Effect of Clinical Phenotype on Yield of Long QT Syndrome Genetic Testing

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OBJECTIVES
The purpose of this study was to examine the effect of clinical phenotype on the yield of genetic testing for congenital long QT syndrome (LQTS).

BACKGROUND
Since the discovery of the first LQTS susceptibility genes in 1995, numerous genotype-phenotype relationships have emerged during the past decade of research genetic testing. In May 2004, LQTS genetic testing became a clinically available molecular diagnostic test.

METHODS
Blinded to genetic test results, analysis of the clinical phenotype was performed in 541 consecutive unrelated patients referred to Mayo Clinic’s Sudden Death Genomics Laboratory for LQTS genetic testing from August 1997 to July 2004.

RESULTS
The yield of genetic testing correlated significantly with the corrected QT interval (QTc) and clinical diagnostic score ranging from 0% when QTc was <400 ms to 62% when QTc was >480 ms (p < 0.0001). Among those with the highest clinical probability, the yield was 72% (89 of 123). The yield fluctuated substantially depending on age at diagnosis in males. Among physicians who referred ≥5 patients, the yield ranged from 0% to 80% (p < 0.0001).

CONCLUSIONS
In this large cohort of unrelated patients referred for LQTS genetic testing, the clinical phenotype strongly correlated with the likelihood of elucidating a pathogenic mutation with the cardiac channel gene screen. (J Am Coll Cardiol 2006;47:764–8) © 2006 by the American College of Cardiology Foundation

Affecting 1 in 5,000 persons, long QT syndrome (LQTS) is the prototypic cardiac channelopathy underscored by profound genetic and phenotypic heterogeneity (1,2). To date, over 400 mutations in five cardiac channel-encoding genes have been identified: LQT1 (KCNQ1-encoded potassium channel [\(I_K1\)] mutations), LQT2 (KCNH2-encoded potassium channel [\(I_K1\)] mutations), LQT3 (KCNSA-encoded sodium channel mutations), and LQT5 and LQT6 (KCNE1- or KCNE2-encoded potassium channel beta subunit mutations) (3–6). The LQT4 stems from ankyrin-B mutations and represents the first nonchannel form of LQTS (7).

Over the past decade, genetic testing for LQTS, particularly the three most common genotypes of LQT1 to LQT3, have revealed relatively gene-specific electrocardiographic profiles, responses to epinephrine, arrhythmogenic triggers and arrhythmogenic temporal states, responsiveness to beta blockers, treatments, and prognosis (8,9). In May 2004, genetic testing for the five LQTS-associated channel genotypes became a commercially available clinical diagnostic test in the U.S. (10).

Clinically, genetic testing has been used to risk-stratify patients, guide treatment decisions, and precisely elucidate the “carrier” status of potential at-risk relatives (11–14). Having completed comprehensive mutational analysis in one of the largest assembled cohorts of unrelated LQTS referrals (6), we scrutinized the effect of clinical phenotype on the yield of LQTS genetic testing.

METHODS

Study population. Between August 1997 and July 2004, 541 consecutive unrelated patients were referred by 103 physicians to Mayo Clinic’s Sudden Death Genomics Laboratory for LQTS genetic testing in accordance with IRB-approved research protocols. Previously, an LQTS-associated channel genotype was established in 272 of 541 cases (6,15,16).

Blinded to genotype, clinical phenotype including ethnicity, sex, age at diagnosis, presence or absence of syncope, seizures, or aborted cardiac arrest, temporally related triggers, family history, and 12-lead electrocardiogram (ECG) was recorded. A cumulative LQTS diagnostic “Schwartz and Moss” score (which is derived in part from the corrected QT interval [QTc], symptoms, and family history) was assigned (17). A cumulative score of ≥4 suggests a robust phenotype and strong probability for LQTS. Sufficient information to derive a clinical score was available in 417 of 541 cases (77%). The QTc data were available for all 417
cases, and an ECG for review and independent calculation was available for 341 cases (63%).

Differences between continuous variables were evaluated using unpaired Student $t$ tests, and nominal variables were analyzed using chi-square analysis. The chi-square test was used for multiple group comparisons. Statistical significance was considered at $p < 0.05$.

**RESULTS**

Table 1 summarizes the clinical phenotype. Figure 1 details the distribution of genotypes: LQT1 ($n = 120$), LQT2 ($n = 93$), LQT3 ($n = 26$), LQT5 ($n = 3$), LQT6 ($n = 1$), and multiples ($n = 29$). Nearly one-half of the cohort (269 of 541) had no LQTS-associated channel mutation and are designated “genotype negative.”

The genotype positive subset ($n = 272$) had a significantly distinct clinical phenotype compared with the genotype negative subset ($n = 269$) in terms of QTc (494 ± 51 ms vs. 470 ± 60 ms; $p < 0.0001$) and cumulative LQTS diagnostic score (Table 1). Forty-one percent of the genotype positive subset had a clinical score of ≥4 compared with 17% of the genotype negative subset ($p < 0.0001$).

There was no difference in sex, ethnicity, age at diagnosis, or history of cardiac arrest between the two subsets. Presence of syncope and family history both trended toward an increase among genotype-positive individuals (46%) compared to genotype-negative individuals (38%, $p = 0.067$).

The yield among white subjects ($n = 483$) was 49% compared with 13 of 19 (68%) Hispanics, 8 of 11 blacks (73%), 2 of 3 Asians, 1 of 1 Native American, and 12 of 24 where the ethnicity was not defined. Although the yield was greater among nonwhites than whites (24 of 34 [71%] vs. 236 of 483 [49%]; $p < 0.02$), ethnicity was not an independent predictor. Rather, the QTc and clinical scores were greater among this small subset of nonwhites (data not shown). Among those with a positive family history of LQTS-attributable symptoms or premature sudden death, the yield was 55%.

Figure 2 depicts the age and gender distribution with an average age at diagnosis of 24 ± 16 years, ranging from 1 day to 78 years (Table 1). Over two-thirds of the patients were under 30 years at diagnosis and males were significantly younger than females (18 ± 16 years vs. 25 ± 15 years; $p < 0.0001$). Overall, gender had no effect on the yield, with 178 of 358 (50%) females and 94 of 183 males (51%) ($p = 0.28$) being genotype positive.

However, the yield fluctuated substantially depending on age at diagnosis in males but not in women: 25 of 30 (83%) males diagnosed at ≤5 years of age were genotype positive compared with only 13 of 39 (33%) males diagnosed ≥6 years of age.

**Table 1.** Demographics of 541 Consecutive Unrelated Patients Referred for Long QT Syndrome Genetic Testing: Comparison of Genotype-Positive and Genotype-Negative Subsets

<table>
<thead>
<tr>
<th></th>
<th>Total Cohort</th>
<th>Genotype-Positive</th>
<th>Genotype-Negative</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of unrelated patients</td>
<td>541</td>
<td>272</td>
<td>269</td>
<td>NS</td>
</tr>
<tr>
<td>Age at diagnosis (yrs) (range)</td>
<td>24 ± 16 (0–78)</td>
<td>23 ± 16 (0–75)</td>
<td>25 ± 16 (0–78)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>183/358</td>
<td>94/178</td>
<td>89/180</td>
<td>NS</td>
</tr>
<tr>
<td>Ethnicity (% white)</td>
<td>93</td>
<td>90</td>
<td>96</td>
<td>NS</td>
</tr>
<tr>
<td>Average QTc (ms) (range)</td>
<td>482 ± 57 (365–759)</td>
<td>494 ± 51 (402–700)</td>
<td>470 ± 60 (365–759)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% with QTc &gt;480 ms</td>
<td>46</td>
<td>57</td>
<td>35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% with syncope</td>
<td>42</td>
<td>46</td>
<td>38</td>
<td>0.067</td>
</tr>
<tr>
<td>% with cardiac arrest</td>
<td>227/541</td>
<td>126/272</td>
<td>101/269</td>
<td>NS</td>
</tr>
<tr>
<td>% with positive family history</td>
<td>12/68/541</td>
<td>13/36/272</td>
<td>12/32/269</td>
<td>0.067</td>
</tr>
<tr>
<td>% with “Schwartz and Moss” score ≥4</td>
<td>29/228/541</td>
<td>41/126/272</td>
<td>17/102/269</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% with “Schwartz and Moss” score ≥4</td>
<td>123/417</td>
<td>89/218</td>
<td>34/199</td>
<td>$1 \times 10^{-9}$</td>
</tr>
</tbody>
</table>

In addition to percentages, absolute numbers for the various clinical parameters are provided as well. QTc = corrected QT interval.
between 11 and 15 years of age (p < 0.0001) (Fig. 3). During the first five years of life, the yield was greater in males (83%) than in females (53%) (p = 0.01). Closer inspection shows that this difference was due to the yield in the subset of 28 patients diagnosed during the first year, wherein 11 of 14 (79%) males were genotype positive compared to 4 of 14 (29%) females (p = 0.02). Unlike ethnicity, age and male gender appear to independently impact the yield of genetic testing, because the QTc and clinical scores were similar between males and females at the various age categories (data not shown).

The yield correlated significantly with QTc and clinical (“Schwartz and Moss”) score (Fig. 4). The yield ranged from 0% for the 7 subjects referred for LQTS genetic testing despite a resting QTc of <400 ms to 62% for the 144 subjects with a QTc of >480 ms (p < 0.0001) (Fig. 4A). Among the 417 unrelated cases with an assigned clinical score, an LQTS genotype was established for 43% of subjects (93/215) when the clinical score was >4. The yield was 44% among the 124 patients with insufficient clinical data to assign a score. In contrast, among those with the highest clinical probability for LQTS (score ≥4), 89 of 123 (72%) were genotype positive: LQT1 (n = 39), LQT2 (n = 30), LQT3 (n = 5), LQT5 (n = 1), and multiple (n = 14) (p < 0.0001) (Fig. 4B).

Finally, we examined the effect of referral source (103 referring physicians and 91 direct patient self-referrals) on the yield. Among the 18 physicians who referred ≥5 patients (290 total), the average yield was 51%, which was no different than the 85 physicians who referred <5 patients (49%) or the self-referrals (49%). However, the yield varied widely among these 18 physicians, ranging from 0% to 80% (p < 0.0001). Within our LQTS clinic, there were 22 unrelated patients assigned a clinical score of ≥4 following their comprehensive and systematic assessment by the same physician. Subsequently, an LQTS-associated mutation was elucidated in all 22 (100% yield).

**DISCUSSION**

Surpassed only by the profound genetic heterogeneity that underlies LQTS, there is pronounced heterogeneity with respect to the yield of genetic testing that was impacted significantly and not surprisingly by the robustness of the clinical phenotype. Clearly, the genotype positive patients had greater QTc intervals and cumulative diagnostic scores than those without an identifiable cardiac channel mutation. Among those having the highest clinical probability for LQTS, the yield was 72%.

Given the nonuniformity of clinical assessment by the nearly 200 different physicians represented in this study, this value (72%) may underestimate the true a priori likelihood of identifying an LQTS channel genotype in the next patient strongly suspected to have LQTS. For example, the
yield reached 83% in the subset of males diagnosed with LQTS during the first five years of life. In addition, following uniform clinical evaluation in our own LQTS clinic, the yield was 100% among those deemed to manifest a robust LQTS phenotype.

Nevertheless, based upon these data, cardiologists can expect the current genetic test to capture approximately three-fourths of LQTS. Thus, a negative genetic test in an index case with definite LQTS (i.e., genotype negative/phenotype positive LQTS) only informs the physician that the five channel genotypes have been excluded. Here, a negative genetic test provides no basis for removing the diagnosis and means that the physician will struggle with the correct classification of relatives (12,14).

In stark contrast, a positive genetic test may influence treatment decisions and will provide the means for precise “carrier” status classification of potentially at-risk relatives. With the increased recognition that a significant minority (25% to 50%) of individuals with genetically proven LQTS have a nondiagnostic QTc, the genetic test has become the new gold standard in the identification of concealed LQTS (18).

In contrast to its role among those with a definite LQTS phenotype, genetic testing may facilitate a move away from the diagnosis of LQTS even in light of its known 25% false negative rate. For example, many patients presently labeled with and treated for LQTS received the diagnosis based upon a clinical phenotype that would only support a low/intermediate clinical probability for the diagnosis. Equipped with an objective test (genetic testing) that effectively rules out 75% of LQTS, physicians may be more willing to consider reclassifying such low probability phenotype individuals as normal rather than persisting with the current default diagnosis of “borderline” LQTS (12).

Indeed, given the overall yield of 50%, this cohort almost certainly contains a spectrum of patients ranging from normal individuals with misdiagnosed vasovagal syncope to LQTS phenocopies such as catecholaminergic polymorphic ventricular tachycardia (CPVT) to correctly suspected LQTS. Among patients where there was an ECG for comparison, 44% of the subset whose clinical phenotype was “borderline” LQTS (12).

Despite the voluntary submission of phenotypic data, there was excellent cooperation from the majority of referring physicians and self-referring patients and we were provided sufficient clinical and ECG information for the majority of cases. Further, the yield (44%) for the subset of patients with insufficient clinical information mirrored the yield of the subset whose clinical phenotype was “borderline.” With this limitation as a caveat, we suspect that the referring physicians with yields >60% were primarily referring patients with a convincing phenotype for LQTS, whereas physicians associated with markedly lower yields were perhaps using this research test with a “rule-out” motive in mind. Alternatively, however, the extremely low yields may suggest that there is ongoing need for continuing medical education directed toward the proper clinical recognition of LQTS.

Conclusions. This study represents one of the largest series of consecutive unrelated patients referred for LQTS genetic testing. The identification of an LQTS channel genotype in one-half the cohort has permitted an in-depth analysis of the clinical parameters exerting the greatest impact on the yield of such genetic testing. In univariate analyses, ethnicity, age at diagnosis, QTc, “Schwartz and Moss” score, and referral source all impacted the yield of the genetic test. The observations described should assist in the proper utilization and diagnostic interpretation associated with LQTS genetic testing.
Acknowledgments
We are particularly indebted to each of the unrelated research subjects for their participation. We trust that families afflicted by LQTS will become the true beneficiaries of the scientific discoveries of the past decade. We also wish to thank all the physicians, the CARE foundation, and the SADS foundation for directing patients to this research program for genetic testing over these past seven years.

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REFERENCES