Atrioventricular Block-Induced Torsades de Pointes With Clinical and Molecular Backgrounds Similar to Congenital Long QT Syndrome

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Background: Atrioventricular block (AVB) sometimes complicates QT prolongation and torsades de pointes (TdP).

Methods and Results: The clinical and genetic background of 14 AVB patients (57±21 years, 13 females) who developed QT prolongation and TdP was analyzed. Electrophysiological characteristics of mutations were analyzed using heterologous expression in Chinese hamster ovary cells, together with computer simulation models. Every patient received a pacemaker or implantable cardioverter defibrillator; 3 patients had recurrence of TdP during follow-up because of pacing failure. Among the ECG parameters, QTc interval was prolonged to 561±76 ms in the presence of AVB, but shortened to 495±42 ms in the absence of AVB. Genetic screening for KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 revealed four heterozygous missense mutations of KCNQ1 or KCNH2 in 4 patients (28.6%). Functional analyses showed that all mutations had loss of functions and various gating dysfunctions of Iks or Ik. Finally, action potential simulation based on the Luo-Rudy model demonstrated that most mutant channels induced bradycardia-related early afterdepolarizations.

Conclusions: Incidental AVB, as a trigger of TdP, can manifest as clinical phenotypes of long QT syndrome (LQTS), and that some patients with AVB-induced TdP share a genetic background with those with congenital LQTS. (Circ J 2010; 74: 2562–2571)

Key Words: Atrioventricular block; Ion channels; Long QT syndrome; Torsades de pointes

The acquired form of long QT syndrome (LQTS) is a major cause of torsades de pointes (TdP), which results from various factors, including drugs, bradycardia or hypokalemia. Regarding bradycardia, Kurita et al demonstrated that patients with bradycardia-induced TdP displayed abnormally prolonged QT intervals at slower heart rates (<60 beats/min) than those without TdP. Some groups have reported the genetic background of bradycardia-induced TdP, as well as congenital LQTS. In 2001, we reported a female with 2:1 atrioventricular block (AVB) and TdP, in whom the KCNH2 A490T mutant was identified as heterozygous. Subsequently, Lupoglazoff et al demonstrated that, in neonates, LQTS with 2:1 AVB is associated with KCNH2 mutations whereas sinus bradycardia-related LQTS is associated with KCNQ1 mutations. Chevalier et al reported that among 29 patients with complete AVB and a QT interval >600 ms, 5 (17%) had mutations on genes encoding K+ channels, and the expression test of these mutations showed functional changes compared with the wild-type (WT) K+ current.

In Japan, some papers on congenital LQTS have been published, but the molecular pathogenesis of AVB-related TdP has not been fully examined, particularly with respect to the relationship between genotype and cellular electrophysiology. The aim of this study was to investigate gene mutations and clarify their functional outcome in con-

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secutive AVB patients complicated with TdP.

Methods

Study Population
The study cohort contained 14 consecutive probands, from unrelated families, who showed a prolonged QT interval and TdP associated with AVB. They were referred to 3 institutes in Japan: Shiga University of Medical Science (Otsu), National Cardiovascular Center (Suita), and Kyoto University Graduate School of Medicine (Kyoto) for LQTS genetic testing between 1996 and 2008.

Clinical Characterization
In each case, we recorded 12-lead electrocardiograms (ECGs) before and after AVB episodes, as well as gathering the results from other cardiovascular examinations and detailed clinical evaluations. Prolonged QT interval was diagnosed by the presence of prolongation of ventricular repolarization (corrected QT interval [QTc] >460 ms in lead V5, according to Bazett’s formula). We excluded cases of TdP caused by AVB with drugs associated with QT prolongation, as well as those with active ischemia detected by noninvasive or invasive tests, including coronary angiography. We also investigated cardiac events in all 14 probands and their family members. Cardiac events were syncpe, TdP, ventricular fibrillation (VF), aborted cardiac arrest (requiring defibrillation) or sudden cardiac death. We also followed the therapies and clinical prognoses of these patients.

Genetic Analysis
Genomic DNA was isolated from venous blood by QIAamp DNA blood midikit (Qiagen, Hilden, Germany). Established primer settings were used to amplify the entire coding regions of the known LQTS genes (KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2). Denaturing high-performance liquid chromatography (WAVE system Model 3500, Transgenomic, Omaha, NE, USA) was performed as described elsewhere, and abnormal conformers were amplified by polymerase chain reaction (PCR), and sequenced with an ABI PRISM-3130 sequencer (Perkin-Elmer Applied Biosystems, Wellesley, MA, USA). If we detected mutations in these genes, family members associated with the probands were also genetically analyzed. Formal informed consent was obtained from each patient or their guardians according to standards approved by local institutional review boards.

Expression Plasmids
The expression plasmids, pRES2-EGFP/KCNQ1 (wild-type; WT/KCNQ1) and pRc-CMV/KCNH2 (WT/KCNH2) were kindly provided by Dr Barhanin (Université de Nice, Sophia Antipolis, Valbonne, France) and Dr Sanguinetti (University of Utah, Salt Lake City, UT, USA), respectively. The mutants were introduced using overlap PCR. The mutant plasmids were constructed by substituting the 838-bp XhoI-BglII for the G272V mutant, 464-bp HindIII-BstXI for the D111V mutant, 1458-bp BstXI-BglII for the A490T mutant, or 592-bp FseI-ShI fragments for the P846T mutant for the corresponding fragments of WT/KCNQ1 or WT/KCNH2. The nucleotide sequence of the construct was confirmed prior to the expression studies.

Electrophysiological Experiments
Whole-cell patch-clamp recordings were conducted at 37.0±1.0°C using an EPC-8 patch-clamp amplifier (HEKA, Lambrecht, Germany) 48–72 h after transfection. No leak subtraction was used. The normal Tyrode solution contained (in mmol/L): NaCl 140, KCl 5.4, CaCl2: 1.8, MgCl2: 0.5, NaH2PO4: 0.33, glucose 5.5, and HEPES 5 (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mmol/L): potassium aspartate 70, KCl 40, KH2PO4: 10, EGTA 5, MgSO4: 1, Na-ATP (Sigma, St Louis, MO, USA) 3, Li-GTP 0.1, and HEPES 5 (pH adjusted to 7.4 with KOH). A coverslip with adherent CHO cells was placed on the bottom of a glass recording chamber (0.5 mL in volume) mounted on the stage of an inverted microscope (TE2000-U, Nikon, Tokyo, Japan). Pipette resistance was 3–5 MΩ when filled with internal solution. Currents and voltages were digitized and voltage commands were generated through an LIH-1600 AD/DA interface (HEKA) controlled by PatchMaster software (HEKA). Current amplitude was divided by membrane capacitance (Cm) to obtain current densities (pA/pF) in each cell. The voltage-dependence of current activation was determined by fitting the normalized tail current (I_{tail}) vs test potential (V_{test}) to a Boltzmann function:

\[ I_{tail} = \frac{1}{1 + \exp\left(\frac{V_{test} - V_{0.5}}{k}\right)} \]

where V_{0.5} indicates the voltage at which the current is half-maximally activated and k is the slope factor.

Computer Simulation of Action Potential Duration (APD)
Ventricular action potentials were simulated by using the dynamic Luo-Rudy model with recent modifications. The ratio of I_{Kr} and I_{Ks} conductance was set at 23:1, 17:1, and 19:1 in the epicardium, endocardium, and M cell layer, respectively. Based on the experimental data of voltage-clamp recordings of KCNH2 channels heterologously expressed in CHO cells, we constructed Markov or Hodgkin-Huxley models for simulated mutant channels as compared with mutants associated with congenital LQTS. In order to construct mutant channel models, we decreased the conductance of each channel as appropriate for the decreased current density, and looked for adequate changes in mutant channels by changing each coefficient value, in turn, for gating states associated with impaired gating defects. The simulation for voltage-clamp experiments was calculated using the 4th-order Runge-Kutta method with a fixed-time step of Rockville, MD, USA) supplemented with 10% fetal bovine serum (Gibco-BRL) and antibiotics (100 U/mL penicillin and 100 μg/ml streptomycin) in a humidified incubator gassed with 5% CO2 and 95% air at 37°C. CHO cells were transiently transfected using 1 μg of WT/KCNQ1 or mutant/ KCNH1, and 1 μg of pRES-CD8/KCNE1 per 35-mm dish, using the LipofectAMINE method according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, USA). In some experiments, 0.5 μg of WT/KCNQ1 was transfected with or without mutant/KCNQ1, instead of 1 μg of WT/ KCNH1. Cells successfully transfected with both KCNQ1 and KCNE1 cDNA were selected by green fluorescent protein (GFP) and decoration with anti-CDB antibody-coated beads (Dynabeads CD8; Dynal Biotech, Oslo, Norway). The cells were transiently transfected with either WT/KCNH2 or mutant/KCNH2, using the LipofectAMINE method according to the manufacturer’s instructions. For a 35-mm dish the amount of plasmid was 2 μg and 0.175 μg of GFP; only GFP-positive cells were used for the patch-clamp study.
Table 1. Clinical Characteristics and Gene Mutations of Probands With Bradycardia-Induced Torsades de Pointes

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<th>ECG at AVB</th>
<th>HR (beats/min)</th>
<th>QTc (ms)</th>
<th>HR (beats/min)</th>
<th>QTc (ms)</th>
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**Results**

**Clinical Characteristics of the Patients in This Study**

The clinical characteristics of the 14 patients enrolled in the study are presented in Table 1. The mean age at the onset of AVB was 57±21 years, and 13 patients (92.8%) were females. All patients showed TdP with AVB: 10 had complete AVB, 3 had 2:1 AVB, and 1 had Wenckebach AVB. No patient had experienced syncope or ventricular arrhythmias prior to the appearance of TdP. One patient (case 6) in Table 1 had atrial fibrillation, mitral regurgitation, and complete AVB without prolonged QT interval, but no TdP.

In most patients with AVB-related TdP, the tachyarrhythmia started from premature ventricular contractions after a long-pause interval following ventricular arrhythmias, so-called “TdP from short–long–short pattern” (Figure 1D).13 The ECGs available at the time of AVB showed severely prolonged QT interval (heart rate 44±11 beats/min and QTc 561±76 ms). On the other hand, the ECGs without AVB available in 7 cases also showed a prolonged QT interval (heart rate 64±27 beats/min, P<0.05, and QTc 495±42 ms, P=NS, vs those in AVB). ECGs in sinus rhythm were obtained in 4 and 3 patients before and after AVB, respectively.

All patients underwent implantation of either an implantable cardioverter-defibrillator (ICD) or permanent pacemaker (PM), together with the administration of several drugs, including β-blockers (ICD n=3; PM n=11). Mean clinical follow-up during advanced therapy was 102±71 months. After the placement of a PM or ICD, 2 patients maintained own ventricular beats but the other 12 depended on ventricular pacing during the follow-up period. Three patients had recurrence of TdP even while receiving treatment. Patient no. 11 suddenly experienced repetitive TdP because of pacing failure and no. 13 also experienced TdP when her own ventricular beats had been set faster than the basal pacing rate. In patient 6, the reappearance of Wenckebach AVB without ventricular pacing caused ventricular tachycardia. In all 3 cases, no gene mutations were detected.

**Molecular Genetics and Clinical Characteristics of Patients With Gene Mutations**

The genetic analysis revealed different heterozygous mutations in 4 (28.6%) of 14 AVB-related TdP cases (Table 1): 1 KCNQ1 mutation, G272V, and 3 KCNH2 mutations, D111V, A490T and P846T (Figure 1A). All were located in the non-pore regions; G272V is located in the N-terminus, S2–S3 inner loop, and C-terminal domains for the KCNQ1 channel; D111V, A490T, and P846T are located in the N-terminus, S2–S3 inner loop, and C-terminal domains for the KCNH2 channel, respectively (Figure 1B). In the remaining 10 patients, we were unable to detect any mutations associated with the 5 major LQTS-related genes.
Figure 1. Molecular discovery and clinical data associated with KCNQ1 and KCNH2 mutations, and the initiation of atrioventricular block-related torsades de pointes (TdP). (A) Denaturing high-performance liquid chromatography patterns and DNA sequence data in normal controls and patients with G272V for KCNQ1 (Left), D111V for KCNH2 (Middle), and P846T for KCNH2 (Right). (B) Schemes showing the topology of cardiac ion channel proteins for KCNQ1 and KCNH2 and the location of mutations identified in this study. (C) Two pedigrees of G272V and D111V families. Circles and squares represent female and male family members, respectively; probands are indicated by arrows. Heterozygous carriers are represented as half-filled symbols, family members in whom no genetic data were available are shown by open symbols, and non-carriers by open symbols with N. QTc intervals corrected by Bazett’s formula in lead V5 are given for each available family member. (D) Representative ECG recordings from case 7 (76-year-old female with G272V-KCNQ1 mutation). TdP during 2:1 AV block started with so-called “short (!)–long (+)–short (#) pattern” which resulted in a long pause (+).
tion was identified in a 76-year-old female who did not have a particularly relevant family history (Figure 1A Left panel). For approximately 10 years, she had taken nilvadipine and gliclazide because of hypertension and diabetes mellitus. Approximately 1 year before hospitalization, her QTc interval was within normal range (424 ms). When she was admitted to hospital because of syncope, her monitoring ECGs displayed 2:1 AVB (50 beats/min), prolonged QTc interval (578 ms), and repetitive TdP (Figure 1D). Her serum K+ level was low (2.5 mEq/L). Because AVB persisted, she underwent DDD PM implantation. After correction of the serum K+ level and PM therapy, her QTc interval shortened and TdP disappeared. She was free from cardiac events for the following 59 months. The genetic analysis revealed 3 children as non-mutation carriers (Figure 1C Left panel).

D111V in KCNH2 (Case 4 in Table 1) The D111V mutation was identified in a 57-year-old female who did not have a particularly relevant family history (Figure 1A Middle panel). She experienced syncope after eating breakfast, and the monitoring ECG in the ambulance documented complete AVB (45 beats/min), prolonged QTc interval (578 ms) and TdP. After external PM therapy was initiated, TdP disappeared. She was free from cardiac events for the following 59 months. The genetic analysis revealed 3 children as non-mutation carriers (Figure 1C Left panel).

P846T in KCNH2 (Case 8 in Table 1) The P846T mutation was found in a 71-year-old female who did not have a particularly relevant family history (Figure 1A Right panel). She experienced syncope after breakfast, and the monitoring ECG in the ambulance displayed complete AVB (45 beats/min) and repetitive TdP with prolonged QT interval. On admission, her AV conduction resumed at 57 beats/min, but her QTc interval remained prolonged (729 ms). After ICD implantation, she was free from cardiac events for 46 months, but her QTc interval remained prolonged (489 ms). We did not conduct a genetic analysis in this family.

A490T in KCNH2 (Case 1 in Table 1) We have previously reported the clinical features of a A490T mutation identified in a 27-year-old female. Briefly, her 12-lead ECG showed severe bradycardia because of 2:1 AVB (50 beats/min) with complete left bundle branch block and remarkable prolongation of QTc interval (600 ms). She fainted and collapsed while talking on the telephone, and the Holter ECG showed TdP associated with 2:1 AVB.
Expression Study

In order to clarify the functional consequences of the G272V mutation of KCNQ1 and the D111V, A490T, and P846T mutations of KCNH2, we assessed the electrophysiological properties of the WT and mutant clones by using CHO cells.

**Biophysical Assay of KCNQ1 Mutant Channel** Figure 2A shows representative examples of whole-cell currents recorded from CHO cells transfected with WT/KCNQ1, G272V/KCNQ1 alone or WT co-expressed G272V/KCNQ1 (WT/G272V) plus KCNE1. CHO cells transfected with WT/KCNQ1 (1 or 0.5 μg) displayed outward currents with slow activation/deactivation kinetics on depolarization, which are typical of I_{Ks} currents, as previously reported.14,15 In contrast, a cell transfected with G272V/KCNQ1 (1 μg) displayed smaller I_{Ks} currents compared with that of the WT (1 μg). WT/G272V at an equimolar ratio (0.5 μg) also showed smaller I_{Ks} currents.

In Figure 2B, the tail current densities at −50 mV measured in multiple cells are plotted as a function of test pulse voltages (between −50 and +50 mV). The tail current densities at −50 mV after depolarizing test pulses to +40 mV were 77.0±11 pA/pF for 1 μg WT (n=18), 49.5±7.9 pA/pF for 0.5 μg WT (n=14), 25.4±4.5 pA/pF for 0.5 μg WT plus 1 μg mutant (green squares; n=10–13), 2 μg mutant (red squares; n=8–12). (C) Normalized activation curves by fitting to Boltzmann function.

**Table 2. Parameters of Inactivation in WT and Mutant KCNH2**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>WT/D111V</th>
<th>D111V</th>
<th>WT/A490T</th>
<th>A490T</th>
<th>WT/P846T</th>
<th>P846T</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{0.5}</td>
<td>−58.3±4.7</td>
<td>−40.1±4.1**</td>
<td>−47.4±7.0</td>
<td>−32.5±3.9*</td>
<td>−44.2±3.3</td>
<td>−38.7±2.4**</td>
<td>−55.3±3.5</td>
</tr>
<tr>
<td>Slope factor</td>
<td>29.2±1.4</td>
<td>33.9±1.3</td>
<td>35.3±1.7**</td>
<td>30.6±1.4†</td>
<td>34.9±1.1**</td>
<td>33.0±0.6†</td>
<td>37.5±1.7*</td>
</tr>
</tbody>
</table>

*P<0.001 vs WT, **P<0.01 vs WT, †P<0.05 vs WT.

WT, wild-type.

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Figure 3. All KCNH2 mutant channels show loss of function associated with decreased density on I_{Kr}. Electrophysiological characteristics of KCNH2 mutant channels in Chinese Hamster ovary cells. (A) Families of representative current traces. Experimental protocol is shown in the inset. Concentrations of cDNAs used for transfection are indicated above each graph. (B) Tail current-voltage relationships for 2 μg wild-type (WT: closed circles; n=20), 1 μg WT (dotted line; n=14), 1 μg WT plus 1 μg mutant (green squares; n=10–13), 2 μg mutant (red squares; n=8–12). (C) Normalized activation curves by fitting to Boltzmann function.
slope factors, G272V alone channel was larger than WT (P<0.05). Overall, the most important finding was the dominant-negative effect for the G272V channel.

Biophysical Assay of 3 KCNH2 Mutant Channels Figure 3A shows representative examples of whole-cell currents recorded from CHO cells transfected with WT/KCNH2 (2 and 1 μg), mutant/KCNH2 (2 μg), or WT co-expressed mutant/KCNH2 (WT/mutant) (1 μg each). CHO cells transfected with WT/KCNH2 (2 or 1 μg, Figure 3A Upper 2 panels) displayed outward currents with inward rectifying properties, which are typical of I\textsubscript{Kr} currents. In contrast, the magnitude of currents from cells expressing all of the WT/mutants and mutant only were remarkably reduced (Figure 3A Lower 6 panels).

In Figure 3B, the tail current densities at −60 mV are plotted as a function of test pulse voltages (between −60 and +50 mV). The mean current densities after depolarization to +20 mV in WT channels were 66.2±11 pA/pF for 2 μg (n=20) and 45.0±9.3 pA/pF for 1 μg (n=14). In contrast, those in the WT/mutant and mutant channels were 25.1±2.9 pA/pF in WT/D111V (n=13), 15.8±6.0 pA/pF in WT/A490T (n=10), 20.5±3.9 pA/pF in WT/P846T (n=12), 18.8±3.6 pA/pF for D111V (n=9), 15.2±3.4 pA/pF for A490T (n=12), 6.1±2.3 pA/pF for P846T (n=8), respectively. They were all significantly smaller than those of the 2-μg WT channels (vs WT 2 μg; P<0.01). Figure 3C shows that all WT/mutant and mutant channels tended to shift to the depolarization side compared with the WT. Overall, all mutant channels showed loss of function associated with a dominant-negative effect and shift of the activation curve to depolarization.

We then examined whether the mutations affected the inactivation kinetics of mutant channels using a double-pulse protocol. V\textsubscript{0.5} and the slope factor of steady-state inactivation differed between WT and WT plus mutant or mutant. All mutant KCNH2 channels showed the shift of inactivation curves to depolarizing direction, and the differences were statistically significant (Table 2). Therefore, we also changed the parameters associated with inactivation states in the following simulation study.

Computer Simulation of APD In order to compare how functional changes caused by mutations affect ventricular action potentials, a simulation study
AVB-Induced TdP

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AVB-Induced TdP was conducted using the Luo-Rudy model, which incorporated the Markov or Hodgkin-Huxley process gating for the mutant channels (Figure 5). Each figure presents (A) action potential duration (APD) at basic cycle length (BCL) of 600 ms, (B) APD at BCL of 1,000 ms, and (C) APD at BCL of 2,000 ms. The longer the BCL in each model was, the more the APD was prolonged. EADs appeared in the models of D111V/WT, A490T/WT, and P846T/WT only at BCL of 2,000 ms, but appeared at all BCLs in the case of G272V/WT.

Table 3. Parameters of Simulation Data in Bradycardia-Induced Long QT Syndrome

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>WT basal parameters</th>
<th>Mutant changed parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>G272V</td>
<td>gsk=0.202*(1+0.6/(1+pow(0.000038/cai),1.4)))</td>
<td>gsk=0.067*(1+0.6/(1+pow(0.000038/cai),1.4)))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>xs1 ss=1/(1+(v-15)/16.7))</td>
<td>xs1 ss=1/(1+(v-6.5)/16.7))</td>
</tr>
<tr>
<td>KCNH2</td>
<td>D111V</td>
<td>gherg=0.0135*pow(Kout,0.59)</td>
<td>gherg=0.331*0.0135pow(Kout,0.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-36))</td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-69))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αi=0.439<em>exp(-0.02352</em>(v+25))*4.5/Kout</td>
<td>αi=0.439<em>exp(-0.02352</em>(v+3))*4.5/Kout</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ββ=2.9375e-3<em>exp(-0.02158</em>v)</td>
<td>ββ=2.9375e-3<em>exp(-0.02158</em>v)</td>
</tr>
<tr>
<td>KCNH2</td>
<td>A490T</td>
<td>gherg=0.0135*pow(Kout,0.59)</td>
<td>gherg=0.1887*0.0135pow(Kout,0.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-36))</td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-69))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αi=0.439<em>exp(-0.02352</em>(v+25))*4.5/Kout</td>
<td>αi=0.439<em>exp(-0.02352</em>(v+3))*4.5/Kout</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ββ=2.9375e-3<em>exp(-0.02158</em>v)</td>
<td>ββ=2.9375e-3<em>exp(-0.02158</em>v)</td>
</tr>
<tr>
<td>KCNH2</td>
<td>P846T</td>
<td>gherg=0.0135*pow(Kout,0.59)</td>
<td>gherg=0.265*0.0135pow(Kout,0.59)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-36))</td>
<td>αα=65.5e-3<em>exp(0.05547153</em>(v-80))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>αi=0.439<em>exp(-0.02352</em>(v+25))*4.5/Kout</td>
<td>αi=0.439<em>exp(-0.02352</em>(v+3))*4.5/Kout</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ββ=2.9375e-3<em>exp(-0.02158</em>v)</td>
<td>ββ=1.3<em>2.9375e-3</em>exp(-0.02158*v)</td>
</tr>
</tbody>
</table>

Discussion

There are 3 major findings in the present study. (1) In 4 of 14 consecutive AVB-associated TdP patients, 3 KCNH2 and 1 KCNQ1 heterozygous missense mutations were identified. (2) Electrophysiological analyses revealed loss of function associated with decreased current densities and various dysfunctions on \( I_{Ks} \) or \( I_{Kr} \) in 4 mutants. (3) Functional changes reconstituted by the computer simulation resulted in a prolonged APD and EAD under condition of bradycardia.

During AVB, our 14 patients showed a prolonged QT interval and TdP. Based on a comparison of ECGs available before and after AVB, we found the QT intervals were lengthened even in the absence of AVB. These clinical characteristics indicate that AVB-related TdP might share a similar genetic background with congenital LQTS: mutations on cardiac ion channel genes could be partially causative. Lupo-
glazoff et al. 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%). 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. 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. 3 KCNH2 mutations, R328C, R696C and R1047L, were shown to have no strong dominant-negative effects on Iks. Another KCNE2 mutation (R77 W), which was identified in an AVB patient while taking flecainide, exerted no effects on Iks. 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 Iks, or Ikr, 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.

Several mutations of SCN5A, coding the α-subunit of Na+ channels, have been found in newborn and infant cases of long QT. 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. 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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References
AVB-Induced TdP


