glazoff et al[5] demonstrated that in neonates that, while LQTS with 2:1 AVB is associated with *KCNH2* mutations, sinus bradycardia-related LQTS is associated with *KCNQ1* mutations. In 9 of 10 cases, 2:1 AVB-induced LQTS could be caused by LQTS-related gene mutations. In contrast, Chevalier et al found 4 K^+ channel gene mutations in 5 of 29 adult patients with AVB-induced LQTS (17.3%).[6] Our cohort also consisted of adult LQTS patients, with a mutation rate of 28.6%. This prevalence rate was similar to Chevalier's report, but lower than that in the 2:1 AVB-related LQTS in neonates. These studies have shown that AVB-induced LQTS in neonates has a stronger genetic association than AVB-induced LQTS in adults. Regarding the diagnostic rate of genetic testing in general, no candidate mutations could be detected in 30–40% of congenital LQTS cases. In contrast, it has been shown recently that genetic polymorphisms modify the QT interval.[18–24] Although we did not check polymorphisms in the present study, it is possible that our subjects might have some modifier-gene mutations. Thus, it remains possible that the remaining 10 patients in our study without apparent genetic variants may have as yet unknown variants.

In our cohort, it was difficult to prove the efficacy of β-blockers because very few patients were taking these drugs. In order to investigate the efficacy of β-blockers it will be necessary to study more cases with AVB-induced TdP. The first step in the treatment of all patients with AVB-induced TdP is the implantation of a device. Although PM implantation as first-line therapy for AVB-induced TdP is not disputed, 3 of our patients had a recurrence of TdP after the device was implanted, because of inadequate ventricular pacing, suggesting that AVB patients with TdP require strict PM management. In cases of persistent QT prolongation, even after PM therapy, it might become necessary to consider ICD implantation.

Several AVB-related gene mutations have been functionally assayed:[6] 3 *KCNH2* mutations, R328C, R696C and R1047L, were shown to have no strong dominant-negative effects on I_{Kr}. Another *KCNE2* mutation (R77W), which was identified in an AVB patient while taking flecainide, exerted no effects on I_{Kr}. Overall, previous analyses of mutations have shown them to cause only mild functional change. Our study showed similar results; all 4 mutants displayed loss of function associated with decreased densities on I_{Ks} or I_{Kr}, which were basically similar to those in congenital LQTS. On average, our patients experienced TdP at 57 years of age, which is older than the mean age of onset reported for those with congenital LQTS. Mutation carriers, who remain asymptomatic well into adulthood, may incidentally have fatal events in the presence of additional triggers, such as AVB.[25]

Several mutations of *SCN5A*, coding the α-subunit of Na^+ channels, have been found in newborn and infant cases of long QT.[26–28] They showed functional 2:1 AVB caused by profound QT prolongation. Therefore, the pathological basis differs between those cases and ours. Irrespective of genetic testing results, our patients who developed TdP in the presence of AVB showed QT prolongation, even in sinus rhythm. Thus, AVB may not be directly associated with QT prolongation, but the bradycardia caused by AVB enhances it and eventually leads to TdP. Our computer simulation study showed that, at a slower heart rate, APD lengthened significantly, suggesting that AVB-related bradycardia could exacerbate QT prolongation.

Study Limitation

Female sex is a predisposing factor for the development of cardiac arrhythmic events in patients with congenital and acquired LQTS, as previous reports have demonstrated.[29–31] In our study, almost all patients (93%) were also female, and therefore it would be possible that not only AVB but female sex affected cardiac repolarization and ventricular irritability in our cohort.

Conclusion

This study showed that incidental AVB as a trigger of TdP could manifest as clinical phenotypes of LQTS, and that some patients with AVB-induced TdP could have genetic backgrounds associated with congenital LQTS-related genes.

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KCNE2 modulation of Kv4.3 current and its potential role in fatal rhythm disorders

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BACKGROUND The transient outward current I_{to} is of critical importance in regulating myocardial electrical properties during the very early phase of the action potential. The auxiliary β subunit *KCNE2* recently was shown to modulate I_{to}.

OBJECTIVE The purpose of this study was to examine the contributions of *KCNE2* and its two published variants (M54T, I57T) to I_{to}.

METHODS The functional interaction between Kv4.3 (α subunit of human I_{to}) and wild-type (WT), M54T, and I57T *KCNE2*, expressed in a heterologous cell line, was studied using patch-clamp techniques.

RESULTS Compared to expression of Kv4.3 alone, co-expression of WT *KCNE2* significantly reduced peak current density, slowed the rate of inactivation, and caused a positive shift of voltage dependence of steady-state inactivation curve. These modifications rendered Kv4.3 channels more similar to native cardiac I_{to}. Both M54T and I57T variants significantly increased I_{to} current density and slowed the inactivation rate compared with WT *KCNE2*. Moreover, both variants accelerated the recovery from inactivation.

CONCLUSION The study results suggest that *KCNE2* plays a critical role in the normal function of the native I_{to} channel complex in human heart and that M54T and I57T variants lead to a gain of function of I_{to}, which may contribute to generating potential arrhythmogeneity and pathogenesis for inherited fatal rhythm disorders.

KEYWORDS Cardiac arrhythmia; M54T variation; I57T variation; *KCNE2*; Kv4.3; Sudden cardiac death

ABBREVIATIONS CHO = Chinese hamster ovary; **HERG** = human ether-a-go-go related gene; **WT** = wild type

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Introduction

Classic voltage-gated K^+ channels consist of four pore-forming (α) subunits that contain the voltage sensor and ion selectivity filter[1,2] and accessory regulating (β) subunits.[3] *KCNE* family genes encode several kinds of β subunits consisting of single transmembrane-domain peptides that co-assemble with α subunits to modulate ion selectivity, gating kinetics, second messenger regulation, and the pharmacology of K^+ channels. Association of the *KCNE1* product minK with the α subunit Kv7.1 encoding *KCNQ1* forms the slowly activating delayed rectifier K^+ current I_{Ks} in the heart.[4,5] In contrast, association of the *KCNE2* product MiRP1 with the human ether-a-go-go related gene (HERG) forms the cardiac rapid delayed rectifier K^+ current I_{Kr}.[6]

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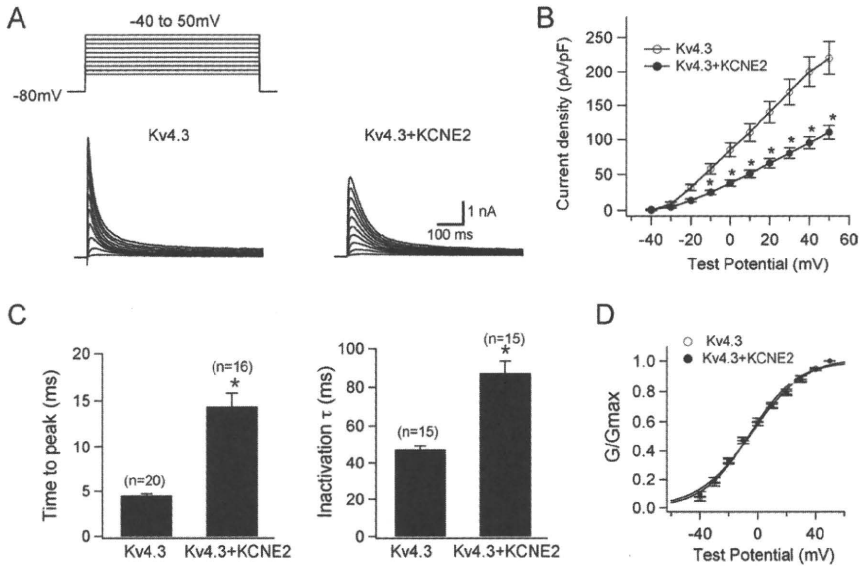

Figure 1 *KCNE2* co-expression with Kv4.3 produces smaller I_{to}-like currents with slower activation/inactivation kinetics. **A:** Representative current traces recorded from Chinese hamster ovary (CHO) cells expressing Kv4.3 **(left)** and Kv4.3 + *KCNE2* **(right).** As shown in the **inset** in 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

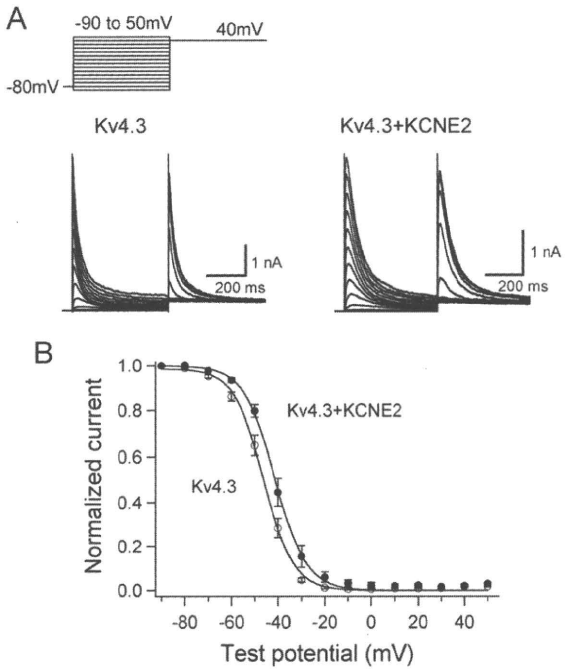

Figure 2 *KCNE2* co-expression with Kv4.3 causes a positive shift of voltage dependence of steady-state inactivation. **A:** Representative Kv4.3 and Kv4.3 + *KCNE2* current traces induced by 500-ms pulses (P1) from −90 to +50 mV applied from the holding potential −80 mV in 10-mV steps followed by a 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]

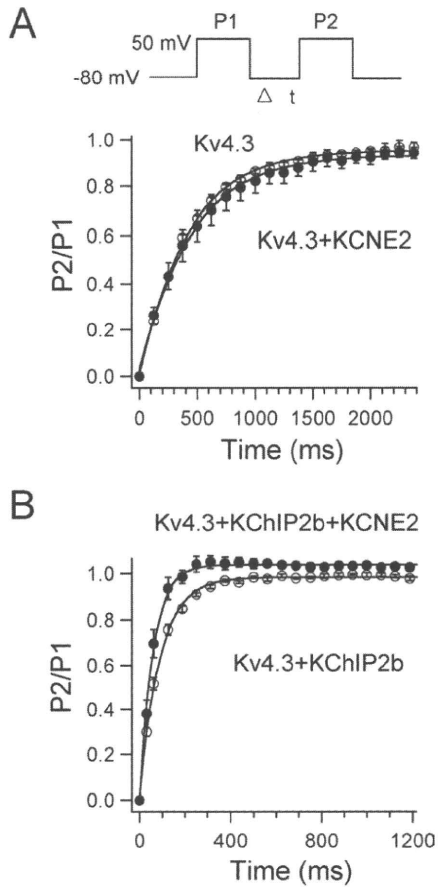

Figure 3 Effects of *KCNE2* co-expression on recovery from inactivation of Kv4.3 **(A)** and Kv4.3 + KChIP2b **(B)** currents. Recovery from inactivation was assessed by a two-pulse protocol **(A, inset):** a 400-ms test pulse to +50 mV (P1) followed by a variable interval at −80 mV, then by a second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

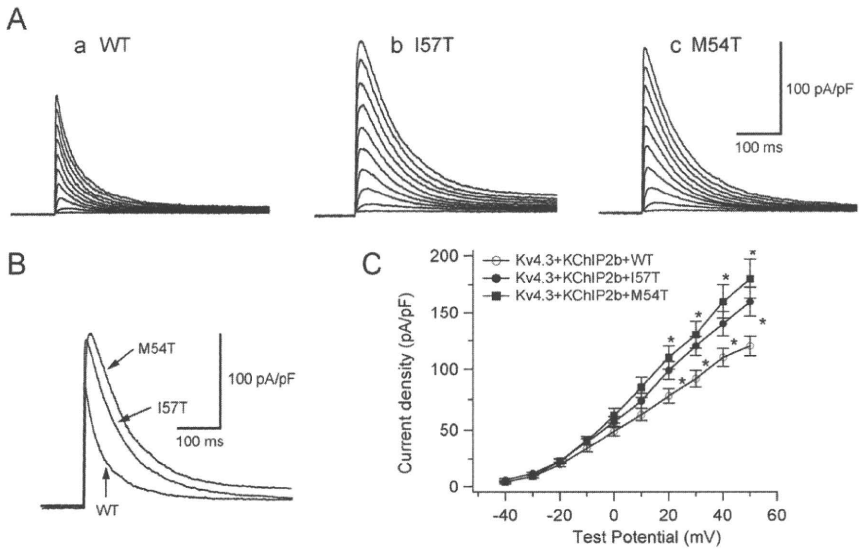

Figure 4 Two *KCNE2* transmembrane variants, I57T and M54T, increase the reconstituted Kv4.3 + KChIP2b channel current and slow its inactivation. **A:** Three sets of current traces elicited by depolarizing pulses for 500 ms from a holding potential of −80 mV to potentials ranging between −40 and +50 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).

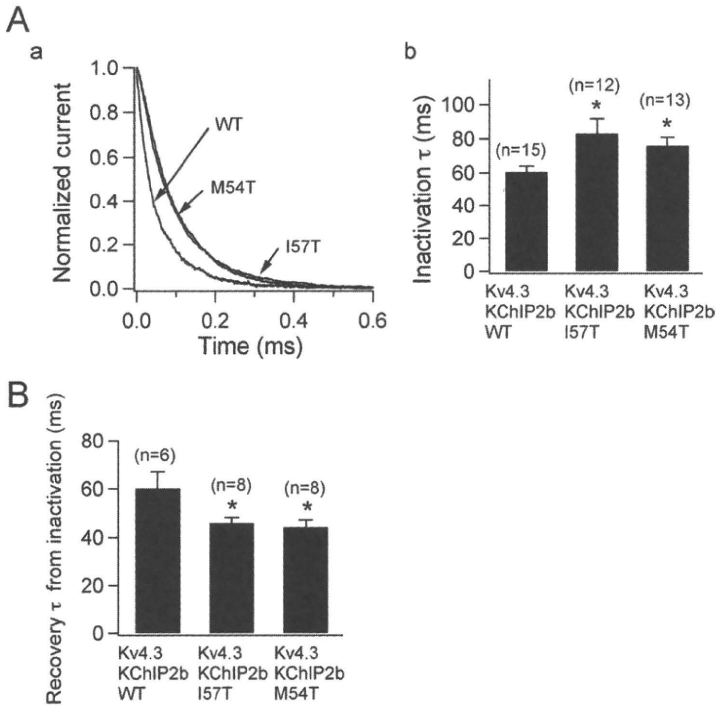

Figure 5 Two *KCNE2* variants slow inactivation kinetics and accelerate recovery from inactivation. **A, a:** Three current traces obtained from Chinese hamster ovary (CHO) cells transfected with wild-type (WT), I57T, and M54T *KCNE2* variant co-expressed with Kv4.3 and KChIP2b. Traces, which are normalized 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]

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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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CLINICAL PERSPECTIVE

The congenital long-QT syndrome (LQTS) diagnosed at perinatal life and through infancy is associated with high morbidity and mortality rates. However, data on the clinical presentation and genotype-phenotype correlation of this youngest age group of LQTS are limited. A nationwide survey was conducted in Japan, and 58 cases (18 fetuses, 31 neonates and 9 infants) were registered. Among them, the peak age at diagnosis was 0 to 2 days, and the 3 most frequent clinical presentations included sinus bradycardia, ventricular tachycardia/torsades de pointes, and atrioventricular block. The genotype was confirmed in 29 (71%) of 41 patients who underwent genotyping; the incidence resembled that of child LQTS. Patients who presented with early-onset ventricular tachycardia/torsades de pointes and atrioventricular block were almost exclusively those with LQT2 and LQT3 among the 3 major genes, but a considerable number of genetically unidentified ones were included. Sudden cardiac death/aborted cardiac arrest were prevalent in the latter. LQT1 patients tended to show only sinus bradycardia or positive family history of LQTS. These results mean that many life-threatening episodes observed in early-onset LQTS should be treated immediately and aggressively even without knowledge of the genotype. On the other hand, the present study was encouraging in that the outcome of patients was favorable with multiple pharmaceutical agents, typically with β-blockers, mexiletine, and magnesium and with pacemaker implantation/ implantable cardioverter-defibrillator, independent of the genotype. Further application of gene testing is needed to establish the most appropriate genotype-specific strategy for these patients.

P wave and the development of atrial fibrillation

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BACKGROUND Terminal P-wave inversion in lead V_1 representing left atrial overload has been considered a precursor of atrial fibrillation (AF).

OBJECTIVE The purpose of this study was to determine whether this P-wave morphologic characteristic can predict the development of AF.

METHODS Digital analysis of 12-lead ECGs was performed to enroll patients with P terminal force ≥0.06 s × 2 mm in lead V_1 from among a database of 308,391 ECG recordings. The prognostic value of ECG characteristics for developing AF was determined.

RESULTS A total of 78 patients (mean age 52 ± 19 years) with left atrial overload were chosen from among 102,065 patients in the database. During mean follow-up of 43 months, 15 (19%) patients developed AF (AF group) versus 63 (81%) patients who did not (non-AF group). No significant difference was noted between the AF and non-AF groups with regard to the area, duration, and amplitude of the P-wave terminal portion in lead V_1. In contrast, the area, duration, and amplitude of the P-wave initial portion in the same lead were significantly greater in the AF group than in the non-AF group (114.6 ± 73.0 μV × ms vs 73.1 ± 59.3 μV × ms, 42.2 ± 12.4 ms vs 35.7 ± 10.1 ms, and 94.0 ± 39.9 μV vs 68.8 ± 49.4 μV, respectively; $P < .05$ for each). Multivariate analysis confirmed that the area of the P-wave initial portion was independently associated with the development of AF (hazard ratio 4.02, 95% confidence interval 1.25–17.8; $P = .018$).

CONCLUSION P-wave initial portion in lead V_1 was an independent risk stratifier of AF development in patients with marked left atrial overload.

KEYWORDS Atrium; Electrocardiography; Fibrillation; Prognosis

ABBREVIATIONS **AF** = atrial fibrillation; **CI** = confidence interval; **ECG** = electrocardiogram; **LA** = left atrium; **RA** = right atrium

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Introduction

The P wave reflects electrical depolarization of both the right atrium (RA) and the left atrium (LA). When the P wave is biphasic in lead V_1, the positive initial portion and the negative terminal portion of the P wave represent depolarization of the RA and the LA, respectively.[1,2] Morris et al[3] reported that the magnitude of the negative terminal potion of the P wave, calculated as the algebraic product of the duration and amplitude (P terminal force) in precordial lead V_1, was significantly larger in patients with various valvular heart diseases than in normal subjects. In their study, the P terminal force was associated with mitral valve area and increased LA pressure. The magnitude of the P terminal force has been shown to be associated with LA enlargement as revealed by transthoracic echocardiography.[4,5] These findings suggest that the negative terminal potion of the P wave in lead V_1 is a sign of pressure and volume overload in the LA, which may lead to structural and functional remodeling in the LA. Because atrial fibrillation (AF) often occurs and/or recurs in the remodeled LA,[6] the increased P terminal force may underlie the generation of AF. The increased P terminal force is observed not only in valvular heart diseases but also in other heart diseases, including hypertension, myocardial infarction, and cardiomyopathy.[7,8] These disorders potentially underlie the generation of AF. However, little is known about whether P terminal force occurring in those disorders is associated with a prognostic risk for the development of AF. Prolonged P-wave duration is a useful predictor of AF development.[9,10] The signal-averaged P-wave electrocardiogram (ECG) has a significant role in identifying patients who are susceptible to paroxysmal AF and in predicting the progression from paroxysmal to permanent AF.[11] Measurement of signal-averaged P-wave duration requires a dedicated system, which is not widely available in general clinical practice. In contrast, standard 12-lead ECGs can be conveniently recorded, and automatic analysis of 12-lead ECG recordings yields information to clinicians. In our university hospital, more than 300,000 ECGs obtained from more than 100,000 patients are available for digital analysis. Using this large database, we performed a retrospective cohort study to investigate whether terminal P-wave inversion in lead V_1 predicts the development of AF.

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Methods

Database

We constructed a database for analyzing resting 12-lead ECGs recorded in our hospital, which is associated with the Shiga University of Medical Science. A total of 102,065 patients (49,286 females and 52,779 males) who had undergone ECG recordings between January 1983 and October 2008 were collected in our database, and a total of 308,391 ECG recordings were performed during this period. Twelve leads were simultaneously acquired. The 12-lead ECG was recorded for 10 seconds at a sweep speed of 25 mm/s and calibrated to 1 mV/cm in the standard leads. ECG signals were recorded at an interval of 2 ms (i.e., 500 Hz). Digital data were stored on a computer server with 12-bit resolution. From the database, patients who fulfilled ECG criteria of LA overload were chosen using the analysis software MUSE7.1 (GE Marquette Medical Systems, Inc., Milwaukee, WI, USA). Computer-processed ECGs defined LA overload criteria as follows. (1) ECGs displaying biphasic P wave in lead V_1 were chosen. (2) The P wave was divided into the positively deflected portion in the initial P wave and the negatively deflected portion in the terminal P wave. (3) The terminal P wave in lead V_1 with duration $\geq$0.06 second and amplitude ≤ -0.2 mV (i.e., P terminal force $\geq$0.12) was considered as meeting LA overload criteria in this study (Figure 1).

Study participants

From our database, 78 participants who had marked LA overload were selected and assessed for the development of AF. A control group of 234 participants who did not have LA overload also was selected (1:3 matching). Individual matching was performed accounting for confounders (age, gender, date when ECG was taken), and when control candidates numbered more than three, the three controls were chosen randomly from among the candidates. The research protocol was approved by the Ethical Committee of Shiga University of Medical Science (19–75).

Digital analysis of ECG

The MUSE7.1 software detected identical P waves using a template matching technique. A point that had an area $\geq$160 μV/ms from the baseline level was considered to be P-wave onset, and a point that had an area $\leq$160 μV/ms from the baseline level was considered to be P-wave offset. The duration, amplitude, and area of total P wave, initial P wave, and terminal P wave in lead V_1 were measured using matrix parameters available in MUSE7.1. P-wave area was constructed by integrating the duration and amplitude. Duration $\times$ amplitude of P-wave initial and terminal portions in lead V_1 were calculated as force values. These variables were composed using the average value of the P wave during 10 seconds of recording time. Because all measurements of 12-lead ECGs were performed digitally using MUSE7.1, neither intraobserver nor interobserver variability occurred in this study.

Statistical analysis

The occurrence of AF was set as an endpoint, and the prognostic factors for developing AF were explored in the analysis. Patients whose ECG exhibited AF during the follow-up period (AF group) were compared with patients who did not (non-AF group). The follow-up period was defined as the interval between the first day when an ECG with LA overload was recorded and the first day when an ECG displaying AF was recorded in the AF group, or the interval between the first day when an ECG with LA overload was recorded and the latest day when an ECG was recorded in the non-AF group. The occurrence of death from any cause during the follow-up period was assessed by mail questionnaire. Written informed consent was obtained from all patients. Data are given as mean $\pm$ SD or percentage, and group comparisons were made using t-test or Mann-Whitney test, as appropriate. Categorical variables were compared using the Fisher exact test. Comparison of AF occur-

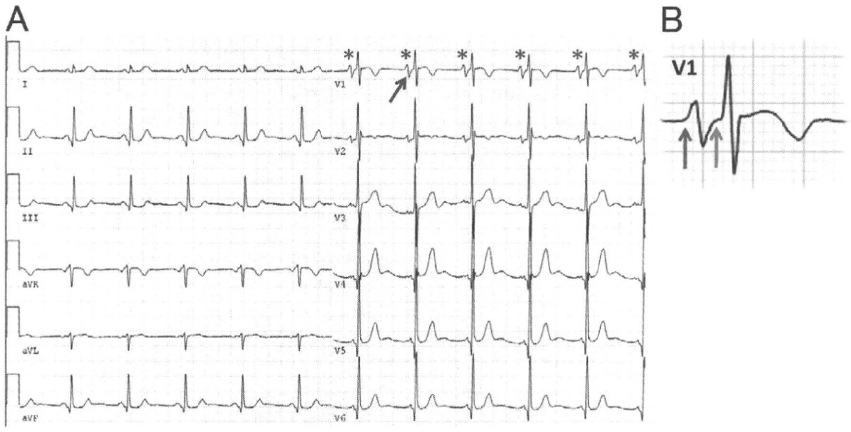

Figure 1 **A:** Twelve-lead ECG showing typical pattern of left atrial overload in lead V_1. *Red arrow* indicates P-wave negative terminal portion in lead V_1. *Asterisks* indicate P waves with identical morphology detected by template matching. **B:** Magnified ECG trace of lead V_1. *Blue arrow* indicates P-wave onset. *Green arrow* indicates P-wave offset.

rence between patients with LA overload and control patients was performed by logistic regression analysis and reported as odds ratio with 95% confidence interval (CI). Kaplan-Meier curves were used for determining the difference between two groups, and log rank test was used for examining the difference. Cox proportional hazard regression was used to estimate multivariate adjusted hazard ratios accounting for confounders (age, sex, cause of heart disease, ECG variables of P wave). All statistical tests were two-tailed, and $P < .05$ was considered significant.

Results

Atrial fibrillation

A total of 78 patients (mean age 52 ± 19 years) who fulfilled ECG criteria of marked LA overload were selected from our database using the GE Marquette 12SL ECG analysis program and enrolled for ECG analysis in this study. Of these patients, 15 (19%) developed AF (AF group), whereas 63 did not present AF (non-AF group). The control group consisted of 234 patients who were well matched for age (52 ± 19 years) and gender (78 women and 156 men; Table 1). AF developed in 3 (1.3%)of 234 control patients. The incidence of AF in patients with marked LA overload was 15-fold higher than that in control patients ($P < .001$). The odds ratio for occurrence of AF in patients with LA overload compared with control patients was 18.3 (95% CI 5.15–65.3). The mean follow-up period of the control patients was significantly longer than that of the patients with LA overload (78 ± 73 months vs 43 ± 52 months; $P < .001$). Kaplan-Meier survival analysis is shown in Figure 2. The AF-free event rate was significantly higher ($P < .001$) in patients with LA overload than in control patients (hazard ratio 24.5, 95% CI 7.94–107.3).

Characteristics of the patients

The clinical characteristics of patients in the AF and non-AF groups are listed in Table 2. The mean follow-up period of the AF group and non-AF group averaged 45 ± 61 months and 43 ± 50 months, respectively ($P = .93$). No significant difference with regard to age and sex was disclosed between the AF and non-AF groups. The average age at ECG documentation of AF was 59 ± 13 years. In the AF group, 14 (93%) of 15 patients had structural heart diseases such as hypertension, myocardial infarction, valvular heart diseases, and nonischemic cardiomyopathy. In contrast, structural heart disease was present in 46 (73%) of 63 patients in the non-AF group ($P = .081$). The presence of hypertension was more frequent in the AF group than in the non-AF

Table 1 Comparison of characteristics of control patients and patients with left atrial overload

	Control	Left atrial overload
No. of patients	214	78
Age (years)	52.4 ± 19.3	52.4 ± 19.3
Male [n (%)]	156 (66.7)	52 (66.7)
Follow-up period (months)	78.0 ± 72.9*	43.3 ± 52.0

Values are given as mean ± SD unless otherwise indicated.
*$P < .001$ vs patients with left atrial overload.

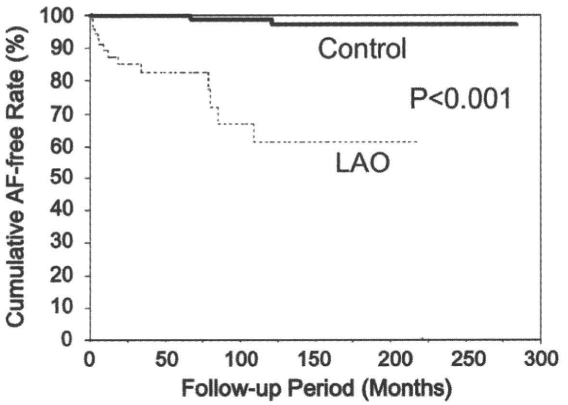

Figure 2 Kaplan-Meier estimates of atrial fibrillation (AF)-free event rate in patients with left atrial overload (LAO) and control patients. The difference between the two groups was significant ($P < .001$ by log rank test).

Table 2 Characteristics of the patients

Characteristic	AF group (n = 15)	Non-AF group (n = 63)	P value
Age (years)	55.8 ± 14.7	51.6 ± 20.3	.22
Gender (male/female)	10/5	42/21	1
Structural heart disease	14 (93)	46 (73)	.063
Hypertension	9 (60)	20 (31)	.045
Valvular heart disease	7 (47)	16 (25)	.12
Myocardial infarction	0 (0)	8 (13)	.06
Nonischemic cardiomyopathy	3 (20)	15 (24)	.66
Hypertrophic cardiomyopathy	3 (20)	7 (11)	.38
Dilated cardiomyopathy	0 (0)	8 (13)	.06
NYHA functional class I/II/III/IV	13/2/0/0	30/28/5/0	.80
Left ventricular ejection fraction (%)	63.2 ± 9.89	54.0 ± 18.5	.04
Antiarrhythmic drug			
Class IA	6	2	.01
Class IC	1	1	.32
Class III	1	0	.07
Diuretic	5	21	.70
Beta blocker	5	9	.32
Calcium antagonist	2	14	.44
Angiotensin II receptor blockade	1	2	.55
Angiotensin-converting enzyme inhibitor	3	8	.48
Nitrate	3	11	.81
Digitalis	5	15	.37
Oral anticoagulant	5	11	.14
Aspirin	3	5	.20

Values are given number, number (%), or mean ± SD.
AF = atrial fibrillation; NYHA = New York Heart Association.