Allelic Variants in Long-QT Disease Genes in Patients With Drug-Associated Torsades de Pointes

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Background—DNA variants appearing to predispose to drug-associated “acquired” long-QT syndrome (aLQTS) have been reported in congenital long-QT disease genes. However, the incidence of these genetic risk factors has not been systematically evaluated in a large set of patients with aLQTS. We have previously identified functionally important DNA variants in genes encoding K⁺ channel ancillary subunits in 11% of an aLQTS cohort.

Methods and Results—The coding regions of the genes encoding the pore-forming channel proteins KvLQT1, HERG, and SCN5A were screened in (1) the same aLQTS cohort (n=92) and (2) controls, drawn from patients tolerating QT-prolonging drugs (n=67) and cross sections of the Middle Tennessean (n=71) and US populations (n=90). The frequency of three common nonsynonymous coding region polymorphisms was no different between aLQTS and control subjects, as follows: 24% versus 19% for H558R (SCN5A), 3% versus 3% for R34C (SCN5A), and 14% versus 14% for K897T (HERG). Missense mutations (absent in controls) were identified in 5 of 92 patients. KvLQT1 and HERG mutations (one each) reduced K⁺ currents in vitro, consistent with the idea that they augment risk for aLQTS. However, three SCN5A variants did not alter I_{Na}, which argues that they played no role in the aLQTS phenotype.

Conclusions—DNA variants in the coding regions of congenital long-QT disease genes predisposing to aLQTS can be identified in ≈10% to 15% of affected subjects, predominantly in genes encoding ancillary subunits. (Circulation. 2002;105:1943-1948.)

Key Words: arrhythmia ▪ genetics ▪ drugs ▪ long-QT syndrome

Marked QT interval prolongation and the morphologically distinctive ventricular tachycardia torsades de pointes (TdP) develop in 1% to 8% of patients receiving QT-prolonging antiarrhythmic drugs, such as quinidine, sotalol, ibutilide, and dofetilide.1 Drug-associated QT prolongation and TdP (the “acquired” long-QT syndrome; aLQTS) are also well recognized during therapy with “noncardiovascular” agents, including certain antihistamines,2,3 antibiotics,4,5 and antipsychotics,6 among others. Risk factors implicated in the development of this complication of drug therapy include female sex, hypokalemia, congestive heart failure, left ventricular hypertrophy, recent conversion from atrial fibrillation, marked elevation of plasma drug concentration (especially with noncardiovascular therapies), and baseline QT prolongation.7-9 Despite identification of such risk factors, however, the development of TdP remains unpredictable in an individual subject. This highly variable response to drug therapy suggests, in turn, that additional as-yet-unidentified risk factors may contribute.

Among known gene carriers for the congenital long-QT syndrome, females are more likely to be symptomatic, and a similar female preponderance is found in aLQTS.7,10 Similarly, the congenital long-QT syndrome includes electrocardiographic features nearly identical to those of aLQTS.11 The recognition of these parallels between the congenital and drug-associated clinical syndromes thus raises the possibility that genetic factors may modulate susceptibility to the drug-associated form.12-15 With the identification of mutations in genes encoding pore-forming ion channels (KvLQT1 [KCQ1], HERG [KCNH2], and SCN5A) and the ancillary proteins minK (KCNE1) and MiRP1 (KCNE2) in the congenital syndrome has come the opportunity to determine whether individuals developing drug-associated TdP harbor allelic variants in these genes. Indeed, sporadic case reports do identify subclinical congenital long-QT syndrome in occasional patients with the drug-associated form. However, the proportion of patients in whom potentially contributory DNA
variants are present has not yet been determined, and this is the goal of the present, and previous,16,17 studies.

Methods

Patient Ascertainment

Drug-associated TdP was diagnosed in patients receiving a recognized culprit drug and developing typical electrocardiographic features, including QT prolongation or deformity, pause-dependent onset, and polymorphic ventricular tachycardia lasting >10 beats in the 150 to 240 beats/min range. Faster polymorphic ventricular tachycardia was classified as ventricular fibrillation, and such patients were not included. Cases are included here both from Vanderbilt University Medical Center (n = 60) and elsewhere (n = 32); in all cases, electrocardiographic documentation of the event and of the inciting drug was required. These 92 patients include 27 patients displaying marked QT prolongation from normal values to >600 ms shortly after initiation of a culprit drug, that renormalized with drug withdrawal. A blood sample was obtained from each patient for extraction of DNA from lymphocytes. DNA was harvested by, and archived by, the Vanderbilt Program in Human Genetics. For Vanderbilt patients, informed consent approved by the Institutional Review Board was obtained. For non-Vanderbilt patients, local Human Subjects approval was obtained. The clinical characteristics of the population included in this study are presented in Table 1.

Screening for Allelic Variants

Single-stranded conformational polymorphism (SSCP) analysis was used to identify polymorphisms in the coding region of the three long-QT disease genes SCN5A, KvLQT1 (KCNH), and HERG (KCNH2). We have previously reported the incidence of allelic variants in minK (KCN1E) and in MiRP1 (KCN1E).16,17

Most PCR primer sets were those previously reported,18–21 derived from intronic regions and designed to generate fragments small enough (<350 bp in all cases) for SSCP analysis. For exons >350 bp (exons 12, 17, and 28 in SCN5A; exons 4, 6, and 7 in HERG; and exons 1 and 16 in KvLQT1), primer sets that generated overlapping smaller fragments were used. New primers were designed to amplify GC-rich coding regions of HERG (exons 1 and 12; nucleotides 184 to 259 and 2876 to 3148 of GenBank accession no. NM_000238). These were, for exon 1, 5'–GCTCAGGATGCGGTGCGGA–3' (forward primer) and 5'–CGCTGCACACACACGTCCGATCCC–3' (reverse primer), and for exon 12, 5'–TTTCCACACAGAGCCAGGATGCCGGTGCGGA–3'.

Values are given as percentages.

| Female | 66 |
| Risk factors | |
| Heart failure | 25 |
| LH | 14 |
| Hypokalemia | 18 |
| Hypomagnesemia | 9 |
| No identifiable risk factor | 11 |

Concurrent therapies

Digoxin 29
Calcium channel-blocker 14
β-Blocker 11

Culprit drugs

Antiarrhythmics 77
Quinidine 32
Sotalol 17
Nonantiarrhythmics 23

Data Analysis

Allelic variants are reported here that resulted in amino acid changes in the encoded proteins. Mutations reported in the Results section were by definition detected only in affected individuals and not in any of the control populations described below. We also identified polymorphisms, defined as variants present in >1% of the population, in the coding region of SCN5A and HERG. The frequency of these polymorphisms was compared with those in the following three reference populations: (1) patients receiving a QT-prolonging antiarrhythmic agent and not developing marked QT prolongation or TdP (n = 67); (2) a set of randomly selected, unrelated individuals with ethnicities representing those of the middle Tennessee area (n = 71; 48 white and 23 black); and (3) a subset of the 450-individual anonymized polymorphism discovery resource22 available from the National Human Genome Research Institute (n = 90). Because polymorphism frequency can vary by ethnicity, only samples obtained from patients identified at Vanderbilt University Medical Center were included in this analysis. Thus, a total of 456 control alleles were compared with 120 alleles in Vanderbilt patients with aLQTS.

Electrophysiology

Mutations identified in aLQTS subjects were engineered into wild-type channels in mammalian expression vectors (pSi), transfected with a bicistronic plasmid also encoding green fluorescent protein (GFP) into tsa-201 cells (SCN5A) or CHO cells (K+ channels) using lipofectamine, and studied using standard whole-cell electrophysiological methods, as previously reported.23,24 The transfection mixture included 2 μg of ion channel plasmid, 1 μg of GFP/prCev, and 12 μL of lipofectamine reagent in 0.5 mL serum-free DMEM for 6 to 8 hours, after which the standard medium was restored for 48 hours. Cells exhibiting green fluorescence were chosen for electrophysiological analysis. Sodium channel constructs were cotransfected with an equimolar ratio of the Na+ channel β1 subunit, and KCN1E (minK) was cotransfected with KvLQT1 constructs to record If.

Results

Mutations were identified in 5 of 92 patients, as follows: 3 in SCN5A, 1 in KvLQT1, and 1 in HERG (Figure 1; Table 2). The SCN5A and HERG mutations have not been previously identified.
TABLE 2. Mutations and Polymorphisms Identified

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide Change*</th>
<th>Amino Acid Change</th>
<th>Region</th>
<th>Culprit Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>KvLQT1</td>
<td>C1343G</td>
<td>P448R</td>
<td>C-terminal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C1747T</td>
<td>R583C</td>
<td>C-terminal</td>
<td>Dofetilide</td>
</tr>
<tr>
<td>HERG</td>
<td>C2350T</td>
<td>R784W</td>
<td>C-terminal</td>
<td>Amiodarone</td>
</tr>
<tr>
<td></td>
<td>A2690C</td>
<td>K987T</td>
<td>C-terminal</td>
<td></td>
</tr>
<tr>
<td>SCN5A</td>
<td>C100T</td>
<td>R34C</td>
<td>N-terminal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G1844A</td>
<td>G615E</td>
<td>DI/DII</td>
<td>Quinidine</td>
</tr>
<tr>
<td></td>
<td>C1852T</td>
<td>L618F</td>
<td>DI/DII</td>
<td>Quinidine</td>
</tr>
<tr>
<td></td>
<td>T3748C</td>
<td>F1250L</td>
<td>DII S2</td>
<td>Sotalol</td>
</tr>
<tr>
<td></td>
<td>A1673G</td>
<td>H558R</td>
<td>DI/DII</td>
<td></td>
</tr>
</tbody>
</table>

*Mutations are identified in bold and polymorphisms in italics.

reported, whereas the KvLQT1 has.25 Of these five mutations, four are located in the intracellular domains of the α-subunits, as follows: two in the DI-II linker of the sodium channel, and two in the C termini of the KvLQT1 and HERG proteins.

The KvLQT1 variant C1343G, leading to P448R in the C terminus, was identified in a Japanese aLQTS subject and was absent in controls used here; however, this variant has now been identified in 22% to 28% of Japanese subjects (M. Horie and W. Shimizu, personal communication, December, 2001) and thus represents a common polymorphism in that population. Three other nonsynonymous coding region polymorphisms were identified in this study; two have been previously reported,26,27 whereas the third, C100T leading to R34C, is new. Table 3 presents the frequency of the polymorphisms in the Vanderbilt University Medical Center population compared with the three reference populations; there were no significant differences in frequency of these polymorphisms in the patient group versus the three reference groups. Three other coding region polymorphisms have been reported rarely in Japanese subjects27 and were not identified here. Figure 1 shows the locations of the mutations and polymorphisms on the ion channel proteins. Those that we and others have previously reported13–17 are also shown.

Expression of the R583C KvLQT1 mutant did result in typical \( I_{K_{\text{aLQTS}}} \) or \( I_{K_{\text{c}}} \) (when KCNE1 was coexpressed). However, \( I_{K_{\text{c}}} \) generated by the mutant (Figure 2) was reduced by >50% compared with wild type (from 60.4±4.6 to 25.6±4.2 pA/pF, pulses to +60 mV, \( P<0.01 \)) and the voltage dependence of activation was shifted positively by 19.6 mV (from 7.5±2.6 to 27.1±2.4, \( P<0.01 \)). Both of these mechanisms result in less repolarizing current at plateau potentials. \( I_{K_{\text{aLQTS}}} \) generated by the mutant was also significantly reduced (24.6±3.0 versus 17.7±4.0 pA/pF, \( P<0.05 \)), and the voltage dependence of activation was also shifted rightward (−9.8±1.2 versus 2.1±1.9 mV, \( P<0.05 \)). The R784W HERG mutation produced similar effects, as follows: although the mutant did generate current, it was reduced by \( \approx75\% \) compared with that of the wild-type HERG (37.6±6.1 versus 9.5±1.1, tail currents at −40 mV after a 1-second pulse to +20 mV, \( P<0.01 \)), and its voltage dependence of activation was shifted positively (Figure 3). By contrast, the three sodium channel mutations did not alter \( I_{K_{\text{c}}} \) peak amplitude or the voltage dependence or the voltage dependence of \( I_{K_{\text{c}}} \) activation or inactivation (Table 4). Further, no “pedestal” of noninactivating current (seen with most other SCN5A LQTS mutations) was observed.

### Discussion

Previous reports have identified isolated cases of congenital long QT-associated mutations in patients presenting with apparent drug-associated TdP. This report is the first to systematically analyze a large data set of patients with drug-associated TdP to determine the frequency with which such mutations occur, and hence their contribution to risk for the long-QT syndrome. We have also analyzed the incidence of allelic variants in \( minK \) and \( MIRP1 \) in this data set. In the case of \( MIRP1 \), we identified three mutations and a fourth individual with a polymorphism (T8A) also detected in 1.5% of control subjects.17 No mutations in \( minK \) were identified; however, preliminary studies indicate that a polymorphism (D85N) is more common (7%) among patients with drug-associated TdP than among controls (2% to 4%).14 Thus, these previous findings, along with the current findings in the pore-forming ion channel proteins (α subunits), indicate that mutations predisposing to aLQTS were identified in 5 of 92 subjects and that polymorphisms in \( MIRP1 \) (and possibly \( minK \)), present in 2% to 7% of the population, may also add to risk. Hence, allelic variants contributing to risk can be identified in 10% to 15% of patients presenting with drug-associated TdP.

Studies of congenital long-QT kindreds have previously identified mutation carriers who nevertheless have normal QT intervals. Priori et al28 reported kindreds that included both individuals with the manifest long-QT syndrome and mutation carriers with normal QT intervals. The conclusion, that a normal QT interval does not rule out the presence of a disease-associated mutation, supports earlier inferences derived from analysis of ECG data in large kindreds in which obligate gene carriers could be identified.29 Another situation in which mutation carriers with apparently normal ECGs have been identified are kindreds with the autosomal recessive Jervell-Lange-Neilsen syndrome.30,31 Here, severe symptoms arise in probands, who inherit two abnormal alleles, one from each parent; thus, although parents are phenotypically normal, they carry long-QT syndrome–associated mutations. It has been speculated that such asymptomatic mutation carriers might be at increased risk for TdP on exposure to drugs or other stressors, and indeed Splawski et al31 reported sudden death in an otherwise healthy young Jervell-Lange-Neilsen parent with severe psychic stress.
Multiple mechanisms have been identified in in vitro studies of allelic variants associated with drug-related TdP. Some mutations in MiRP1 appear to reduce current expressed at the cell surface, even in the absence of drugs. By contrast, two variants in MiRP1 (T8A and Q9E) increase sensitivity of the resultant channels to drug block. R555C, in the C terminus of KvLQT1, has been previously identified in LQTS as a “forme fruste” of the congenital long-QT syndrome; in vitro, it does generate current, but its voltage dependence of activation is markedly shifted to positive potentials, suggesting it generates less $I_{Ks}$ at usual plateau voltages. The R583C variant we identified here produced a very similar in vitro phenotype. These changes are modest compared with those observed in patients with manifest long-QT syndrome, consistent with the idea that functionally “mild” mutations may only manifest QT prolongation with additional pharmacological or other stress. Similarly, HERG R784W decreased currents, but did not abolish them. The lack of a discernible in vitro phenotype with the three sodium channel mutations studied here (although activation of the F1250L variant did trend toward a significant shift) highlights the conclusion that identification of a DNA variant does not automatically indicate that the variant is responsible for a clinical phenotype. The inclusion of as-yet-uncharacterized mutations in the catalogue of mutations causing the congenital long-QT syndrome may thus need to be reconsidered.

Polymorphisms in the human genome are common, and many have been implicated in variability in physiology or in drug response, in a wide range of diseases. Although the

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**Figure 2.** Functional consequences of R583C in KvLQT1. A, Currents recorded with coexpression of wild-type KvLQT1 with KCNE1 (minK), using clamp protocol shown in inset. B, Currents recorded with coexpression of R583C KvLQT1 with KCNE1. C, Voltage dependence of activation. D, Voltage dependence of tail currents.

**Figure 3.** Functional consequences of R784W in HERG. A, Currents recorded with expression of wild-type (wt) HERG. B, Currents recorded with R784W HERG. C, Voltage dependence of activation. D, Voltage dependence of tail currents.
frequency of polymorphisms identified here was no different among patients and multiple control groups, further study will be required to establish whether ion channel polymorphisms, such as these, might contribute to variability in the baseline QT interval or in the long-QT phenotype, particularly in the background of other allelic variants. Polymorphisms are also well recognized in noncoding regions, where they may contribute to regulation of gene function. Cardiac repolarization is a highly complex process, depending on the function of multiple gene products, including not only the ion channels studied here, but also other ion channels and multiple other proteins, such as those subserving transport, anchoring, phosphorylation, or other regulatory functions. Thus, allelic variants in any of these might contribute to variability in baseline QT interval and the extent to which the QT interval is prolonged by drug therapy. One emerging view suggests that prediction of such a drug response may require identification of large subsets of polymorphisms (dozens or hundreds) that, together, predict high or low risk. Very large numbers of affected subjects, and appropriate controls, will be required to test this hypothesis.

Although multiple drugs have been implicated in the genesis of TdP, virtually all act by blocking a specific cardiac ion current, \( I_{\text{Kr}} \). The extent to which this results in QT prolongation and TdP is highly variable among subjects. We have suggested that such variability arises because of redundancy of repolarizing currents, a phenomenon we have termed “repolarization reserve.” The multiple risk factors that have been implicated in drug-associated TdP all likely act by inhibiting function of these redundant repolarizing mechanisms. Thus, we suggest that apparently similar patients respond differently to challenge by a QT-prolonging drug because of differences in repolarization reserve. The present study identifies subclinical mutations in long-QT disease genes as a further mechanism reducing repolarization reserve, and hence increasing risk for TdP.

Even a small risk for TdP can modify the regulatory view of risks versus benefits for noncardiovascular therapies, and indeed the postmarketing recognition of TdP has resulted in multiple drug withdrawals or relabeling in the last 5 years. This represents one of a handful of situations in which studies of an unusual genetic disease are being used to identify mechanisms in rare, but nevertheless clinically important, forms of drug toxicity. The algorithm we lay out here, testing genes associated with a congenital disease as candidates for mediating drug toxicity, is one that may well be repeated in studies of other forms of drug toxicity, such as hepatitis, thrombocytopenia, or bone marrow suppression. In general, such reactions have been termed “idiopathic,” and further efforts to understand a molecular and genetic basis are therefore highly desirable.

In summary, analysis of a relatively large set of patients with drug-associated TdP has revealed mutations in the three genes encoding cardiac ion channels in 5 of 92 patients. Taken together with our previous findings in genes encoding function-modulating subunits, our findings indicate that non-synonymous coding region variants in the five long-QT disease genes identified to date are present in 10% to 15% of patients with drug-associated TdP. The current estimate is that LQTS mutation carriers are present in 1 of 1000 to 3000 individuals. Thus, the present study suggests that preprescription genotyping for these allelic variants may well markedly reduce the incidence of drug-associated TdP. Prospective studies will be required to establish this point.

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**References**


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**TABLE 4. Characteristics of Wild-Type and Mutant Sodium Channels**

<table>
<thead>
<tr>
<th>n</th>
<th>Amplitude (pA/pF at -30 mV)</th>
<th>( V_{1/2} ) Activation, mV</th>
<th>( k_{ax} )</th>
<th>( V_{1/2} ) Inactivation, mV</th>
<th>( k_{inact} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>7</td>
<td>260±48</td>
<td>-54.3±3.5</td>
<td>6.7±3.1</td>
<td>-98.3±0.3</td>
</tr>
<tr>
<td>G615E</td>
<td>7</td>
<td>244±32 (P=0.77)</td>
<td>-53.8±4.7 (P=0.90)</td>
<td>5.5±4.0</td>
<td>-95.9±0.5 (P=0.63)</td>
</tr>
<tr>
<td>L618F</td>
<td>7</td>
<td>272±54 (P=0.86)</td>
<td>-59.2±5.5 (P=0.24)</td>
<td>5.5±5.0</td>
<td>-94.4±0.3 (P=0.36)</td>
</tr>
<tr>
<td>F1250L</td>
<td>7</td>
<td>247±49 (P=0.84)</td>
<td>-46.8±4.9 (P=0.06)</td>
<td>7.4±4.3</td>
<td>-92.1±0.6 (P=0.12)</td>
</tr>
</tbody>
</table>

\( P \) values are vs wild type.


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