**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 **KCNQ1** or **KCNQ1**. In the experiments with **KCNQ3**, 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** and 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.³ 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.³ **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.⁴ Additional candidates **GPD1L**,⁵ **CACNA1C**, **CACNB2**, **SCN1B**,⁶ **KCNQ3**,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 **KCNQ3** 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, 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

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affect $I_{rev}$. 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, GPDL1, 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, GPDL1, and SCN1B) and KCNE5 using previously described primers. A similar study was performed on each variant construct before the expression study. Full-length cDNA encoding the short isoform of human KCNQ1/KCNE1 was identified in a proband. DHPLC analysis for KCNE5 was performed by mixing 2 samples to detect hemizygous or homozygous mutations. Denaturing temperatures of DHPLC were 65°C and 67°C. The cDNA sequence was as follows (Equation 1):

$$I_{rev} = A_i \exp(-t/\tau_i) + A_r \exp(-t/\tau_r)$$

where $A_i$ and $A_r$ represent amplitudes, and $\tau_i$ and $\tau_r$ represent time constants for the fast and slow components, respectively. Voltage dependence of Kv4.3 current activation was studied by fitting the mean values of the relation $V_g$ to a double-exponential function as follows (Equation 2):

$$G = 1/(V - V_{rev})$$

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):

$$Y(V) = 1/(1 + \exp[(V - V_i)/k])$$

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 studied by a standard paired-pulse protocol as follows: A 500-ms test pulse to $+30$ mV ($P_T$) 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 ($P_R$). The time constant for recovery from inactivation was determined by fitting the mean values of the relation $P_T/P_R$ versus recovery time to a single exponential function, as follows (Equation 4):

$$P_T/P_R = A \exp(-t/\tau)$$

where $P_T/P_R$ is the ratio between Kv4.3 current amplitudes elicited by $P_T$ and $P_R$, respectively; $\tau$ 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).
The ratio of $I_{Kr}$ and $I_{Ks}$ conductance of each layer was set as $80/1$ for the cycle length of 400 ms for 5 minutes. The dynamic Luo-Rudy model (Clancy and Rudy 2001 model) of a ventricular cell was used with recent modifications. Epicardial and endocardial APs were simulated using a previously reported model. 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 a 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 clinical symptoms.

**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 clinical symptoms.
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 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. At

**Figure 2.** Clinical characterizations of patients with Y81H variant. A, Twelve-lead ECG of patient 1. Arrow indicates coved-type ST-segment elevation. B, ECG recording of patient 2. Top shows ventricular fibrillation recorded in automatic electric defibrillation, and bottom shows a 12-lead ECG for patient 2. Bars indicate 1 mV and 400 ms. C, Family tree of patient 2. Patient 2 (proband) is indicated by arrow (II-2). Roman numerals refer to the different generations, and numbers 1 through 3 identify individuals within each generation. Circles indicate female family members and squares, male family members. The affected family members are filled, and Y81H carriers are indicated with an asterisk. V1 lead recording in the inset is of II-3.

**Figure 3.** Clinical characterizations of patient 4. A, Twelve-lead ECG of patient 4. Right-side ECGs (3rd) were recorded in third costal position. Bars indicate 1 mV and 400 ms. B, Family tree of patient 4. Patient 4 (proband) is indicated by an arrow (III-2). Roman numerals refer to the different generations, and numbers 1 through 9 identify individuals within each generation. Circles indicate female family members and squares, male family members. Slashes indicate deceased family members, and S.D. indicates individuals who died suddenly. The affected family members are filled and D92E;E93X carriers are indicated with an asterisk.
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 Ito, we first coexpressed the KCND3 gene product (Kv4.3, the α-subunit of Ito channel) and KChIP2 (the principle β-subunit for human native cardiac Ito) 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.

KCN5 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-WT (n = 19), 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 ($\tau_{f}$) 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 $\tau_{f}$ for Y81H (but not D92E;E93X) was significantly

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

<table>
<thead>
<tr>
<th>Variant</th>
<th>Peak Current Density at +50 mV, pA/pF</th>
<th>Activation</th>
<th>Inactivation</th>
<th>Inactivation Time Constant ($\tau$)</th>
<th>Recovery of Inactivation, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{1/2}$, mV</td>
<td>Slope Factor, $\kappa$</td>
<td>$V_{1/2}$, mV</td>
<td>Slope Factor, $\kappa$</td>
<td>Fast ($\tau_{f}$), ms</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>
<td>15.3±2.7</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>
<td>24.5±2.4*</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>
<td>14.7±2.2</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 \(\tau\), 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 [IKs] 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-
Table 2. Electrophysiological Analysis of $I_{Ks}$

<table>
<thead>
<tr>
<th>Variant</th>
<th>Tail Current Density at +50 mV, pA/pF</th>
<th>Activation $V_{1/2}$, mV</th>
<th>Slope Factor, $\kappa$</th>
</tr>
</thead>
<tbody>
<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. $I_{Ks}$ indicates KCNQ1+KCNE1 channel. Other abbreviations as in Table 1.

dome pattern similar in the simulation study with Na channel mutation. The model with D92E;E93X showed a loss-of-dome pattern (Figure 8C). These patterns would be anticipated to lead to larger voltage gradients in the ventricular transmural models, increasing the likelihood of a proarrhythmic substrate.

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 $I_{Ks}$ compared with WT KCNE5. KCNE5 was first reported as 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 $I_{Ks}$, but the L65F mutation failed to suppress $I_{Ks}$, therefore exerting a gain of function effect on $I_{Ks}$ and predisposing to atrial fibrillation.

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 $I_{Ks}$ 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 $I_{Ks}$ 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 $I_{Ks}$ 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 miotic 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 $I_{Ks}$ currents.

In contrast, D92E;E93X-KCNE5 identified in the family of patient 4 (Figure 3) appeared to serve as a genetic modifier because sudden death accumulated in the proband’s paternal side (Figure 3B, pedigree). D92E;E93X was inherited from his mother and then transferred to his 2 daughters. Therefore, we could not completely exclude the possibility of the presence of another genetic background predisposing to IVF, which was inherited from his father’s side, although he tested negative for previously reported candidate genes for BrS. Our experimental study implicated that his 2 daughters are at a
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 electrophysiologic and arrhythmogenic 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\(_{\text{Kr}}\) channel, has been believed plausible to cause transmural repolarization dispersion and arrhythmogenicity. However, there are no reports on KCND3 mutations in patients with these pathologic conditions. More recently, a mutation of KCNE3, was found in a BrS family and has been shown to increase I\(_{\text{Ks}}\) current and accelerate its inactivation by interacting with Kv4.3 and KChIP2. The 2 KCNE5 mutations described in the present study increased I\(_{\text{Ks}}\). Thus, KCNE5 variants appear to be modifiers of phenotype and arrhythmogenicity. 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$^+$ current (Ito). 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 Ito 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 Ito 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.
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