Low Penetrance in the Long-QT Syndrome
Clinical Impact

Silvia G. Priori, MD, PhD; Carlo Napolitano, MD; Peter J. Schwartz, MD

Background—It is still currently held that most patients affected by the long-QT syndrome (LQTS) show QT interval prolongation or clinical symptoms. This is reflected by the assumption in linkage studies of a penetrance of 90%. We had previously suggested that a larger-than-anticipated number of LQTS patients might be affected without showing clinical signs. We have now exploited the availability of molecular diagnosis to test this hypothesis.

Methods and Results—We identified 9 families with “sporadic” cases of LQTS, ie, families in which, besides the proband, none of the family members had clinical signs of the disease. Mutation screening by conventional single-strand conformational polymorphism and sequencing was performed on DNA of probands and family members to identify mutation carriers. Of 46 family members considered on clinical grounds to be nonaffected, 15 (33%) were found instead to be gene carriers. Penetrance was found to be 25%. In these families, conventional clinical diagnostic criteria had a sensitivity of only 38% in correctly identifying carriers of the genetic defect.

Conclusions—This study demonstrates that in some families, LQTS may appear with a very low penetrance, a finding with multiple clinical implications. The family members considered to be normal and found to be silent gene carriers are unexpectedly at risk of generating affected offspring and also of developing torsade de pointes if exposed to either cardiac or noncardiac drugs that block potassium channels. It is no longer acceptable to exclude LQTS among family members of definitely affected patients on purely clinical grounds. Conversely, it now appears appropriate to perform molecular screening in all family members of genotyped patients. (Circulation. 1999;99:529-533.)

Key Words: arrhythmia • genetics • molecular biology • torsade de pointes • sudden death

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Several of the genes responsible for the long-QT syndrome (LQTS)1–3 have been identified.4–7 Novel possibilities exist not only to understand how genetically mediated alterations in the ionic control of repolarization affect the manifestations of this often lethal disease but also to define its elusive epidemiology. The latter point derives from the ability to combine clinical and molecular diagnosis when studying potentially affected families.8

For many years, it was taken for granted that each patient affected by LQTS had a prolonged QT interval; this implied a penetrance of or close to 100%. Penetrance represents the probability for an individual with an affected genotype to manifest the clinical signs of the disease, and it has important implications for clinical management. The most reliable approach to define penetrance is to perform mutation screening in family members of affected and genotyped individuals.

Even now, most laboratories, when performing linkage analysis,9–12 assume that the disease has a penetrance ≥90%. This concept was challenged as early as 1980 by Schwartz,13 who proposed, on theoretical grounds, that the spectrum of the disease might have been larger than previously thought and that it might have included patients with a normal QT interval. This hypothesis implied that the penetrance of LQTS in some families might have been well below the traditional 90%; this was supported first by the evidence that 6% of LQTS family members with a normal QT interval had syncope or cardiac arrest14 and later by the evidence that among the gene carriers of 3 LQT1 families, a few (6%) had a normal QT interval.15

To definitely prove or dismiss this hypothesis and to truly estimate the range of penetrance in LQTS, we selected kindreds in which only the proband was diagnosed clinically as being affected by LQTS. On purely clinical grounds, these patients are traditionally defined as “sporadic cases.” They may actually represent either “de novo” mutations or instances of truly low penetrance. Accordingly, to discriminate between these possibilities, we performed mutation screening in all available family members to identify potential silent gene carriers.

Methods

Study Population

Among the population of LQTS patients followed by our group since 1975, we selected those Italian families for which we had first-hand

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information spanning 3 generations. There were 82 such families. Among these 82 families, there were 32 (39%) in which only 1 member was clinically affected, thus fulfilling the description of sporadic cases. The probands of 9 of these families were successfully genotyped, thus making possible the molecular diagnosis of the available family members.

Nine genotyped probands without clinically affected family members entered the study. The living family members (n=52) were offered the opportunity to enter the study. Four individuals live in Canada and were not interested, and 2 other subjects refused to participate because they were afraid of the potential diagnosis, whereas 46 accepted and are included in the present analysis. LQTS-affected individuals were defined on the basis of current diagnostic criteria, including a QTc >470 ms in asymptomatic individuals and a QTc >440 ms for males and >460 ms for females associated with ≥1 of the following: (1) stress-related syncopal episodes, (2) documented torsade de pointes, or (3) family history of early (<35 years of age) sudden cardiac death. Peripheral venous blood (5 mL), two 12-lead ECGs with >4 QRS complexes in each lead, and a detailed family and personal history were obtained for each family member. Each individual was offered the option to refuse testing. Family 5 has been presented elsewhere.

Mutation Analysis
DNA was extracted from peripheral blood lymphocytes. Synthetic oligonucleotides were used to amplify genomic DNA by polymerase chain reaction. For each reaction, we used 100 ng of genomic DNA. Published primer pairs were used to amplify fragments of HERG and KvLQT1. Identification of mutations was performed by a standard single-strand conformational polymorphism and sequencing protocols performed on DNA of probands, family members, and 100 control individuals, as previously described. All mutations were demonstrated to be unique to the patients and were absent in control individuals.

Mutated amino acids are numbered from the starting methionine according to clones AF 000571 and U04270.

Statistical Analysis
QTc values between probands and family members who were or were not gene carriers were compared by ANOVA and Scheffé’s post hoc analysis; statistical significance was accepted for values of P<0.05.

Results

Study Population
Among the 9 probands (5 females), 6 (66%) were symptomatic for syncopal episodes. The mean age at first episode was 21±21 years (range, 8 to 62 years; median, 10.5 years). Mean QTc in lead II was 504±48 ms. Two asymptomatic probands were diagnosed because of prolongation of QT interval at routine investigation (473 and 540 ms, respectively), and the third was diagnosed after a cardiological examination prompted by her 13-year-old brother’s sudden death while swimming. Genotyping revealed that 5 probands had missense mutations in the HERG gene resulting in an amino acid substitution and 4 had missense mutations in the KvLQT1 gene (Table 1).

Forty-six family members entered the study. All of them had been considered not to be affected by LQTS on the basis of the traditional single 12-lead ECG recording performed by the cardiologist in charge of the family. At entry into the study, they all underwent 3 ECGs on separate days, and blood samples were obtained for mutation screening.

Table 1. Mutations Identified in KvLQT1 and HERG Genes

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Gene</th>
<th>Mutation</th>
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</thead>
<tbody>
<tr>
<td>F1</td>
<td>KvLQT1</td>
<td>S225L</td>
</tr>
<tr>
<td>F2</td>
<td>KvLQT1</td>
<td>Y315C</td>
</tr>
<tr>
<td>F3</td>
<td>HERG</td>
<td>A561V</td>
</tr>
<tr>
<td>F4</td>
<td>KvLQT1</td>
<td>Y281C</td>
</tr>
<tr>
<td>F5</td>
<td>KvLQT1</td>
<td>A300T</td>
</tr>
<tr>
<td>F6</td>
<td>HERG</td>
<td>A561V</td>
</tr>
<tr>
<td>F7</td>
<td>HERG</td>
<td>A614V</td>
</tr>
<tr>
<td>F8</td>
<td>HERG</td>
<td>T436M</td>
</tr>
<tr>
<td>F9</td>
<td>HERG</td>
<td>S428STOP</td>
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Molecular Versus Clinical Diagnosis
We were in the position of comparing clinical and molecular diagnoses. In 55 individuals, molecular diagnosis revealed that 9 probands (mean age, 23±17 years) and 15 family members (43±15 years) were mutation carriers (total, 24 individuals, 12 females). The ECG criteria currently used in linkage studies (see Methods) correctly identified all probands as affected but missed all gene carriers among family members. The morphology of the T wave in family members was normal and did not present notches when any of the 12 leads. The distribution of QTc duration in lead II (Figure 1) as well as the diagnostic criteria proposed by Schwartz et al showed overlap between family members who were and were not gene carriers. All 9 probands and none of the family members had a score ≥4 (high probability of LQTS); all 9 probands and 4 gene carrier family members had a score ≥2 (intermediate probability of LQTS). Therefore, the clinical diagnostic criteria performed as well as the ECG criteria (sensitivity, 38%; specificity, 100%) when a score ≥4 was used for the identification of “affected individuals.” By contrast, less strict criteria (score ≥2) increased sensitivity (54%) at the expense of specificity (90%) (Table 2).

The availability of molecular diagnosis proved that the traditional clinical approach would have missed the identifi-
cation of at least 11 gene carriers who had no obvious signs of LQTS (Figure 2) and would have incorrectly labeled as affected 3 healthy individuals.

Penetrance of LQTS

The parents of 4 probands were not carriers for the mutation, and paternity was confirmed by 7 highly polymorphic markers (not shown). These individuals were therefore considered to be carrying de novo mutations. By contrast, in the remaining 5 families, we identified at least 1 gene carrier among family members. This made it possible to calculate “penetrance,” defined as the ratio between patients with the clinical phenotype (QT prolongation according to the above-mentioned criteria) and the total number of family member carriers of the mutation identified in the proband. Average penetrance in these families was found to be 25%, because, of a total of 20 gene carriers, 5 patients had clinical signs of the disease. The penetrance in family 3 (Figure 2), with the A561V mutation in HERG, is 25%; interestingly, other families with the same mutation have a complete penetrance.19

The analysis of the family history of each proband allowed the identification of 3 individuals who died suddenly at the age of 12, 13, and 14 years, respectively; 2 were within the same family. On the basis of the reasonable assumption that these victims of sudden death also were gene carriers who manifested the disease in the most tragic way, we recalculated the penetrance and obtained an average value of 35%, because, of a total of 23 gene carriers, there were 8 clinically affected individuals. The penetrance for each family was 33%, 33%, 25%, 14%, and 33% when the premature sudden deaths were not included, and 33%, 33%, 25%, 25%, and 60% when they were.

Asymptomatic gene carriers were present among both upstream and downstream generations with respect to the proband. Within the same generation, or among siblings, carriers of the same mutation had no concordance of phenotype (Figure 3).

### Discussion

Contrary to current assumptions, the present study demonstrates that LQTS may appear in some families with an extremely low penetrance. This finding has implications for the understanding of the disease, for its diagnosis, and for its clinical management.

### From Hypothesis to Evidence

In 1980, Schwartz proposed that the spectrum of LQTS might have been much larger than expected at that time and that it was likely to include individuals with a normal QT interval.13

The analysis of 1300 family members with a normal QT interval (<440 ms) of the probands of LQTS families enrolled in the International Registry supported this concept; 6% of them experienced syncope or cardiac arrest.14 This strongly suggested that they were affected by LQTS even though their QT was normal. In 1992, Vincent et al15 reported

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>Molecular Diagnosis</th>
</tr>
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<tbody>
<tr>
<td>Fam: 1 - penetrance 33%</td>
<td></td>
</tr>
<tr>
<td>Fam: 2 - penetrance 33%</td>
<td></td>
</tr>
<tr>
<td>Fam: 3 - penetrance 25%</td>
<td></td>
</tr>
<tr>
<td>Fam: 4 - penetrance 14% (25%)</td>
<td></td>
</tr>
<tr>
<td>Fam: 5 - penetrance 33% (46%)</td>
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Figure 2. Family trees of 5 families in which clinical evaluation (left) has led to identification of proband as only affected member within each family. Results of molecular screening are depicted on right. Solid symbols represent affected individuals identified with 2 approaches. Hatched symbols represent premature (<35 years) sudden death. Family 5 has been described in detail elsewhere.17 Two values of penetrance are shown for families 4 and 5: value within brackets was obtained including premature sudden deaths (see text).

<table>
<thead>
<tr>
<th>TABLE 2. Diagnostic Accuracy of Clinical Parameters</th>
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<tr>
<td>Sensitivity, %</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>ECG criteria9</td>
</tr>
<tr>
<td>Score ≥418</td>
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<tr>
<td>Score ≥216</td>
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Figure 3. ECGs (lead II) recorded in probands and in family members of family 3 (see Figure 2). Abnormal prolongation of QT interval is present only in proband; other family members with same mutation show ECG pattern within normal limits.
that of 82 gene carriers from 3 LQT1 families, 5 (6%) had a normal QT interval. This significant step proved that it is possible to be a gene carrier without a prolonged QT interval; conversely, because most gene carriers in these 3 families had the full phenotype, the leading laboratories continued to assume a penetrance of 90%.9–12 The present study ends the question by identifying gene carriers with a normal QT and normal phenotype in 5 different families and by demonstrating the existence of very low penetrance in LQTS.

To test our hypothesis, we had to focus our study on those families in which the existence of only 1 clinically affected individual was suggestive of a sporadic case, according to the traditional classification.1 However, when faced with families with only 1 clinically affected individual, there are 2 possibilities. One is that the individual is indeed the only affected member in his or her family and that no other gene carriers exist (de novo mutation); the other is that he or she is indeed the only clinically affected individual but that some other family members are gene carriers and as such may transmit the disease; in this case, we would be dealing with low penetrance. Molecular diagnosis allowed us to demonstrate which of the 2 possibilities was correct.

At variance with Vincent’s report,15 only the proband in our families had the clinical phenotype. It follows that silent gene carriers may be expected not only within families with many clinically affected individuals but also within families thought to harbor only 1 sporadic case. This represents the final evidence that the spectrum of the disease is indeed very large and that it includes an unforeseen number of silent gene carriers.

The incidence of these sporadic cases has remained undefined over the years. Our own previous gross estimate, based only on clinical experience, was approximately 35%.2 The present quantitative assessment, based on the families referred to our center, gives an incidence of 39%. It thus seems reasonable to assume that approximately one third of families in which the diagnosis of LQTS has been made include these sporadic cases.

**Implications for Molecular Screening**

Molecular diagnosis is slowly entering clinical practice.8 Its widespread use is delayed by the high cost of the screening process, still performed only by research centers, combined with the long time required to obtain results. Its implementation as clinical routine with reimbursements requires clear definition of the criteria for genetic screening.

Clinically affected individuals should be genotyped, because this may have implications for therapy and management.20–22 Still unclear is who else in the family should be tested, once the gene is identified in the proband. Borderline cases could also benefit, because molecular screening would provide a definitive diagnosis, thus ending their doubts. The present findings conclusively demonstrate that penetrance may be so low that in certain families, several members would escape clinical diagnosis despite being gene carriers. In our opinion, performance of molecular screening in these family members as well should no longer be optional. As proponents of a more widespread clinical use of molecular diagnosis, it seems appropriate for us to indicate the availability of our group to perform these tests free of charge in the family members of genotyped probands.

The limitations manifested here by the clinical diagnostic criteria9–16 are the direct consequence of the design of the present study, which has selected families with apparently only 1 member affected. These limitations are not encountered when we are dealing with families in which LQTS+ has a high penetrance.23 For families with few obviously affected individuals, the use of other diagnostic criteria not yet entered into widespread clinical practice may be useful.24–28

Our data also show that some individuals previously considered to be affected by LQTS are not gene carriers. All 3 were diagnosed as affected on the basis of their being family members of a typical LQTS patient (prolonged QT and syncope) and of being males, all with a QTc of 450 ms. This finding calls for caution before diagnosing LQTS in family members with just a modest QT prolongation.

It is conceptually and practically important to realize that the low penetrance demonstrated here in some families affected by LQTS is not dictated by the localization of the mutations involved. Indeed, although a “forme fruste” of LQTS has been reported with a mutation in the C-terminal domain of KvLQT1,29 it now appears that this phenomenon may be also associated with mutations located in the S1–S6 regions of both KvLQT1 and HERG. Furthermore, the mutation A561V, originally described as being associated with high penetrance and recognized later as being a mutational hot spot,19 has now also been found to be associated with a penetrance as low as 25% (Figures 2 and 3). This points to the fact that penetrance does not depend solely on the specific mutations and that it can be profoundly modified by other factors not yet identified. Finally, the possibility that symptomatic patients carry 2 mutations (“compound heterozygotes”) cannot be ruled out.

**Implications for Clinical Management**

The existence of LQTS families with a very low penetrance has multiple clinical implications, all stemming from the fact that an individual who was, and without molecular biology always would have been, considered perfectly healthy is suddenly recognized as a gene carrier for a potentially lethal disease. This individual has the risk of developing a life-threatening arrhythmia and of transmitting the disease to 50% of his or her offspring.

Among our 15 family members found to be gene carriers, only 3 were <30 years old, and all the others were >40 years old. Because LQTS usually manifests itself at a young age, this suggests that these individuals are likely to remain asymptomatic. Conversely, the alteration in repolarizing currents produced by their mutations, even without overt ECG changes, clearly predisposes to the possible occurrence of drug-induced torsade de pointes.30,31 These individuals should be informed about this possibility and be alerted to avoid all drugs, cardiac and noncardiac, that block potassium currents and also situations in which hypokalemia might occur.32 Accordingly, they should receive a comprehensive, updated list of these compounds. With rare exceptions,17 LQTS is an autosomal dominant disease, and these silent gene carriers should be informed that 50% of their offspring
may be expected to carry the same mutations. Molecular diagnosis therefore has to be scheduled for their newborn infants, unless they have obvious QT prolongations.

Should any of these infants be diagnosed as a gene carrier, no assumptions on their being at low risk could be derived from the fact that the affected parent was asymptomatic. In our families, asymptomatic gene carriers were found upstream and downstream with respect to the proband. Thus, parents without the LQTS phenotype may generate highly symptomatic individuals, and symptomatic parents may generate gene carriers with a silent disease.

This report should affect the way physicians deal with LQTS families and has potential medicolegal implications. Contrary to current practice, it is no longer possible to reassure family members of LQTS probands that they are not disease carriers if they have a normal ECG. LQTS should no longer be excluded on purely clinical grounds. Such a significant statement will require the support of a molecular screening negative for the mutations present in the affected patient.

References


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