result	Neonate (0 d)	<1 mo (aborted)	111	638	Mexil	Lido, Mexil, BB, Pheny
7	17	LQT2 (Ala561Val)	Neonate (1 d)	10 y (aborted)	86	520	BB, Mexil	Lido, Mexil, Mg, DC
8	...	No gene test (possible LQT8)*	Neonate (1 d)	57 d	70	640	BB	...
9	...	No gene test	Neonate (4 d)	5 y, 4 mo	60	590	... (refused)	...
10	...	No gene test	Infant (1 mo)	2 y	130	640	BB, Mexil	Lido, Mg
11	...	No gene test	Infant (1 mo)	1 y, 10 mo	60	740	BB, Mexil, PM	Lido, Mexil, BB, Mg, Pacing
12	10	LQT1 (Gly643Ser)	Infant (1 mo)	1 mo (aborted)	109	554	Mexil	Lido
13	39	Negative result	Infant (2 mo)	4 mo (aborted)	160	470	BB, Mexil, ICD	(aborted by ICD)
14	40	Negative result	Infant (2 mo)	2 mo (aborted)	100	774	Mexil	Mexil
				median 10.5 mo	97±31	617±81		

ACA indicates aborted cardiac arrest; BB, β-blocker; ICD, implantable cardioverter-defibrillator; Isp, isoproterenol; Lido, lidocaine; Mexil, mexiletine; Mg, magnesium; Nifed, nifedipine; Pheny, phenytoin; SCD, sudden cardiac death.

*LQT8 was retrospectively possible because phenotype included syndactyly.

school age,[2] we speculate a considerable number of patients are considered to go through infancy uneventfully.

Garson et al[4] reported 287 patients with LQTS age <21 years; their mean±SD age at presentation was 6.8±5.6; and 9% presented with cardiac arrest, 26% with syncope, and 10% with seizures. Although 20% of their subjects were <1 month of age, they did not investigate that age group separately. In the present study, confined to the subjects age <1, clinical features were largely different; that is, the incidence of malignant arrhythmias and bradycardia was high[6,7] whereas that of syncope and seizures was low.

Regarding genotype-phenotype correlations, Zareba et al[18] investigated child and adult LQTS and reported that LQT1 was associated with the highest risk of first cardiac event among the 3 most typical genotypes (LQT1–3). By the age of 15, syncope, ACA, or SCD was noted in 53% of their patients with LQT1 compared with 29% of LQT2 and 6% of LQT3, although cardiac events occurred in LQT3 were more lethal compared with those in LQT1 or LQT2. In contrast, the present study demonstrated that patients complicated by VT/TdP or AVB were almost exclusively those with LQT2 or LQT3 (and LQT8). LQT3 patients in the present study showed the most severe clinical course, similar to those in later-presenting LQT3. Further, patients with LQT1 mostly showed an uneventful clinical course apart from sinus bradycardia,[6] and the reason for diagnosis was bradycardia or prolonged QT interval itself on ECG identified on family screening. Another remarkable feature in our young age group was that a considerable number of patients with malignant arrhythmias were mutation-negative as far as LQT1–3 genes were typically examined. This suggests that this age group includes individuals with rare known mutations that were not examined in the present study as well as those with currently unidentifiable mutations.

Table 4. Comparison of Parameters Among the Groups

Parameter	LQT1 (n=11)	LQT2 (n=11)	LQT3 (n=6)	Negative (n=12)	Global Test	Pairwise Comparison
HR, bpm	109±12 (n=10*)	95±34	100±31	104±32	NS	
QTc, ms	560±24 (n=10*)	538±74	592±79	575±86	NS	
Proportion with family history, %	82	27	50	17	$P<0.05$	LQT1–Negative, $P<0.05$
Proportion with sinus bradycardia, %	73	82	83	75	NS	
Proportion with VT/TdP, %	0	91	100	42	$P<0.05$	LQT1–LQT2, $P<0.001$ LQT1–LQT3, $P<0.005$
Proportion with AVB, %	9	55	83	25	$P<0.05$	(LQT1–LQT3, $P=0.068$)

Data are mean±SD or %. One-way ANOVA was used to compare mean values of HR and QTc. χ^2 test was used to test differences in proportions of subjects with family history, sinus bradycardia, VT/TdP, and AVB among the 4 groups. Pairwise comparisons were conducted using Bonferroni adjustment and Bonferroni inequality of P value. NS indicates not significant; Negative, gene mutation-negative group.

*No. of cases is 10 because data were not available in 1 case.

Notably, many patients in the present study showed sinus bradycardia, although HR was not significantly different among LQT1, LQT2, and LQT3. Sinus bradycardia has been considered a significant presentation of LQTS, especially in the fetal-neonatal period,[3,19,20] and is often a clue to the diagnosis of LQTS. The present study verified that sinus bradycardia is common among all types of LQTS in this age group, especially in fetal-neonatal periods.

Another remarkable feature of the present study was the high incidence of AVB (55% in LQT2, 83% in LQT3), compared with 5% or less in child or adult LQTS.[4,20] It is intriguing that mutations in our LQT2 patients were almost exclusively located at the pore region of HERG gene (amino acid residues 550 through 650),[21] as mutations in that region are related to high risk for cardiac events.[21,22] Lupoglazoff et al[6] reported similar phenotype tendency for neonates with LQTS, that AVB is associated with LQT2 and sinus bradycardia with LQT1. Most of their LQT2 cases also had a mutation in the pore region of the HERG gene, although this was not mentioned in their report. AVB in neonates with an SCN5A mutation have also been reported in single case reports.[8,11,23,24] Considering the implication of sodium channel dysfunction in many other hereditary arrhythmias,[25] the association between LQT3 and AVB is an important finding.

SCD/ACA was seen in 14 cases (24% of all subjects) (7 SCD, 7 ACA), even though 12 of them were under treatment with β-blockers, mexiletine, or both when the events occurred (Table 3). The direct trigger of SCD/ACA remains to be determined, but the mean QTc interval of those patients was apparently prolonged (617±81 ms), and patients with no gene test (6 cases) were included as well, possibly making the selection of drugs inappropriate, such that only β-blockers were given to a possible LQT3 patient. Furthermore, 4 other cases had no known mutation on genotyping. It is possible that the cryptogenic mutations unidentifiable in the current era could be resistant to many drugs.

Therapy

Because individuals with LQT3 showed serious clinical disorders, they were treated aggressively with multiple antiarrhythmic drugs including mexiletine, β-blockers, lidocaine, Mg, and PM/ICD, and only 1 definite LQT3 patient showed ACA. For LQT2, malignant arrhythmias were a little more controllable with the same kind of pharmacotherapy than for LQT3. Again, only 1 definite LQT2 patient showed ACA. Thus, no death was ultimately observed in LQT2 and LQT3. This favorable clinical course might be derived from implicit strategy prevalent among pediatric cardiologists in our country that early-onset LQTS should be treated with the combination of β-blockers and mexiletine at the start of therapy because the genotype is not easy to confirm immediately. In other words, treatment strategies in Japan have been driven more by the clinical symptoms than by the genotype. Nevertheless, the response to the multiple antiarrhythmic pharmacotherapy and the long-term outcome presented in this study are encouraging.

It should be noted that the number of patients who underwent PM/ICD was small in the present cohort compared with other reports.[5,6] It is known that TdP tends to follow a prolonged R-R interval in LQT2 and LQT3, in which conduction disturbances or sinus node dysfunction are common features.[25,26] Thus, PM/ICD should be considered without delay even when the patient who shows drug-resistant, bradycardia-induced VT/TdP is a small baby.[27]

Study Limitations

Because of the retrospective nature of the present survey using questionnaires, the extent of clinical data that could be obtained varied among cases. Although approximate tendency in genotype-phenotype correlations for infants with LQT1, LQT2, and LQT3 was determined, most cases registered in the present study did not undergo genetic analysis for genes other than the 3 typical types. One case with LQT8 was registered in addition to LQT1–3, but no cases with the other types (LQT4–7) were found. Also, decision of treatment strategy depended on the in-charge physician in each case without the use of a uniform protocol for VT/TdP and/or AVB, making it difficult to evaluate the effects of pharmacotherapy and to determine the event rate beyond infancy for each genotype other than the last outcome, alive or death. Therefore, we should wait for accumulation of more cases for establishment of the genotype-specific strategy.

Conclusion

Our nationwide survey indicates that early-onset malignant LQTS are mostly those with LQT2 and LQT3 among the 3 major genes, and the most vulnerable age to life-threatening arrhythmias is from 0 to 2 days of age. A combination pharmacotherapy with a β-blocker and mexiletine sometimes combined with Mg and PM/ICD is recommended as the initial therapy. Prospective study of a large number of patients with LQTS diagnosed from fetal to infantile periods and further application of gene testing are needed to establish the most appropriate treatment strategies for those patients.

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Disclosures

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References

1. Moss AJ, Kass RS. Long QT syndrome: from channels to cardiac arrhythmias. *J Clin Invest.* 2005;115:2018–2024.
2. Priori SG, Napolitano C, Schwartz PJ, Grillo M, Bloise R, Ronchetti E, Moncalvo C, Tulipani C, Veia A, Bottelli G, Nastoli J. Association of