Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene

Arthur J. Moss, MD*; Wataru Shimizu, MD, PhD*; Arthur A.M. Wilde, MD, PhD*; Jeffrey A. Towbin, MD*; Wojciech Zareba, MD, PhD; Jennifer L. Robinson, MS; Ming Qi, PhD; G. Michael Vincent, MD; Michael J. Ackerman, MD, PhD; Elizabeth S. Kaufman, MD; Nynke Hofman, MSc; Rahul Seth, MD; Shiro Kamakura, MD, PhD; Yoshihiro Miyamoto, MD, PhD; Ilan Goldenberg, MD; Mark L. Andrews, BBA; Scott McNitt, MS

Background—Type-1 long-QT syndrome (LQTS) is caused by loss-of-function mutations in the KCNQ1-encoded I<sub>Ks</sub> cardiac potassium channel. We evaluated the effect of location, coding type, and biophysical function of KCNQ1 mutations on the clinical phenotype of this disorder.

Methods and Results—We investigated the clinical course in 600 patients with 77 different KCNQ1 mutations in 101 proband-identified families derived from the US portion of the International LQTS Registry (n=425), the Netherlands’ LQTS Registry (n=93), and the Japanese LQTS Registry (n=82). The Cox proportional hazards survivorship model was used to evaluate the independent contribution of clinical and genetic factors to the first occurrence of time-dependent cardiac events from birth through age 40 years. The clinical characteristics, distribution of mutations, and overall outcome event rates were similar in patients enrolled from the 3 geographic regions. Biophysical function of the mutations was categorized according to dominant-negative (>=50%) or haploinsufficiency (>=50%) reduction in cardiac repolarizing I<sub>Ks</sub> potassium channel current. Patients with transmembrane versus C-terminus mutations (hazard ratio, 2.06; P<0.001) and those with mutations having dominant-negative versus haploinsufficiency ion channel effects (hazard ratio, 2.26; P<0.001) were at increased risk for cardiac events, and these genetic risks were independent of traditional clinical risk factors.

Conclusions—This genotype–phenotype study indicates that in type-1 LQTS, mutations located in the transmembrane portion of the ion channel protein and the degree of ion channel dysfunction caused by the mutations are important independent risk factors influencing the clinical course of this disorder. (Circulation. 2007;115:2481-2489.)

Key Words: electrocardiography ■ genetics ■ long-QT syndrome

Clinical Perspective p 2489

Functional I<sub>Ks</sub> channels result from the coassembly of 4 subunits into a tetrameric protein channel that is transported to the myocyte membrane. Each subunit contains 6 membrane-spanning domains (S1 to S6) flanked by amino (N-) and carboxyl (C)-terminus regions. Two distinct biophysical mechanisms mediate the reduced I<sub>Ks</sub> current in patients with KCNQ1 mutations: (1) coassembly or trafficking defects in which mutant subunits are not transported...
properly to the cell membrane and fail to incorporate into the
tetrameric channel, with the net effect being a $\pm50\%$
reduction in channel function (haploinsufficiency)$^{6}$; and (2) forma-
tion of defective channels involving mutant subunits with the
altered channel protein transported to the cell membrane,
resulting in a dysfunctional channel having $>50\%$ reduction
in channel current (dominant-negative effect).$^{6}$

Limited prior studies involving relatively small numbers of
patients with type-1 LQTS have been reported with conflicting
results on the relationship between various KCNQ1
mutations and the clinical outcome.$^{7,8}$ We hypothesized that
the location, coding type, and functional effect of the channel
mutation would have important influence on the phenotypic
manifestations and clinical course of patients with this disor-
der. To test this hypothesis, we investigated the clinical
aspects of a large cohort of subjects having a spectrum of
KCNQ1 mutations categorized by their location, coding type,
and type of biophysical ion channel dysfunction.

**Methods**

**Study Population**

The study population of 600 subjects with genetically confirmed
KCNQ1 mutations was derived from 101 proband-identified families
with the type-1 LQTS disorder. The proband in each family had QTc
prolongation not due to a known cause. The subjects were drawn
from the US portion of the International LQTS Registry ($n =$ 425),
the Netherlands’ LQTS Registry ($n =$ 93), and the Japanese LQTS
Registry ($n =$ 82). All subjects or their guardians provided informed
consent for the genetic and clinical studies.

**Phenotype Characterization**

Routine clinical and ECG parameters were acquired at the time
of enrollment in each of the registries. Follow-up was censored at age
41 years to avoid the influence of coronary disease on cardiac events.
Measured parameters on the first recorded ECG included QT and
R-R intervals in milliseconds, with QT corrected for heart rate by
Bazett’s formula. The QTc interval was expressed in its continuous
form and categorized into 3 levels: $<500$, $500$ to $530$, and $>530$ ms.
Clinical data were collected on prospectively designed forms with
information on demographic characteristics, personal and family
medical history, ECG findings, therapy, and end points during
long-term follow-up. LQTS-related cardiac events included syncope,
aborted cardiac arrest, or unexpected sudden death without a known
cause. Data common to all 3 LQTS registries involving genetically
identified patients with type-1 genotype were electronically merged
into a common database for the present study.

**Genotype Characterization**

The KCNQ1 mutations were identified with the use of standard
genetic tests performed in academic molecular-genetic laboratories
including the Functional Genomics Center, University of Rochester
Medical Center, Rochester, NY; Baylor College of Medicine, Houston,
Tex; Mayo Clinic College of Medicine, Rochester, Minn; Boston
Children’s Hospital, Boston, Mass; Laboratory of Molecular
Genetics, National Cardiovascular Center, Suita, Japan; and Depart-
ment of Clinical Genetics, Academic Medical Center, Amsterdam,
Netherlands.

Genetic alterations of the amino acid sequence were characterized
by location and by the specific mutation (missense, splice site,
in-frame insertions/deletions, nonsense, stop codon, and frameshift).
The transmembrane region of the KCNQ1-encoded channel was
defined as the coding sequence involving amino acid residues from
120 through 355 ($S5$-pore-$S6$ region 285 to 355), with the
N-terminus region defined before residue 120 and the C-terminus
region after residue 355. Nineteen study patients had intron muta-
tions predicted to disrupt the canonical splice-site domains. Fifty-one
subjects died of sudden cardiac death at a young age but did not have
genotype studies. These 51 subjects were assumed to have the same
KCNQ1 mutation as other affected members of their respective family.
Twelve subjects had 2 mutations, one in the KCNQ1 gene and a second mutation in another LQTS ion channel gene; these 12
subjects are described separately and are not included in any of the
tables or outcome analyses. Subjects with Jervell and Lange-Nielsen
syndrome with deafness and 2 KCNQ1 mutations as well as those
with 1 known KCNQ1 mutation and congenital deafness are not
included in the present study.

The biophysical function of the mutant channels was classified as
having dominant-negative effect ($>50\%$ reduction in function) or
haploinsufficiency ($\approx50\%$ reduction in function) on the basis of the
following: (1) cellular expression studies for those with missense
mutations reported in the literature, with the functional information derived exclusively from heterologous
expression studies; and (2) assumed loss of function for identified
nonsense, splice site, in-frame deletion, and frameshift mutations
that have not been functionally reported in cellular expression studies were categorized
as unknown in terms of type of functional perturbation.

**Statistical Analysis**

Differences in the univariate characteristics by specific groupings
were evaluated by standard statistical methods. The primary end
point was time to syncope, aborted cardiac arrest, or sudden death,
whichever occurred first. The cumulative probability of a first
cardiac event was assessed by the Kaplan-Meier method with
significance testing by the log-rank statistic. The Cox proportional
hazards survivorship model was used to evaluate the independent
contribution of clinical and genetic factors to the first occurrence
of time-dependent cardiac events from birth through age 40 years.$^{9}$
Stratified and unstratified Cox regression models, allowing for
time-dependent covariates, were fit to estimate the adjusted hazard
ratio of each factor as a predictor of first cardiac events. We observed
that sex was not proportional as a function of age with crossover in
risk at age 13 years on univariate Kaplan-Meier analysis. To relax
the assumption of proportional hazards for sex over the entire age
range, separate nonparametric baseline hazard functions were al-
lowered for male and female subjects via the stratified Cox model;
then, to summarize the sex effect, sex was modeled in an unstratified
Cox model as a time-dependent covariate (via an interaction with
time), allowing for different hazard ratios by sex before and after age
13 years.

Because almost all the subjects were first- and second-degree
relatives of probands, the effect of lack of independence between
subjects was evaluated in the Cox model with grouped jackknife
estimates for family membership.$^{10}$ All grouped jackknife standard
errors for the covariate risk factors fell within $5\%$ of those obtained
from the unadjusted Cox model, and therefore only the Cox model
findings are reported.

 Patients who died suddenly at a young age from suspected LQTS
and who did not have an ECG for QTc measurement were identified
in the Cox models as “QTc missing.” Prespecified covariate inter-
actions were evaluated. The influence of time-dependent $\beta$-blocker
therapy (the age at which $\beta$-blocker therapy was initiated) on
outcome was determined by adding this variable to the final Cox
model containing the various covariates.

The authors had full access to and take full responsibility for the
integrity of the data. All authors have read and agree to the
manuscript as written.

**Results**

**Total Study Population**

The spectrum and number of KCNQ1 mutations by location,
type of mutation, and functional effect are presented in Table
1, with the location frequency of the mutations presented
diagrammatically in Figure 1. A total of 77 different KCNQ1
<table>
<thead>
<tr>
<th>Location and Coding*</th>
<th>No. of Subjects†</th>
<th>Type of Mutation</th>
<th>Functional Effect‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1V</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G57V</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>Transmembrane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W120C</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>T144A</td>
<td>7</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>A150fs/133 [del CT 451-452]</td>
<td>2</td>
<td>Frameshift</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>E160K</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G168R</td>
<td>44</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y171X [513 C-&gt;G]</td>
<td>6</td>
<td>Nonsense</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>R174H</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>A178P</td>
<td>5</td>
<td>Missense</td>
<td>Dominant-negative effect (a)</td>
</tr>
<tr>
<td>Y184S</td>
<td>18</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G185S</td>
<td>10</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G189E</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G18OR</td>
<td>4</td>
<td>Missense</td>
<td>Dominant-negative effect (b)</td>
</tr>
<tr>
<td>R190Q</td>
<td>4</td>
<td>Missense</td>
<td>Haploinsufficiency (b, c)</td>
</tr>
<tr>
<td>L191fs/90 [del TGGCG 572-576]</td>
<td>8</td>
<td>Frameshift</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>R195fs/40 [del G 585]</td>
<td>2</td>
<td>Frameshift</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>S225L</td>
<td>13</td>
<td>Missense</td>
<td>Dominant-negative effect (d)</td>
</tr>
<tr>
<td>A226V</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>R237P</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>D242N</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>R243C</td>
<td>13</td>
<td>Missense</td>
<td>Haploinsufficiency (e)</td>
</tr>
<tr>
<td>V254 mol/L</td>
<td>59</td>
<td>Missense</td>
<td>Dominant-negative effect (b, f)</td>
</tr>
<tr>
<td>R258C</td>
<td>1</td>
<td>Missense</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>R259C</td>
<td>1</td>
<td>Missense</td>
<td>Haploinsufficiency (g)</td>
</tr>
<tr>
<td>L266P</td>
<td>15</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G269D</td>
<td>35</td>
<td>Missense</td>
<td>Dominant-negative effect (h)</td>
</tr>
<tr>
<td>G269S</td>
<td>25</td>
<td>Missense</td>
<td>Haploinsufficiency (i)</td>
</tr>
<tr>
<td>L273F</td>
<td>6</td>
<td>Missense</td>
<td>Dominant-negative effect (a)</td>
</tr>
<tr>
<td>L274V</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>S277L</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>Y278H</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>E284K</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G292D</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>F296S</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G306R</td>
<td>2</td>
<td>Missense</td>
<td>Dominant-negative effect (b, j)</td>
</tr>
<tr>
<td>V310I</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>T312I</td>
<td>14</td>
<td>Missense</td>
<td>Dominant-negative effect (a)</td>
</tr>
<tr>
<td>G314S</td>
<td>8</td>
<td>Missense</td>
<td>Dominant-negative effect (h, k, l, m)</td>
</tr>
<tr>
<td>Y315C</td>
<td>10</td>
<td>Missense</td>
<td>Dominant-negative effect (d, n)</td>
</tr>
<tr>
<td>Y315S</td>
<td>1</td>
<td>Missense</td>
<td>Dominant-negative effect (h, m)</td>
</tr>
<tr>
<td>D317G</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>P320H</td>
<td>1</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>T322 mol/L</td>
<td>2</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>G325R</td>
<td>3</td>
<td>Missense</td>
<td>Unknown</td>
</tr>
<tr>
<td>delF340 [del CTT 1017-1019]</td>
<td>7</td>
<td>In-frame deletion</td>
<td>Haploinsufficiency</td>
</tr>
<tr>
<td>A341E</td>
<td>9</td>
<td>Missense</td>
<td>Dominant-negative effect (b)</td>
</tr>
<tr>
<td>A341V</td>
<td>20</td>
<td>Missense</td>
<td>Dominant-negative effect (o)</td>
</tr>
</tbody>
</table>
mutations were identified. A majority of the mutations were localized to the S1 to S6 transmembrane domains (66%), and missense (single amino acid substitutions) accounted for 81% of all the mutations.

The phenotypic characteristics of patients enrolled in each of the 3 registries and by location and type of mutation are presented in Table 2. The clinical characteristics of the patients were similar among the 3 registries except for QTc duration and frequency of β-blocker use. The QTc interval was longer and cardiac events and β-blocker use were more frequent in patients with mutations in the transmembrane than in the C-terminus location. β-Blockers were used less frequently in patients from the Japanese registry than in patients from the other 2 registries. The frequency of first cardiac events was higher in those with than without missense mutations. The clinical characteristics of the 19 subjects possessing intron mutations resembled those with transmembrane and missense mutations.

The QTc interval was significantly longer in the 12 patients with 2 mutations than in those with only single KCNQ1 mutations (570 ± 70 versus 480 ± 60 ms; \(P < 0.01\)). All 12 patients with 2 mutations experienced at least 1 cardiac event. The cumulative probabilities of first cardiac event by location and type of mutation are presented in Figure 2A and 2B, respectively. Significantly higher event rates were found in subjects with transmembrane than C-terminus mutations and in those with than without missense mutations, with the most rapid increase in event rates occurring during ages 7 to 2484 Circulation May 15, 2007
20 years. In patients with transmembrane-localized mutations, the event rates for patients with mutations localized to the pore region (S5-pore-S6) were nearly identical to those with nonpore mutations (data not shown).

The findings from the Cox regression analysis for location and type of mutation are presented in Table 3. The clinical risk factors associated with first cardiac events involved males before age 13 years, females after age 13

### TABLE 2. Phenotypic Characteristics by Source of Subjects, Location of Mutation, and Type of Mutation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>United States (n=425)</th>
<th>Netherlands (n=93)</th>
<th>Japan (n=82)</th>
<th>Trans Membrane (n=452)</th>
<th>C-Terminus (n=127)</th>
<th>Yes (n=483)</th>
<th>No (n=98)</th>
<th>Intron Mutation (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, %</td>
<td>57</td>
<td>53</td>
<td>54</td>
<td>57</td>
<td>51</td>
<td>54</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>ECG at enrollment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QTc†‡, ms</td>
<td>488±58</td>
<td>450±45</td>
<td>472±46</td>
<td>485±53</td>
<td>460±61</td>
<td>481±59</td>
<td>471±38</td>
<td>478±60</td>
</tr>
<tr>
<td>Therapy, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers†‡</td>
<td>45</td>
<td>34</td>
<td>26</td>
<td>45</td>
<td>28</td>
<td>42</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Pacemaker</td>
<td>2.4</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>2.4</td>
<td>1.4</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Defibrillator</td>
<td>6.4</td>
<td>3.2</td>
<td>0</td>
<td>5.8</td>
<td>3.1</td>
<td>5.2</td>
<td>5.1</td>
<td>0</td>
</tr>
<tr>
<td>First cardiac event*‡§, %</td>
<td>41</td>
<td>37</td>
<td>38</td>
<td>45</td>
<td>21</td>
<td>43</td>
<td>26</td>
<td>42</td>
</tr>
<tr>
<td>Syncope‡ (n=200)</td>
<td>35</td>
<td>31</td>
<td>29</td>
<td>38</td>
<td>17</td>
<td>36</td>
<td>21</td>
<td>32</td>
</tr>
<tr>
<td>Aborted cardiac arrest (n=15)</td>
<td>1.9</td>
<td>1.1</td>
<td>7.3</td>
<td>2.9</td>
<td>0.8</td>
<td>2.5</td>
<td>2.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Death (n=23)</td>
<td>4.0</td>
<td>5.5</td>
<td>1.2</td>
<td>4.0</td>
<td>3.1</td>
<td>4.2</td>
<td>2.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Ever cardiac event, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syncope‡§</td>
<td>35</td>
<td>31</td>
<td>31</td>
<td>39</td>
<td>17</td>
<td>37</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>Aborted cardiac arrest†</td>
<td>2.4</td>
<td>1.5</td>
<td>8.8</td>
<td>5.3</td>
<td>3.2</td>
<td>5.4</td>
<td>2.0</td>
<td>11</td>
</tr>
<tr>
<td>Death</td>
<td>11</td>
<td>14</td>
<td>2.4</td>
<td>10</td>
<td>6.3</td>
<td>11</td>
<td>4.1</td>
<td>26</td>
</tr>
</tbody>
</table>

Plus-minus values are mean±SD. Percentages >10 are rounded to a whole number. The 600 subjects in this table include 51 subjects who died suddenly at a young age, were from families with known KCNQ1 mutation, and were assumed to have the family mutation. Patients with intron mutations are categorized separately and are not included in the location or missense categories. Seven subjects with transmembrane mutations and 1 with C-terminus mutations had missing data about the date of the first cardiac event. Eight subjects with missense mutations had missing data about the date of the first cardiac event. Numbers in parentheses indicate the total number of specific first cardiac events from the 3 sources of patients.

*First cardiac event was syncope, aborted cardiac arrest, or sudden death, whichever occurred first.
†P<0.01 for the comparison of characteristics among the 3 sources of subjects.
‡P<0.01 for the comparison of characteristics between the 2 locations of the mutations.
§P<0.01 for the comparison of characteristics between missense yes and no.
years, and longer QTc intervals. Mutations located in the transmembrane region of the channel made significant and independent contributions to the risk model, but missense mutations were not an independent risk factor. Three different intron mutations were present in 19 subjects from 4 families, and these intron mutations made a meaningful but nonsignificant contribution to the risk model. Prespecified interactions were investigated for their effect on cardiac events, and no significant interactions were found for transmembrane location by type of mutation, transmembrane location by QTc, or mutation type by QTc.

Time-dependent β-blocker use was associated with a significant 74% reduction in the risk of first cardiac events (P<0.001).

### Biophysical Function and Outcome

The clinical implications of disordered biophysical function of the mutant KCNQ1 channels were investigated in a subset of 356 subjects with known or suspected alteration in ion channel function (see Methods for functional categorization). The clinical characteristics of patients with dominant-negative and haploinsufficiency ion channel dysfunction are presented in Table 4. Patients with mutations having dominant-negative ion current effects had a longer QTc interval and a higher frequency of cardiac events than subjects with mutations resulting in haploinsufficiency. The cumulative probabilities of a first cardiac event by the biophysical function of the mutations are presented in Figure 2C. As shown in Table 5, patients with mutations having
TABLE 5. Cox Regression With Multiple Predictor Variables Including Ion Channel Dysfunction for First Cardiac Events

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands:United States</td>
<td>2.78</td>
<td>1.48-5.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Japan:United States</td>
<td>1.63</td>
<td>1.02-2.63</td>
<td>0.04</td>
</tr>
<tr>
<td>Male &lt;13 y/female &lt;13 y</td>
<td>1.94</td>
<td>1.29-2.91</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Female 13-40 y/male 13-40 y</td>
<td>1.95</td>
<td>0.99-3.87</td>
<td>0.06</td>
</tr>
<tr>
<td>QTC 500–530 ms:QTC &lt;500 ms</td>
<td>1.88</td>
<td>1.18-2.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>QTC &gt;530 ms:QTC &lt;500 ms</td>
<td>3.22</td>
<td>2.06-5.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>QTC missing*:QTC &lt;500 ms</td>
<td>2.07</td>
<td>1.29-3.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Dominant-negative:haploinsufficiency</td>
<td>2.26</td>
<td>1.56-3.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time-dependent β-blocker use</td>
<td>0.21</td>
<td>0.09-0.48</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The analysis involved 354 subjects with known or suspected ion channel dysfunction; 2 subjects were not included because of missing data about the date of their first cardiac event.

*The QTC missing category involves 26 patients who died suddenly at a young age without a prior ECG.

dominant-negative functional effects experienced a significantly greater risk for cardiac events than those with haploinsufficiency (hazard ratio, 2.26; 95% CI, 1.56 to 3.25; P<0.001) after adjustment for relevant covariates including QTc and gender effects by age group. β-Blocker use was associated with a significant 79% reduction in first cardiac events in this subset of patients. Because substantial colinearity exists for transmembrane mutations, missense mutations, and mutations with dominant-negative biophysical function, the individual effects of these 3 mutation parameters could not be ascertained reliably in the same Cox model.

Discussion

The main results of the present study from 600 patients having a spectrum of KCNQ1 mutations derived from 3 LQTS registries are significantly higher cardiac event rates in patients with transmembrane mutations and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effect of these genetically determined factors is independent of traditional clinical risk factors and of β-blocker therapy.

Since 1995, when the first 2 genes responsible for LQTS were identified,11,12 molecular genetic studies have revealed a total of 9 forms of congenital LQTS caused by mutations in genes involving potassium channel (LQT-1, -2, -5, -6, and -7), sodium channel (LQT-3, -9), and calcium channel proteins (LQT-8) as well as a membrane-adapter protein (LQT-4).2,13 Genotype–phenotype studies have enabled us to stratify risk and to treat more specifically patients with LQT-1, LQT-2, and LQT-3 subtypes of this genetic disorder. LQT-1, the most common form of LQTS, accounts for ~50% of genotyped patients8,14 and has more variable expressivity and incomplete penetrance than the other forms.15 Mutation location and knowledge of the functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for LQT-1, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.

Mutations in KCNQ1 are responsible for defects in the slowly activating component of the delayed rectifier current I_{Ks}.16 This current is the main repolarizing current at increased heart rate and is highly sensitive to catecholamines.3 We speculate that I_{Ks} channels with transmembrane mutations might have reduced responsiveness to the regulatory β-adrenergic signaling of the ion-conduction pathway with more impairment of shortening of the QTc with exercise-related tachycardia than mutations in the C-terminus region.

Functional I_{Ks} channels result from the coassembly of 4 KCNQ1-encoded subunits. A mutated gene encodes a protein with aberrant function, and the presence of both normal and abnormal proteins in the ion channel contributes to a >50% reduction in ion channel function (dominant-negative effect).

An alternative mechanism of reduced repolarizing KCNQ1 K+ current is the inability of mutated subunits to coassemble with normal gene products, such as occurs with a trafficking defect, resulting in a ≤50% reduction in channel function (haploinsufficiency). With only 1 exception,17 this is the case for all studied truncating mutations leading to incomplete proteins. Our assumption that truncated proteins (based on frameshift nonsense mutations) lead to haploinsufficiency seems justified. The biophysical effect of missense mutations is unpredictable, and both haploinsufficiency and dominant-negative effects have been described. In the absence of reported biophysical studies, missense mutations were classified as unknown.

Previous attempts to identify a genotype–phenotype relationship for KCNQ1 mutations failed to reach consensus on the clinical outcome of the type and site of mutations.7,8 Relatively small numbers and different ethnic background of the previously reported patients with the LQT-1 genotype might be responsible for the discrepant results. The present larger study allows us to demonstrate for the first time that the biophysical effect clearly affects the clinical outcome (ie, dominant-negative mutations are associated with a more severe phenotype than are mutations conferring haploinsufficiency [Figure 2C], even after adjustment for relevant covariates [Table 5]). The risk observed in 19 subjects with 3 different intron mutations was not quite significant (P=0.06), possibly because of small numbers, but the magnitude of the risk effect was similar to the risk accompanying transmembrane mutations. Although these intron mutations produced splice-site alterations predicted to affect the transmembrane portion of the ion channel, we used a separate categorization of intron mutations in view of the limited understanding of the structural alterations and functional effects resulting from these exon-skipping intron mutations.

A few additional findings from this large genotype–phenotype study of type-1 LQTS patients emphasize high risk for first cardiac events during adolescence, a crossover in risk by sex at approximately age 13 years, and a lower rate of first cardiac events in the adult years than in the younger years. These findings are not especially new,18,19 but the present study highlights their presence in type-1 LQTS.

Study Limitations

The present study used the biophysical function of mutations reported in the literature in only a portion of the mutations.
that were included (see references associated with Table 1 in the online-only Data Supplement). The published studies were from many different laboratories with the use of different cellular heterologous expression systems involving *Xenopus* oocytes and other cells at both room and physiological temperatures. Although such nonuniform testing may have contributed to some inconsistency in the categorized biophysical function, the finding of a significantly higher event rate in mutations with dominant-negative than with haploinsufficient effects (hazard ratio, 2.26; \(P<0.001\)) is unlikely to have resulted from the nonuniform testing. Unfortunately, we did not have the resources to perform such uniform testing in all 77 mutations presented in the present study.

Once a mutation was identified in KCNQ1, thorough genetic sequencing was not performed routinely in all the ion channel genes to look for second mutations. Thus, some of the patients included in the analysis may have had a second mutation in addition to the identified KCNQ1 mutation. It is estimated that \(\approx 10\%\) of genotype LQTS patients may carry a second mutation, and those with \(>1\) mutation could contribute to some of the findings in our study. In addition, it is possible that some of the reported mutations (Table 1) are simply uncommon sequence mutations, but this is relatively unlikely because all the subjects in the present study were derived from families in which the proband had QTc prolongation not due to a known cause.

The outcome analyses included subjects from families with a known KCNQ1 mutation who died suddenly and unexpectedly at a young age and were classified as LQTS-related death with the same mutation that was present in the family. It is possible that a few of these subjects could have died from a non-LQTS cause or had an LQTS mutation different from the family mutation, but we think the error rate is likely to be small. The number of deaths and aborted cardiac arrest events is small, and there is insufficient power to evaluate the risk association of the genotype characteristics with these endpoint events in a multivariate time-dependent model.

**Conclusions**

The present study confirms that in patients with type-1 LQTS, longer QTc intervals are associated with higher cardiac event rates and that male patients are generally younger than female patients at first cardiac events.\(^{20,21}\) The new findings from the present study are that transmembrane mutations and mutations with dominant-negative functional effect adversely influence the outcome of this disorder independent of traditional clinical risk factors and \(\beta\)-blocker therapy. The present study was not designed to assess the effectiveness of different therapies in patients with KCNQ1 mutations. The findings presented do not provide justification for using specific genotype characteristics to identify patients for implanted defibrillator therapy.

**Note Added in Proof**

After this article was accepted for publication, we noted the recent article by Tsuji et al, in which the A344A/sp [1032G>A] mutation that we categorized as haploinsufficient (Table 1) was reported to have a weak dominant-negative effect.\(^{22}\) We reran the KCNQ1 data reclassifying the 27 A344A/sp [1032 G>A] mutations as dominant-negative. Negligible changes occurred in the results as presented in Table 5 and Figure 2C; the hazard ratio for dominant-negative:haploinsufficiency (Table 5) was unchanged at 2.26 (\(P<0.001\)).

**Acknowledgment**

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**Disclosures**

Dr Ackerman is a consultant for Clinical Data (formerly Genainenance Pharmaceuticals) with respect to the FAMILION genetic test for cardiac ion channel mutations. The other authors report no conflicts.

**References**


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**CLINICAL PERSPECTIVE**

Type-1 long-QT syndrome is caused by loss-of-function mutations in the KCNQ1-encoded I_{Ks} cardiac potassium channel. In the present study involving 600 patients having a spectrum of KCNQ1 mutations derived from 3 long-QT syndrome registries, we found that cardiac event rates are increased significantly in patients with mutations located in the transmembrane region of the potassium channel and in patients with mutations having a putative dominant-negative effect on the repolarizing I_{Ks} current. The effects of these genetically determined factors are independent of traditional clinical risk factors and of β-blocker therapy. Mutation location and knowledge of functional effects of the mutation provide additional risk information beyond the clinical risk factors and the genotype, at least for type-1 long-QT syndrome, and this information should contribute to improved risk stratification and more focused management of these higher-risk patients.
Clinical Aspects of Type-1 Long-QT Syndrome by Location, Coding Type, and Biophysical Function of Mutations Involving the KCNQ1 Gene


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