**KCNE5 (KCNEIL) Variants Are Novel Modulators of Brugada Syndrome and Idiopathic Ventricular Fibrillation**

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**Background**—Brugada syndrome (BrS) has a significantly higher incidence among the male sex. Among genes coding ion channels and their modulatory proteins, *KCNE5 (KCNEIL)* is located in the X chromosome and encodes an auxiliary β-subunit for K channels. *KCNE5* has been shown to modify the transient outward current (*Ito*), which plays a key role in determining the repolarization process in the myocardium. This study investigated whether *KCNE5* mutations could be responsible for BrS and other idiopathic ventricular fibrillation (IVF).

**Methods and Results**—In 205 Japanese patients with BrS or IVF who tested negative for *SCN5A* mutation, we conducted a genetic screen for *KCNE5* variants. We identified 2 novel *KCNE5* variants: p.Y81H in 3 probands and p.[D92E;E93X] in 1 proband from 4 unrelated families. Y81H was identified in 1 man and 2 women; D92E;E93X was found in a 59-year-old man. All probands received implantable cardioverter-defibrillators. Functional consequences of the *KCNE5* variants were determined through biophysical assay using cotransfection with *KCND3* or *KCNQ1*. In the experiments with *KCND3*, which encodes Kv4.3, *Ito* was significantly increased for both *KCNE5* variants compared to wild type. In contrast, there were no significant changes in current properties reconstructed by *KCNQ1*+/wild type *KCNE5* and the 2 variants. With the simulation model, both variants demonstrated notch-and-dome or loss-of-dome patterns.

**Conclusions**—*KCNE5* modulates *Ito*, and its novel variants appeared to cause IVF, especially BrS, in male patients through gain-of-function effects on *Ito*. Screening for *KCNE5* variants is relevant for BrS or IVF. *(Circ Arrhythm Electrophysiol. 2011;4:352-361.)

**Key Words:** *KCNE5* protein human ■ *KCNE1L* protein human ■ Brugada syndrome ■ idiopathic ventricular fibrillation

Brugada syndrome (BrS) is a clinical entity presenting ST-segment elevation in the right precordial ECG leads and a high incidence of sudden death due to idiopathic ventricular fibrillation (IVF).1 Its incidence rate is 8- to 10-fold higher in the male sex.1 However, the mechanism underlying this sex difference remains unknown. In 1998, *SCN5A*, a gene encoding the α-subunit of cardiac Na channels, was reported as the causative gene for BrS.2 *SCN5A* mutations cause a reduction of cardiac Na current, which increases transmural dispersion of action potential (AP) duration in the right ventricle, thereby inducing the characteristic ST elevation seen on ECG and arrhythmogenicity. However, *SCN5A* mutations were identified in only 20% to 30% of patients.3 Additional candidates *GPD1L*,4 *CACNA1C, CACNB2,6 SCN1B,7 KCNE3,8 and *KCNQ1*9 have been reported, but supposedly, they are not major causative genes in BrS. We identified *SCN5A* mutations in only 4 (10.5%) of 38 patients with BrS,10 and none in *GPD1L,11 KCNQ1, or KCNE3* in the same patient group. Therefore, some genes responsible for BrS may remain unidentified.

**Clinical Perspective on p 361**

According to previous studies, the characteristic ST elevation in right precordial leads could be induced either by reduction in Na current or L-type calcium channel current or increased in transient outward currents (*Ito*). Blockade of *Ito* by 4-aminopyridine causes a loss of AP dome in the epicardium of canine wedge preparation,12 and activation of *Ito* by NS5806 has been shown to recapitulate the electrographic and arrhythmic manifestation of BrS.13 In humans, *Ito* supposedly comprises Kv4.3 and Kv channel-interacting protein (KChIP2),14 and β-subunits encoded by the *KCNE* family...
affect $I_{Na}$. Among KCNE family members, KCNE5 (KCNE1L) is located on the X chromosome and, therefore, may be involved in sex differences. Indeed, a polymorphism, 97T, in KCNE5 may afford protection against atrial fibrillation (AF) and contribute to sex differences in AF. Therefore, we screened extensively for KCNE5 variants in 205 consecutive probands with BrS, IVF, or both and identified 2 novel KCNE5 variants in 4 unrelated families. Both variants were studied using heterologous coexpression with Kv4.3/KChIP2 in Chinese hamster ovary (CHO) cells.

**Methods**

**Patients**

Study patients comprised 205 probands with BrS or IVF from unrelated families. They were referred consecutively to either of our laboratories for genetic evaluation. All subjects submitted written informed consent in accordance with the guidelines approved by each institutional review board. Each underwent detailed clinical and cardiovascular examinations.

**Genotyping**

Genomic DNA was isolated from venous blood lymphocytes as previously described. Through polymerase chain reaction, denaturing high-performance liquid chromatography (DHPLC), and direct DNA sequencing, we performed a comprehensive open reading frame and splice-site mutational analysis of known BrS-related genes ( KCNQ1, SCN5A, KCNE2, KCNE3, GPD1L, and SCN1B) and KCNE5 using previously described primers. Through polymerase chain reaction, denaturing high-performance liquid chromatography (DHPLC), and direct DNA sequencing, we performed a comprehensive open reading frame and splice-site mutational analysis of known BrS-related genes ( KCNQ1, SCN5A, KCNE2, KCNE3, GPD1L, and SCN1B) and KCNE5 using previously described primers. Although we did not examine CACNA1C and CACNB2 because the patients had no QT shortening in their ECGs, we checked known mutations in the Ca channels once a change in 

**Plasmid Construction**

The complete coding region of KCNE5 was amplified using polymerase chain reaction from human genomic DNA of patients and controls and cloned into a pIRES2-EGFP vector that we previously constructed. Nucleotide sequence analysis of the KCNE5 coding region was performed on each variant construct before the expression study. Full-length cDNA encoding the short isoform of human KCND3 subcloned into the pIRES-GFP (Clontech; Palo Alto, CA) expression vector was provided by Dr G. F. Tomaselii (The Johns Hopkins University; Baltimore, MD). Full-length cDNA encoding KChIP2 subcloned into the PCMV-IRS expression vector was a gift from Dr G.-N. Tseng (Virginia Commonwealth University; Richmond, VA). Full-length cDNAs for human KCNQ1 and KCNE1 were provided by Dr. J. Barhanin (Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Valbonne, France) and subcloned into pIRE2-EGFP and pIRE2-CD8 vectors, respectively, as previously reported.

**Mammalian Cell Line and cDNA Transfection**

CHO cells were transiently cotransfected with cDNA of KCND3, KChIP2, and wild type (WT) or variants (Y81H or D92E/E93X) of KCNE5 at equimolar concentrations (1.5 μg KCND3, 1 μg KChIP2, and 1.5 μg KCNE5) using Lipofectamine (Invitrogen Life Technologies; Carlsbad, CA). In a subset of experiments, WT or KCNE5 variants also were cotransfected into cells with 0.5 μg KCNQ1 and 0.5 μg KCNE1 (subcloned to pCI vector).

**Patch-Clamp Recordings and Data Analysis**

Whole-cell membrane currents were recorded with an EPC-8 patch-clamp amplifier (HEKA Elektronik; Lambrecht, Germany). Data were low-pass filtered at 1 kHz, acquired at 5 kHz through an LIH-1600 analog-to-digital converter (HEKA Elektronik), and stored on a computer hard drive using Patchmaster software (HEKA Elektronik). The extracellular solution contained 140 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L CaCl2, 0.5 mmol/L MgCl2, 0.33 mmol/L NaH2PO4, 5.5 mmol/L glucose, and 5.0 mmol/L Hepes (pH 7.4 with NaOH). Patch pipettes were fabricated from borosilicate glass capillaries (Narishige; Tokyo, Japan) on a horizontal microelectrode puller (P-97; Sutter Instrument Co; Novato, CA), and the tips were fire-polished using microforge. Patch pipettes had a resistance of 3.0 to 4.0 MΩ when filled with the pipette solution containing 70 mmol/L potassium aspartate, 50 mmol/L KCl, 10 mmol/L KH2PO4, 1 mmol/L MgSO4, 3 mmol/L Na2-ATP (Sigma), 0.1 mmol/L Li2-GTP (Roche Diagnostics GmbH; Mannheim, Germany), 5 mmol/L EGTA, and 5 mmol/L Hepes (pH 7.2).

Forty-eight hours after transfection, cells on coverslips were transferred to a 0.5-mL bath chamber mounted on the stage of an inverted microscope (Nikon Eclipse) and constantly superfused with external solution kept at 25°C. In patch-clamp experiments with reconstituted Kv4.3 or KCNQ1, only CHO cells that emitted green fluorescence were chosen for current recording. KCNE5 (WT or variant) protein expression was detected by polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450; Dynal; Oslo, Norway). Kv4.3 (Iκ) currents were elicited from a holding potential of −80 mV by 300-ms depolarizing voltage commands. Current amplitudes were measured as the difference between the levels of peak current and that at the end of depolarizing pulse. The time course of Kv4.3 current inactivation was evaluated by fitting the declining phase of the current trace with a double-exponential function as follows (Equation 1):

$$I_{Kv4.3} = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$$

where $A_1$ and $A_2$ represent amplitudes, and $\tau_1$ and $\tau_2$ represent time constants for the fast and slow components, respectively. Voltage dependence of Kv4.3 current activation was studied by constructing mean conductance/voltage (G/V) relations. The value of G for every voltage was calculated from the following expression (Equation 2):

$$G = I/(V - V_{rev})$$

where $I$ is Kv4.3 the current amplitude, and $V_{rev}$ is the reversal potential for $K^+$. The steady-state activation/inactivation kinetics were fitted to the following Boltzmann equation as follows (Equation 3):

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

where $V_{rev}$ is the potential for half-maximal inactivation and activation, respectively; $k$ is the slope factor; and $Y$ is the normalized conductance or current.

Recovery from inactivation of Kv4.3 current was assessed by a standard paired-pulse protocol as follows: A 500-ms test pulse to +30 mV ($P_2$) was followed by a variable recovery interval (1 to 600 ms) at −80 mV then by a second 300-ms test pulse to +30 mV ($P_1$). The time-constant for recovery from inactivation was determined by fitting the mean values of the relation $P_2/P_1$ versus recovery time to a single exponential function, as follows (Equation 4):

$$P_2/P_1 = A \exp(-t/\tau)$$

where $P_2/P_1$ is the ratio between Kv4.3 current amplitudes elicited by $P_2$ and $P_1$, respectively; $t$ is the interpulse interval; $A$ is constant; and $\tau$ is the recovery time constant.

KCNQ1/KCNE1 currents were evoked by various depolarizing voltage steps with a duration of 2 seconds from holding potential of −80 mV, and tail currents were recorded at −50 mV. Voltage dependence of KCNQ1/KCNE1 activation and inactivation was evaluated by fitting the mean current-voltage ($I-V$) relationship of tail currents to the Boltzmann equation (Equation 3).
Results

In 4 of 205 index patients, we identified 2 novel variants in KCNE5 (Figure 1). The first variant was a single-nucleotide alternation (c.241T>C) (Figure 1A), resulting in an amino acid substitution from a tyrosine at residue 81 with a histidine (p.Y81H). This Y81H variant was identified in 3 probands. The second variant was found in a male proband and had 2 nucleotide changes (c.[276C>A;277G>T]) (Figure 1B), causing an amino acid substitution from an aspartate at residue 92 with a glutamate and from a glutamate at residue 93 with a stop codon (p.[D92E;E93X]). They are located in the transmembrane (Y81H) and C-terminus (D92E;E93X) and in highly conserved regions (Figure 1C). In 300 unrelated healthy individuals with normal ECGs from the general Japanese population, Y81H was identified in 3 healthy women, and D92E;E93X was absent.

Phenotypic Characterization

Patient 1

The first proband with Y81H was a 41-year-old man who lost consciousness for a few minutes after suffering a convulsion while drinking alcohol. Although he was conscious in the ambulance, he lost consciousness in the hospital emergency department. The monitor ECG at that time displayed VF, and DC shock was performed. His ECGs (Figure 2A) displayed type 1 Brugada pattern, and he received an implantable cardioverter-defibrillator. There was no family history of syncope or sudden cardiac death. The patient’s older sister carried the same mutation, but she was healthy and had no

Figure 1. Molecular discovery of KCNE5 mutations. Denaturing high-performance liquid chromatography and DNA sequence analyses of control and patients are shown. Abnormal denaturing high-performance liquid chromatography data (inside the frame) was acquired from the mixed sample of a patient and control. A, Analyses of patients 1, 2, and 3. DNA sequencing chromatograms demonstrate a Tyr-to-His substitution at residue 81.

B, Analyses of patient 4. DNA sequencing chromatograms demonstrate an Asp-to-Glu substitution at residue 92 and a Glu-to-stop codon at residue 93.

C, Alignment of amino acid sequence of KCNE5 in various species and comparison with human KCNE1–4. Red font indicates variant sites and homologies. Asp indicates aspartate; Glu, glutamate; His, histidine; Tyr, tyrosine.
A history of syncope. Her ECG did not show Brugada pattern (not shown).

**Patient 2**
The second proband with Y81H was a 47-year-old woman who fell down suddenly while working at the cash desk of a supermarket. She immediately underwent cardiopulmonary resuscitation and was transported to the hospital. The recording of automatic external defibrillation showed VF (Figure 2B, top). Although there was no sign of ischemic heart disease through cardiac angiography examination, VF was induced during electrophysiological examination. The patient received an implantable cardioverter-defibrillator. Her ECG showed no ST elevation in the right precordial leads (Figure 2B, bottom) and did not show Brugada pattern for the next follow-up period. In the flecainide infusion test, ST-segment elevation in the right precordial leads was not produced. Figure 2C shows the patient’s family tree. Her younger brother (II-3) had a history of syncope and carried the same mutation, although his ECG did not show Brugada pattern (Figure 2C inset), even in the superior-costal position. His ECG also showed no conduction or depolarizing disturbance (PR, 170 ms; QRS, 100 ms; QT, 388 ms; QTc, 433 ms). The patient’s mother (I-2) and daughter (III-3) were carrying the same mutation but remain asymptomatic and showed no Brugada pattern on ECG.

**Patient 3**
The third proband with Y81H was a 54-year-old woman who had syncope when she was drinking alcohol and watching television. After a few minutes, she recovered consciousness. Her ECG showed ST elevation in the V1 and V2 leads recorded a few days after the event (data not shown); therefore, cardiac electrophysiology testing was performed. In the test, VF could be induced, and the patient received an implantable cardioverter-defibrillator. The patient had no family history of syncope and sudden cardiac death. Her relatives did not give consent for genetic analysis.

**Patient 4**
A novel variant, D92E;E93X, was identified in a 59-year-old man who was a postal worker. When he started a ride on his motorcycle after a postal delivery, he suddenly lost consciousness. He recovered consciousness in the ambulance.
that time, he had a fever and his body temperature was 38.0°C. His ECG (Figure 3A) showed saddle-back-type ST elevation in the right precordial leads and coved-type ST elevation at the third costal position. We therefore diagnosed symptomatic BrS, and the patient received an implantable cardioverter-defibrillator. The patient’s grandfather (I-1), father (II-5), uncle (II-4), and paternal cousin (III-1) all died suddenly at the ages of 45, 52, 45, and 31, respectively (Figure 3B). In genetic analysis, his mother and 2 daughters carried this mutation in a heterozygous manner; however, all were asymptomatic and had no Brugada pattern on ECG. Four probands tested negative for the causative genes of BrS as described in the Methods. Also, they were negative for the reported Ca channel mutations.

Electrophysiological Analysis

Effects of KCNE5 on Kv4.3/KChIP2 Current Amplitude and Kinetics
To examine the effects of the KCNE5 variants on I_{to}, we first coexpressed the KCND3 gene product (Kv4.3, the α-subunit of I_{to} channel) and KChIP2 (the principle β-subunit for human native cardiac I_{to}15,26,27) in CHO cells. Figure 4A shows representative whole-cell current traces recorded from cells cotransfected with KCND3, KChIP2, and without (left) or with (right) KCNE5. Cells expressing Kv4.3/KChIP2 channels alone showed rapidly activating and inactivating currents. Coexpression of KCNE5 reduced peak current densities as summarized in Figure 4B but not to a statistically significant extent. Figure 4C shows 2 current traces recorded from cells cotransfected with or without KCNE5 elicited by +50 mV. Two peak levels were adjusted to show the time course of inactivation. This was significantly more rapid in the presence of KCNE5 (P<0.05), as shown by the plots of inactivation time constants (obtained using Equation 1) shown in Figure 4D.

KCNE5 Variants Increase Kv4.3/KChIP2 Current
We then examined the functional effect of 2 novel KCNE5 variants on Kv4.3/KChIP2 currents. The 5 parts of Figure 5A show current traces elicited by depolarizing pulses from a holding potential of −80 mV in cells cotransfected with KCNE5-WT, Y81H, D92E;E93X, Y81H WT, and D92E;E93X WT. Both KCNE5 variants significantly increased Kv4.3/KChIP2 currents (○ and ▴). However, when cotransfected with WT and respective variants at a 1:1 ratio, this stimulatory action of KCNE5 variants was completely abolished (● and ◇). Figure 5B summarizes peak current-membrane potential relations. Each symbol indicates the mean current density calculated from pooled data at respective test potentials, and vertical bars indicate SEs. With a depolarization to +50 mV, for example, the current densities...
were 98.9±9.9 picoamperes per picofarad (pA/pF), 156.5±15.9 pA/pF, and 178.0±20.0 pA/pF in the presence of KCNE5–Y81H (n=22), Y81H (n=22), and D92E;E93X (n=20), respectively (P<0.05 versus WT). In contrast, when coexpressed with WT and KCNE5 variants, they were 112.5±11.1 pA/pF and 109.8±10.2 pA/pF for the WT/Y81H (n=18) and WT/D92E;E93X (n=27), respectively (P>0.05 versus WT).

Effects of KCNE5 variants on voltage dependence for Kv4.3/KChIP2 currents were studied in terms of current activation/inactivation (Figure 5C, Table 1). The values of $V_{1/2}$ and $k$ for KCNE5-WT were not statistically different from those for variant KCNE5 proteins, thus indicating that variants did not affect either activation or inactivation kinetics of Kv4.3/KChIP2 currents.

Inactivation time courses also were assessed using Equation 1 and are summarized in Figure 6A and Table 1. The fast time constant for inactivation ($\tau_i$) was 15.3±2.7 ms, 24.5±2.4 ms, and 14.7±2.2 ms; the slow time constant ($\tau_s$) was 64.2±4.2 ms, 63.8±4.0 ms, and 57.1±2.1 ms; and the fraction of the amplitude of the fast inactivation component was 0.46±0.04, 0.38±0.04, and 0.42±0.02 for WT (n=18), Y81H (n=18), and D92E;E93X (n=21), respectively. The value of $\tau_i$ for Y81H (but not D92E;E93X) was significantly

**Table 1. Electrophysiological Analysis of I_{to}**

<table>
<thead>
<tr>
<th></th>
<th>Activation</th>
<th>Inactivation</th>
<th>Inactivation Time Constant (s)</th>
<th>Recovery of Inactivation, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{1/2}$, mV</td>
<td>Slope Factor, $k$</td>
<td>$V_{1/2}$, mV</td>
<td>Slope Factor, $k$</td>
</tr>
<tr>
<td>WT</td>
<td>98.9±9.9</td>
<td>−2.0±2.0</td>
<td>18.2±1.9</td>
<td>−46.2±1.2</td>
</tr>
<tr>
<td>Y81H</td>
<td>156.5±15.9</td>
<td>−5.7±0.9</td>
<td>14.2±0.6</td>
<td>−45.8±0.9</td>
</tr>
<tr>
<td>D92E;E93X</td>
<td>178.0±20.0</td>
<td>−3.5±1.3</td>
<td>16.6±1.3</td>
<td>−45.9±0.9</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. $A_i$ indicates amplitude; I_{to}, transient outward current; pA/pF, picoamperes per picofarad; $V_{1/2}$ potential for half-maximal inactivation and activation; WT, wild type.

*P<0.05 vs WT.
larger than that for WT. There were no significant differences between the values of $\tau_r$ or the fraction of fast inactivating current for WT and variant KCNE5.

In the next series of experiments, a 2-pulse protocol was used to investigate the recovery of inactivation of Kv4.3/KChIP2 currents in CHO cells (see Methods section). Figure 6B shows typical examples of time-dependent recovery of the Kv4.3/KChIP2 current amplitudes when WT or variant KCNE5 was coexpressed. In Figure 6C, the mean $P_2/P_1$ ratios are plotted as a function of interpulse interval and are fitted to Equation 4. The time constants for recovery of inactivation thus calculated were not significantly different, suggesting that 2 variants did not affect the recovery of Kv4.3/KChIP2 channel activity from inactivation (Table 1).

**KCNE5 Mutants and KCNQ1+KCNE1 Channel**

Recently, novel mutation in a conserved residue of KCNQ1 (gene coding $\alpha$-subunit of the KCNQ1+KCNE1 $I_{Ks}$ channel), V162M, was reported to cause BrS by accelerating current activation.9 Therefore, we tested the effect of WT and variant KCNE5 on reconstituted $I_{Ks}$ current in CHO cells. Figure 7A shows representative current traces of $I_{Ks}$ with or without KCNE5. As previously reported,28 cotransfection of WT KCNE5 reduced $I_{Ks}$ without changing activation gating kinetics. Numeric data obtained from pooled experiments are summarized in Table 2. Two KCNE5 variants exerted effects analogous to WT KCNE5 on $I_{Ks}$ currents, reducing them without changing the activation gate kinetics, and there was no statistical difference between WT and the 2 mutants.

Finally, deactivation time constants for tail currents were calculated by fitting a single exponential equation and are summarized in Table 2. They were not significantly different, suggesting that the 2 KCNE5 variants did not affect $I_{Ks}$ channel function in a way different from that of WT KCNE5.

**Simulation Study**

AP simulations were conducted to determine the effects of the KCNE5 variants on ventricular AP profiles. Figure 8 shows representative results. Simulated APs from epicardial and endocardial layers are overlapped. Compared to WT (Figure 8A), the myocardium model with Y81H (Figure 8B) demonstrated a notch-and-dome AP morphology and loss-of-
because of homology with KCNE1, coding a single-WT KCNE5 gene. In patients with BrS or IVF and demonstrated that KCNE5 is located in the X chromosome, the upregulation of Ito currents may occur preferentially in male (XY) than in female mutation carriers (XX). In other words, the male sex would be anticipated to have more lethal phenotypes than the female sex. Indeed, we identified 11 mutant carriers in the 4 families; 3 men and 8 women. All the male cases were symptomatic, and 2 of them showed a typical Brugada pattern on ECG, whereas 6 of 8 female cases remained asymptomatic. Interestingly, all the female carriers, including 2 symptomatic probands, did not show typical Brugada patterns on ECG. In total, only 2 men among 11 carriers had a Brugada pattern on ECG, and both developed VF. This sex difference in ECG features also may partially result from the sex-dependent difference in Ito current density.

In the present functional assay of 2 novel KCNE5 variants, Y81H and D92E;E93X (Figure 5), the coexpression of variant with WT KCNE5 subunits (to mimic the heterozygous condition) abolished the stimulatory action of the mutants. Given that KCNE5 is located in the X chromosome, the upregulation of Ito currents may occur preferentially in male (XY) than in female mutation carriers (XX). In other words, the male sex would be anticipated to have more lethal phenotypes than the female sex. Indeed, we identified 11 mutant carriers in the 4 families; 3 men and 8 women. All the male cases were symptomatic, and 2 of them showed a typical Brugada pattern on ECG, whereas 6 of 8 female cases remained asymptomatic. Interestingly, all the female carriers, including 2 symptomatic probands, did not show typical Brugada patterns on ECG. In total, only 2 men among 11 carriers had a Brugada pattern on ECG, and both developed VF. This sex difference in ECG features also may partially result from the sex-dependent difference in Ito current density.

In 300 controls, we identified 3 female heterozygous Y81H carriers but no D92E;E93X carriers. Both cases (obligatory heterozygous because they are female [XX]) showed normal ECG and were asymptomatic. Y81H-KCNE5, therefore, could be a genetic polymorphism but not a mutation; however, the functional assay of both variants showed that they cause a larger Ito in case of male carriers (XY), which would be expected to influence transmural dispersion of repolarization and, thereby, arrhythmogenicity. It remains unknown why 2 female Y81H-KCNE5 carriers were symptomatic because they have 2 X chromosomes. However, X chromosome inactivation may be related to this phenomenon because it results in the random silencing of 1 of the paired X chromosomes in mammalian females to achieve the dosage equivalency with males. Once an X is to be active or inactive during late blastocyst stage, this state is stably inherited through subsequent somatic mitotic divisions, resulting in females being mosaics for cells with each parental X active. Recently, Carrel and Willard reported that ∼15% of X-linked genes escape from the inactivation phenomenon to some degree, therefore explaining the expression heterogeneity among the female sex. In our 2 female cases with symptomatic VF, similar mechanisms, such as gene inactivation and its escape phenomenon, may underlie the arrhythmogenicity by causing diverse levels of mutant KCNE5 expression. This in turn may change the densities and properties of resultant Ito currents.

Table 2. Electrophysiological Analysis of Ito

<table>
<thead>
<tr>
<th>Variant</th>
<th>Tail Current Density (pA/pF) at +50 mV</th>
<th>Activation V_{1/2}, mV</th>
<th>Slope Factor, k</th>
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<tr>
<td>Q1/E1</td>
<td>76.0±5.7</td>
<td>0.7±1.6</td>
<td>11.5±0.4</td>
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<tr>
<td>Q1/E1/E5WT</td>
<td>54.6±5.9</td>
<td>1.9±1.4</td>
<td>12.7±0.4</td>
</tr>
<tr>
<td>Q1/E1/E5Y81H</td>
<td>51.2±8.9</td>
<td>3.7±2.0</td>
<td>13.0±0.6</td>
</tr>
<tr>
<td>Q1/E1/E5D92E;E93X</td>
<td>53.0±5.4</td>
<td>2.6±2.2</td>
<td>13.6±0.3</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. Ito indicates KCNQ1+/KCNE1 channel. Other abbreviations as in Table 1.

Discussion

In the present study, we identified 2 novel variants in KCNE5 (KCNE1L) in patients with BrS or IVF and demonstrated that these variants upregulated reconstituted Ito compared with WT KCNE5. KCNE5 was first reported as a KCNE1-like gene because of homology with KCNE1, coding a single-transmembrane-domain protein. As a member of KCNE family (1 through 5), it can function as an ancillary subunit of Kv channels. Recently, a KCNE5 mutation, L65F, was found to be associated with AF. WT KCNE5 suppresses Ito, but the L65F mutation failed to suppress Ito, therefore exerting a gain of function effect on Ito and predisposing to atrial fibrillation.

Figure 8. Simulation study of KCNE5-WT and mutants. A, WT models. Gray lines indicate the action potential in epicardium, and black indicates action potential endocardium. B, Y81H model displays notch-and-dome and loss-of-dome pattern in epicardium. C, D92E;E93X model displays loss-of-dome pattern in epicardium.
higher risk of IVF and have a potential to transmit the arrhythmogenicity to their offspring, especially male ones. These poor genotype-phenotype correlations indicate that these KCNE5 variants do not confer final causality but are more likely to modulate the electrocardiographic and arrhythmia manifestations of BrS or IVF secondary to other causes.

With regard to candidate genes responsible for BrS or IVF, Kv4.3, encoded by KCND3 and an α-subunit for the I_{to} channel, has been believed plausible to cause transmural arrhythmogenicity to their offspring, especially male ones. More recently, a mutation of KCNE3 (R99H), another β-subunit for voltage-gated K channels, was found in a BrS family and has been shown to increase I_{to} current and accelerate its inactivation by interacting with Kv4.3 and KChIP2.8 The 2 KCNE5 mutations described in the present study increased I_{to}. Thus, KCNE5 variants appear to be modifiers of phenotype and arrhythmogeneity. Screening for KCNE5 mutations may help to detect patients at risk for sudden cardiac death.

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Disclosures
None.

References


CLINICAL PERSPECTIVE
Brugada syndrome (BrS) and idiopathic ventricular fibrillation (IVF) are malignant cardiac disorders that cause syncope and sudden death without structural abnormality. Their frequency is much higher in male sex than in female sex, although the reason remains unclear. In electrophysiological analyses, BrS was shown to be associated with reductions in Na or Ca currents or increases in transient outward K^{+}/H^{+} current (I_{to}). In light of sex differences, we focused on the KCNE5 gene because it is located on the X chromosome. In the present cohort of patients with BrS or IVF (N = 205), we found 2 novel KCNE5 variants in 4 symptomatic probands. A similar KCNE5 variant also was identified in 3 asymptomatic female controls. In patch-clamp analysis using a heterologous expression system, both KCNE5 variants increased the I_{to} current. In a computer simulation study, both variants demonstrated notch-and-dome or loss-of-dome patterns, which could lead to larger voltage gradients in the ventricular transmural models. Thus, KCNE5 variants modulate I_{to} current and, thereby, the phenotypes of BrS and IVF more predominantly in male patients. Although further study in a larger cohort of patients with BrS or IVF is needed, KCNE5 screening in these patients may be useful to predict prognosis.
**KCNE5 (KCNE1L) Variants Are Novel Modulators of Brugada Syndrome and Idiopathic Ventricular Fibrillation**

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