Most LQT2 Mutations Reduce Kv11.1 (hERG) Current by a Class 2 (Trafficking-Deficient) Mechanism

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Background—The KCNH2 or human ether-a-go-go related gene (hERG) encodes the Kv11.1 α-subunit of the rapidly activating delayed rectifier K⁺ current (I_K) in the heart. Type 2 congenital long-QT syndrome (LQT2) results from KCNH2 mutations that cause loss of Kv11.1 channel function. Several mechanisms have been identified, including disruption of Kv11.1 channel synthesis (class 1), protein trafficking (class 2), gating (class 3), or permeation (class 4). For a few class 2 LQT2-Kv11.1 channels, it is possible to increase surface membrane expression of Kv11.1 current (I_Kv11.1). We tested the hypotheses that (1) most LQT2 missense mutations generate trafficking-deficient Kv11.1 channels, and (2) their trafficking-deficient phenotype can be corrected.

Methods and Results—Wild-type (WT)-Kv11.1 channels and 34 missense LQT2-Kv11.1 channels were expressed in HEK293 cells. With Western blot analyses, 28 LQT2-Kv11.1 channels had a trafficking-deficient (class 2) phenotype. For the majority of these mutations, the class 2 phenotype could be corrected when cells were incubated for 24 hours at reduced temperature (27°C) or in the drugs E4031 or thapsigargin. Four of the 6 LQT2-Kv11.1 channels that had a wild-type–like trafficking phenotype did not cause loss of Kv11.1 function, which suggests that these channels are uncommon sequence variants.

Conclusions—This is the first study to identify a dominant mechanism, class 2, for the loss of Kv11.1 channel function in LQT2 and to report that the class 2 phenotype for many of these mutant channels can be corrected. This suggests that if therapeutic strategies to correct protein trafficking abnormalities can be developed, it may offer clinical benefits for LQT2 patients. (Circulation. 2006;113:365-373.)

Key Words: arrhythmia ■ ion channels ■ long-QT syndrome ■ hERG ■ protein trafficking

The KCNH2 or human ether-a-go-go related gene (hERG1) encodes the Kv11.1 protein α-subunits that underlie the rapidly activating delayed rectifier K⁺ current (I_K) in the heart, and mutations in KCNH2 cause a loss of function phenotype to result in the proarrhythmic type 2 long-QT syndrome (LQT2). Genetic analyses have identified ~200 LQT2-associated KCNH2 mutations (see http://pc4.fsm.it:81/cardmoc/index.html), with missense (single amino acid substitution) mutations being the dominant predicted protein abnormality (~67% frequency). Functional expression studies in several laboratories have identified multiple mechanisms that underlie the loss of function phenotype, including abnormalities in Kv11.1 synthesis (class 1 mechanism), intracellular transport (protein trafficking) to the cell surface membrane (class 2 mechanism), channel gating (class 3 mechanism), or permeation (class 4 mechanism). One purpose of the present study was to determine whether a single mechanism is dominant in causing the loss of Kv11.1 function associated with LQT2.

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Trafficking-deficient ion channel proteins (class 2) are postulated to result from abnormalities in protein biogenesis and are retained in the endoplasmic reticulum (ER) until they are degraded. This is increasingly being recognized as an important disease-causing mechanism. The trafficking-deficient ion channel phenotype can be corrected to increase functional expression at the cell surface membrane. For example, in cystic fibrosis, several drugs correct the trafficking-deficient phenotype of the cystic fibrosis transmembrane conductance regulator (CFTR) deletion mutant, ΔF508-CFTR, which is potentially clinically important because this mutation is observed in ~70%
of these patients. Similarly, a few trafficking-deficient LQT2-Kv11.1 channels also can undergo pharmacological correction to increase their functional expression at the cell surface membrane. Because there is no dominant mutation associated with LQT, the potential clinical relevance of these findings remains uncertain. Thus, a second purpose of the present study is to test the hypothesis that the trafficking-deficient phenotype of most class 2 LQT2-Kv11.1 channels can be corrected.

We studied 34 missense mutations identified in patients with LQT2. We found that 28 of these LQT2-Kv11.1 channels resulted in a trafficking-deficient phenotype, and of the 28 class 2 LQT2 mutations, 19 (68%) could undergo correction by 1 or more of the different cell culture conditions tested. This is the first study to identify a dominant mechanism linked to the loss of LQT2-Kv11.1 channel function, and it demonstrates that correction of defective protein trafficking occurs in many LQT2-Kv11.1 channels.

**Methods**

**Cell Lines and Drug Exposure**

Wild-type Kv11.1 (WT-Kv11.1) and 34 Kv11.1 missense mutations associated with LQT2 (LQT2-Kv11.1) were studied (Table 1). The LQT2 mutations span the length of the Kv11.1 protein, including the N- and C-terminus; transmembrane spanning domains S1, S2, S4, S5, and S6; the pore region; and extracellular and intracellular linkers. Constructs encoding these mutations were generated and expressed in HEK293 cells with transient and stable transfection as described previously.28,32–34 The transfection was performed with 3 μg of cDNA (heteromeric channels) or 1.5 μg of WT and LQT2 cDNA (heteromeric channels) with Superfect (Qiagen).

**Electrophysiology**

Kv11.1 current (I<sub>Kv11.1</sub>) was measured by the whole-cell patch-clamp technique as described previously.32 The extracellular bath solution contained (in mmol/L) 137 NaCl, 4 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 glucose, and 10 HEPES (pH 7.4 with KOH). The intracellular pipette solution contained (in mmol/L) 130 KCl, 1 MgCl<sub>2</sub>, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). The holding potential was −80 mV, and the baseline (zero current) is indicated as a dotted line in the figures. All voltage-clamp experiments were performed at 22°C to 23°C within 1 to 2 hours after cells were removed from their culture conditions. I<sub>Kv11.1</sub> was normalized to cellular capacitance, and in some experiments, data were fit by the Boltzmann function,35 with maximal activation normalized to 1.0 to give the relative I<sub>Kv11.1</sub>. Voltage protocols and data analysis were done with pCLAMP 8.0 (Axon Instruments) and Origin (6.0, Microcal) computer software.

**Western Blot**

The Western blot procedure was described previously.30 Briefly, whole-cell lysates of similarly confluent cultures were generated by solubilizing cells in NP-40 lysis buffer (1% NP-40, 150 mmol/L NaCl, 10% glycerol, 5 mmol/L EDTA, and 50 mmol/L Tris-HCl, pH 7.4). Equal amounts of protein and sample buffer with a final [DTT] of 100 mmol/L were mixed and heated for 10 minutes at 60°C and subjected to 7.5% SDS-PAGE, and then the proteins were electrophoretically transferred onto nitrocellulose membranes. The nitrocellulose membranes were incubated with a Kv11.1 antibody directed against the C-terminus.30 The antibody was then detected with the ECL detection kit (Amersham).

**Statistical Analysis**

Data are presented as mean±SE. ANOVA and Student t test were used for statistical analysis, with P<0.05 considered significant.
Most LQT2 Mutations Are Trafficking Deficient

Results

Most LQT2-Kv11.1 Channels Have a Trafficking-Deficient Phenotype

Figure 1A shows representative Western blot analyses of HEK293 cells transiently expressing WT-Kv11.1 or LQT2-Kv11.1 channels. The top row shows WT- and 6 LQT2-Kv11.1 channels that contain both the 135- and 155-kDa protein bands. The middle and bottom rows show 28 LQT2-Kv11.1 channels that lack or have only a faint 155-kDa protein band. B, Normalized peak tail \( I_{K_{\text{11.1}}} \) measured from HEK293 cells transiently expressing WT- or 22 LQT2-Kv11.1 channels. The solid bars show the mean peak tail \( I_{K_{\text{11.1}}} \), measured from cells expressing a Western blot pattern similar to WT-Kv11.1 channels (Figure 1A, top row). Shaded bars show the mean peak tail \( I_{K_{\text{11.1}}} \) measured from cells expressing protein bands that have a class 2 phenotype (Figure 1A, middle and bottom rows).

Figure 1. A, Representative Western blot analyses of HEK293 cells transiently expressing WT-KV11.1 or LQT2-KV11.1 channels. The top row shows WT- and 6 LQT2-KV11.1 channels that contain both the 135- and 155-kDa protein bands. The middle and bottom rows show 28 LQT2-KV11.1 channels that lack or have only a faint 155-kDa protein band. B, Normalized peak tail \( I_{K_{\text{11.1}}} \) measured from HEK293 cells transiently expressing WT- or 22 LQT2-KV11.1 channels. The solid bars show the mean peak tail \( I_{K_{\text{11.1}}} \), measured from cells expressing a Western blot pattern similar to WT-KV11.1 channels (Figure 1A, top row). Shaded bars show the mean peak tail \( I_{K_{\text{11.1}}} \) measured from cells expressing protein bands that have a class 2 phenotype (Figure 1A, middle and bottom rows).

The 135- and 155-kDa bands. In contrast, the Western blot analyses of the 28 LQT2-KV11.1 channels show protein bands at 135 kDa, whereas the 155-kDa protein band is absent or faint. These patterns were confirmed in a minimum of 3 Western blots for each mutation. These data are summarized in Table 1 in the column labeled class 2, where a “Y” indicates a trafficking-deficient (class 2) phenotype with an absent or faint 155-kDa protein band and an “N” indicates the presence of a 155-kDa protein band similar to WT-KV11.1 channels. These data suggest that most LQT2-KV11.1 channels are trafficking deficient.

We next measured \( I_{K_{\text{11.1}}} \) recorded from cells transiently expressing WT-KV11.1 or 22 LQT2-KV11.1 channels. From the holding potential, cells were depolarized to 50 mV for 5 seconds, and then the peak tail \( I_{K_{\text{11.1}}} \) was measured at a test pulse to \(-120\) mV for 3 seconds. Figure 1B shows the peak tail \( I_{K_{\text{11.1}}} \) amplitudes (\( n \geq 4 \) cells per group) normalized to the mean peak value recorded from cells expressing WT-KV11.1 channels. The solid bars show the mean peak tail \( I_{K_{\text{11.1}}} \) measured from cells expressing WT-KV11.1 or from cells expressing R328C-, P347S-, T436M-, and R922W-KV11.1, all of which had a protein trafficking phenotype similar to WT-KV11.1 channels (Figure 1A, top row). In cells expressing WT-KV11.1 channels, the mean peak tail \( I_{K_{\text{11.1}}} \) was \(-151.9 \pm 13.1\) pA/pF, which was not different from the mean peak tail \( I_{K_{\text{11.1}}} \) values recorded for the 4 LQT2-KV11.1 channels \((-137.8 \pm 17.6, -170.5 \pm 26.8, -181.0 \pm 23.7, \) and \(-146.9 \pm 30.6\) pA/pF, respectively, \( P > 0.05 \) compared with WT \( K_{\text{11.1}} \) for each). We did not study T421M- or G628S-KV11.1 channels, which also have protein trafficking phenotypes similar to WT-KV11.1 channels (Figure 1A, top row), because these channels have been reported to cause loss of KV11.1 channel function by altering gating and permeation, respectively.\(^{4,35}\) The shaded bars show the normalized mean peak tail \( I_{K_{\text{11.1}}} \) measured from the other 18 class 2 LQT2-KV11.1 channels studied. As shown in Figure 1B, these mutations expressed a small \( I_{K_{\text{11.1}}} \), and the normalized mean peak tail \( I_{K_{\text{11.1}}} \) values typically were reduced by 80% to 90%.

Because LQT2 follows a dominant inheritance pattern, patients have both mutant and WT KCNH2 alleles. We tested whether coexpression of WT with class 2 LQT2 cDNAs would alter the trafficking-deficient phenotype. Figure 2A shows representative Western blot analyses of cells expressing WT-KV11.1 channels or coexpressing WT- and the 28 class 2 LQT2-KV11.1 channels. Because some patients who carried the F640V KCNH2 allele were heterozygous with the common polymorphism K897T, we also expressed K897T-carrying WT-KV11.1 was normalized to the mean peak values.
Four Putative LQT2 Mutations Do Not Alter \( I_{Kv11.1} \)

We studied in greater detail the electrophysiology of the R328C-, P347S-, T436M-, and R922W-Kv11.1 channels, which have a protein trafficking phenotype and \( I_{Kv11.1} \) density similar to WT-Kv11.1 channels. Figure 3A shows representative families of \( I_{Kv11.1} \) traces. From the holding potential, cells were prepulsed in 10-mV increments to \(-70 \) to 70 mV for 4 seconds, and then a test pulse was applied to \(-50 \) mV for 5 seconds to measure tail current. These data demonstrate that \( I_{Kv11.1} \) traces recorded from cells expressing WT-, R328C-, P347S-, T436M-, or R922W-Kv11.1 channels had similar properties. Figure 3B shows the mean normalized peak tail \( I_{Kv11.1} \) recorded during the test pulse to \(-50 \) mV plotted as a function of the prepulse voltage. The data were fit with a Boltzmann function (solid line). Figure 3C shows the mean slope factor \((k, \text{mV/e-fold change})\) and V1/2 (mV) values calculated with the individual Boltzmann fits to I-V relations recorded from cells expressing WT-, R328C-, P347S-, T436M-, or R922W-Kv11.1 channels. There were no differences in the \( k \) or V1/2 values (\( n = 4 \), 5, \( P > 0.05 \)). The deactivation rate of \( I_{Kv11.1} \) \((\tau_{\text{deactivation}})\) was determined by fitting the decay of \( I_{Kv11.1} \) recorded during the test pulse as a double exponential process. Figure 3D shows the mean fast and slow time constants \( (\tau_{\text{fast}} \text{ and } \tau_{\text{slow}}) \) were not different (\( n = 4 \), 5, \( P > 0.05 \)). The relative amplitudes of the slow exponential component \((A_{\text{slow}}/A_{\text{slow}} + A_{\text{fast}})\) also were not different (data not shown). Our findings (Figure 1B and Figure 3) suggest that compared with cells expressing WT-Kv11.1 channels, the R328C-, P347S-, T436M-, or R922W-Kv11.1 channels did not reduce \( k_{\text{Kv11.1}} \) or alter \( V_{1/2} \) channel function.

Class 2 LQT2 Phenotype for Most Class 2 LQT2-Kv11.1 Channels Is Corrected in Different Cell Culture Conditions

For a few class 2 LQT2-Kv11.1 channels, incubation of cells at reduced culture temperatures, in drugs that block \( I_{Kv11.1} \) or in the sarcoplasmonic/endooplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) inhibitor thapsigargin, has been shown to improve Golgi processing and surface membrane expression. We therefore studied whether protein processing could be corrected in the 28 class 2 LQT2-Kv11.1 channels. Figure 4A shows representative Western blot analyses of cells stably expressing the I31S-, A422T-, D456Y-, V612L-, F640V-, or R752W-Kv11.1 channels cultured in control conditions \((37^\circ\text{C}), 27^\circ\text{C} \) (24 hours), or in E4031 \((10 \text{ \text{mM}}, 24 \text{ hours})\), and in thapsigargin \((1 \text{ \text{mM}}, 24 \text{ hours})\). In control conditions, only the immature protein band at 135 kDa was present, as expected for class 2 channel mutations. For the I31S-Kv11.1 channels, protein processing was not corrected in any of the culture conditions. The processing of the A422T-Kv11.1 channels was selectively corrected in cells incubated at 27°C or in E4031 with the appearance of the mature protein band at 155 kDa. The processing of the D456Y-Kv11.1 channels was corrected in cells incubated at 27°C, in E4031 or in thapsigargin. The processing of the V612L-Kv11.1 channels was corrected in cells incubated in E4031 and weakly corrected in thapsigargin. The processing of the F640V-Kv11.1 channels was corrected in cells incubated in E4031. The processing of the R752W-Kv11.1 channels was only corrected by incubation in 27°C. For each mutation, a minimum of 3 Western blot analyses were performed. Figure 4B shows representative \( I_{Kv11.1} \) traces (voltage-clamp protocol the same as in Figure 1B) recorded from the same cell lines stably expressing the I31S-, A422T-, D456Y-, V612L-, F640V-, or R752W-Kv11.1 channels 1 to 2 hours after removal from culture conditions. These results demonstrate that conditions that increase \( V_{1/2} \) processing to the mature channel also increase \( I_{Kv11.1} \). Data for the mean peak tail \( I_{Kv11.1} \) are summarized in Table 2. WT \( I_{Kv11.1} \) was not significantly increased by any of the culture conditions. In contrast, \( I_{Kv11.1} \) for the different mutations was selectively increased by the culture conditions that corrected protein processing. All 28 class 2 channels were studied similarly with Western blot.
analyses, and these data are summarized in Table 1 for culture at 27°C, in E4031, or in thapsigargin. An increase in the 155-kDa protein band density is indicated with a (H11001). These data show that the protein processing of 16 of 28 class 2 channels increased in cells incubated in 27°C, the protein processing of 17 of 28 class 2 channels increased in cells incubated in E4041, and the protein processing of 9 of 28 class 2 channels increased in cells incubated in thapsigargin. None of the different culture conditions were able to correct protein processing for 9 of the class 2 LQT2-Kv11.1 channels.

Thapsigargin has been reported to correct the protein-processing defect of specific mutations in both cystic fibrosis and LQT2. The drug curcumin is a SERCA inhibitor that has been reported to correct the trafficking of the common cystic fibrosis–causing mutation ΔF508-CFTR.40–42 We tested whether cell culture in curcumin (up to 20 μmol/L, 24 hours) would alter the trafficking abnormality in the LQT2-Kv11.1 mutations G601S and F805C, which respond to cell culture in thapsigargin with increased trafficking. Curcumin did not correct protein processing or increase $I_{\text{Kv11.1}}$ in cells expressing these mutations (data not shown).

**Discussion**

One purpose of the present study was to determine whether there is a dominant mechanism that underlies the loss of function of missense LQT2-Kv11.1 channels. Our findings demonstrate that 28 LQT2-Kv11.1 channels have a trafficking-deficient phenotype (class 2), 1 LQT2-Kv11.1 channel (T421M) disrupts gating (class 3), 1 LQT2-Kv11.1 channel (G628S) disrupts permeation (class 4), and 4 LQT2-Kv11.1 channels do not appear to alter function. Of the 34 LQT2 missense mutations studied, 24 cause a nonconserved change in the hydrophobicity of the amino acid residue side chain, and there is no apparent correlation between the type of amino acid substitution and the Kv11.1 trafficking phenotype. In contrast, the location of amino acid substitutions in putative Kv11.1 α-helical or β-sheet domains appears to correlate with the class 2 phenotype. These findings are summarized in the Kv11.1 α-subunit cartoon in Figure 5, which shows putative secondary structures as cylinders (α-helices) or bars (β-sheets). The placement of the Per-ARNT-Sim (PAS) domain structures in the N-terminus is based on the crystal structure of the Kv11.1 N-terminal segment, the placement of the transmembrane segments is based on...
In the present studies, we found 6 channel mutations that on Western blot analyses expressed both the immature and mature protein bands similar to WT-Kv11.1 channels and thus had a similar trafficking phenotype. Of these, T421M-Kv11.1 expresses $I_{Kv11.1}$ that has altered gating, and G628S-Kv11.1 inserts correctly into the cell surface membrane but does not generate $I_{Kv11.1}$ presumably because of its location within the K$^+$ selectivity filter. The other 4 (R328C-, P347S-, T436M-, and R922W-Kv11.1) have $I_{Kv11.1}$ densities and gating properties that are similar to WT-Kv11.1 channels. These findings raise the question of whether these 4 channels are disease-causing mutations in the patient kindreds in which they were identified, or whether these are uncommon sequence variants with no functional significance. In fact, P347S-Kv11.1 was recently identified with a heterozygous frequency of 0.5% in a control patient population. This supports our conclusion that P347S-, along with R328C-, T436M-, and R922W-Kv11.1 channels are likely to represent uncommon sequence variants. The present data emphasize the role of functional expression studies in correctly identifying putative disease-causing mutations and the need for extensive control group studies to understand the population burden of genetic variation.

There has been increasing interest in recent years directed toward identifying techniques that correct trafficking of class 2 LQT2-Kv11.1 mutations. Only a small number of these mutations have been studied. The data in Table 1 (see also Figure 4) show that correction of LQT2-Kv11.1 trafficking occurs in the majority of class 2 LQT2 mutations we studied. Furthermore, the different cell culture conditions produced different patterns of correction. For example, D456Y-Kv11.1 channels showed correction by culture at 27°C, in E4031, or in thapsigargin, whereas I31S-Kv11.1 channels did not undergo correction in any culture condition. The most common pattern was that correction occurred with both culture in E4031 or culture at reduced temperature. A total of 8 patterns were present (Table 1). It is tempting to speculate that these different patterns of correction represent distinct steps at which mutations disrupt Kv11.1 channel biogenesis, and correction improves channel biogenesis by stabilizing the channel protein in configurations that facilitate proper trafficking.

### TABLE 2. Summary of Mean Peak Tail $I_{Kv11.1}$ (pA/pF) Recorded From HEK293 Cells Stably Expressing WT-Kv11.1 Channels or Class 2 LQT2-Kv11.1 Channels in Control Conditions (37°C), at 27°C (24 Hours), in E4031 (10 μmol/L; 24 Hours) With 1-Hour Washout, or in Thapsigargin (1 μmol/L; 24 Hours)

<table>
<thead>
<tr>
<th>LQT2</th>
<th>Control 27°C</th>
<th>E4031 24 Hours</th>
<th>Thapsigargin 24 Hours</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>83.0 ± 10.7</td>
<td>103.1 ± 14.3</td>
<td>80.2 ± 2.2</td>
<td>5</td>
</tr>
<tr>
<td>I31S</td>
<td>5.7 ± 0.5</td>
<td>6.0 ± 0.4</td>
<td>5.2 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>A422T</td>
<td>6.5 ± 1.2</td>
<td>46.8 ± 12.9†</td>
<td>26.6 ± 5.1†</td>
<td>4–5</td>
</tr>
<tr>
<td>D456Y</td>
<td>7.8 ± 0.7</td>
<td>52.3 ± 10.6†</td>
<td>21.5 ± 2.4†</td>
<td>9–12</td>
</tr>
<tr>
<td>V612L</td>
<td>7.0 ± 3.0</td>
<td>7.0 ± 8.0</td>
<td>26.9 ± 3.6†</td>
<td>7–9</td>
</tr>
<tr>
<td>F640V</td>
<td>7.6 ± 0.9</td>
<td>5.7 ± 0.6</td>
<td>45.9 ± 3.6†</td>
<td>4</td>
</tr>
<tr>
<td>R752W</td>
<td>4.9 ± 0.8</td>
<td>7.0 ± 16.1†</td>
<td>5.0 ± 0.5</td>
<td>8–9</td>
</tr>
</tbody>
</table>

*P < 0.05 and †P < 0.01.
The present data also show that coexpression of class 2 LQT2 cDNAs with WT-Kv11.1 cDNA retained the trafficking-deficient phenotype. Recently, pharmacological correction was studied in a heteromeric channel (coassembled WT-Kv11.1 and N470D-Kv11.1 subunits) in a HEK293 expression system. Similar to homomeric N470D-Kv11.1 channels, heteromeric channels were trafficking deficient, and culture in E4031 resulted in correction of the trafficking-deficient phenotype. Thus, the presence of WT subunits does not prevent correction of the class 2 LQT2-Kv11.1 phenotype.

There are potential limitations to this work. We studied 34 LQT2 missense mutations that span the length of the Kv11.1 channel, which represents only ~25% of the missense mutations presently associated with LQT2. We did not study less common types of mutations (eg, truncations, deletions, etc.). We used a human heterologous expression system to study ion channel protein trafficking abnormalities and their correction, and thus, extrapolation to human disease must be done with caution. These results are not specific to HEK293 cells, however, because we have observed similar trafficking-deficient phenotypes and correction patterns in COS-1 (transformed African Green Monkey fibroblast-derived cell line) cells (data not shown).

In summary, we studied 34 missense mutations to determine whether there was a dominant mechanism that resulted in the loss of Kv11.1 channel function associated with LQT2. Of these, 30 channels showed loss of function, with 28 (93%) having a trafficking-deficient or class 2 phenotype. Thus, in this LQT2 model, the dominant mechanism by which missense mutations cause loss of function is to generate trafficking-deficient channels. For the majority of these class 2 LQT2-Kv11.1 channels, their protein processing could be corrected by 1 or more cell culture conditions. The finding that many class 2 mutations undergo correction to improve channel expression in the surface membrane suggests that further studies to develop clinically relevant strategies to correct protein trafficking abnormalities may benefit families with LQT2.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Congenital long-QT syndrome (LQTS) type 2 involves mutations in the gene *hERG* that encodes the Kv11.1 potassium channel. The loss of its ionic current has been attributed to several mechanisms, including disruption of channel synthesis (class 1), protein trafficking (class 2), gating (class 3), or permeation (class 4). We studied 34 missense (single amino acid substitution) mutations identified in patients with type 2 LQTS. When expressed in a human cell line, 28 channels had a trafficking-deficient (class 2) phenotype, 1 channel had abnormal gating, 1 channel had abnormal permeation, and 4 channels had a normal phenotype (which suggests that these channels are uncommon sequence variants). Thus, abnormal protein trafficking is the most common mechanism for loss of ionic current in these *hERG* mutations. These data suggest that therapeutic strategies to correct protein trafficking abnormalities, if they can be developed, may offer clinical benefits for some patients with LQTS.
Most LQT2 Mutations Reduce Kv11.1 (hERG) Current by a Class 2 (Trafficking-Deficient) Mechanism
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