Editorial

Is long QT syndrome entering the era of molecular diagnosis?

The idiopathic long QT syndrome is a congenital disease characterised by prolongation of the QT interval and by stress-induced syncopal episodes caused by the occurrence of "torsades de pointes".¹ The understanding of the pathophysiological mechanisms of LQTS has been the subject of speculation for several years.² Because LQTS is a familial disease it became evident that major developments in the understanding of the disease were likely to come from the rapid developments in molecular biology. Whenever the genetic defect of a familial disease is identified, major advances are produced immediately at two levels: the pathophysiological substrate is conclusively determined and unequivocal identification of gene carriers becomes possible.

As a result of these advances, the Investigators of the International Registry of the LQTS and molecular biologists are collaborating in an attempt to identify the genetic defect in LQTS. The major achievements in the molecular understanding of LQTS will be briefly summarised in order to set the stage for the discussion of the clinical impact of these discoveries.

The genetic approach

In 1991 Keating *et al*³ demonstrated linkage between LQTS and chromosome 11. This was followed in 1994 by the finding that other LQTS families were linked to chromosomes 3 and 7⁴ and by a report suggesting linkage with chromosome 4 in one family.⁵ By the end of 1994 there was definitive evidence of the genetic heterogeneity of LQTS.⁶ This evidence implies that what we used to regard as one disease is the common phenotypic manifestation of at least five different diseases.

In 1995 the genes for chromosomes 3 (LQT3), 7 (LQT2), and 11 (LQT1) were identified⁷⁻¹⁰ respectively as *SCN5A* (the cardiac human sodium channel) and *HERG* and *KVLQT1* which encode for two different potassium channels. Research was then directed toward the characterisation (identification, expression, and development of gene-based cellular models) of the specific mutations present in the gene of affected individuals.¹¹⁻¹⁶ It is not uncommon for different mutations within a given gene to account for the diverse clinical manifestations (phenotype) in affected families. Hypertrophic cardiomyopathy is a striking example of a disease in which mutations not only in

several genes, such as cardiac myosin heavy chain, tropomyosin, and cardiac troponin T, but also, most importantly, different mutations within the same gene have been associated to forms of the disease with very different clinical outcome.¹⁷

In the case of LQTS several different mutations have been identified in each gene. Sixteen LQT1 families⁹ showing ten different mutations have been studied; five LQT2 kindred were reported by Keating *et al*,⁸ two by Scholtze-Bahr *et al*,¹⁵ and two by Priori *et al* (personal communication): each family presented different mutations. Finally, three mutations have been identified in six LQT3 families.^{7,10}

Current perspectives in molecular diagnosis of LQTS

Not only is each genetic variant likely to cause a different syndrome, but each mutation has the potential to cause a disease with its own natural history. This conclusion has practical consequences in terms of what we can expect from molecular diagnosis of LQTS and from clinical exploitation of molecular knowledge.

First of all, the evidence that no single mutation accounts for a high percentage of clinical cases of LQTS makes it unlikely that population screening will be fruitful. Therefore molecular diagnosis should be limited to those families in which diagnosis of LQTS has been made or at least suspected on sound clinical grounds. In these cases the identification of gene carriers has practical consequences. Unlike disorders in which early diagnosis is of limited clinical value because no treatment is available, LQTS can be treated effectively and molecular diagnosis if it extended treatment to gene carriers could prevent the occurrence of sudden death. Currently there are no data to define which gene carriers for each LQT variant are at risk of developing a full-blown disease, and we do not know whether antiadrenergic treatment in all gene carriers would improve survival. Because β blockade is an effective, relatively well tolerated, and low-cost treatment the option of prophylactic treatment should at least be discussed with patients.

How to approach clinical molecular diagnosis of LQTS

The molecular techniques used for research can also be used for clinical screening. Linkage analysis can be used to identify the chromosome on which the disease-causing mutation is located. Linkage analysis is based on the use of polymorphic chromosomal markers positioned close to the disease gene. Two allelic forms of a marker (one of maternal and the other of paternal origin) are present in each individual; the haplotype of each family member is defined and if all the affected individuals share the same haplotype, there will be a high degree of probability that the marker is cosegregating with the disease and linkage can be proven. Statistical analysis can be used to calculate the odds that the DNA marker and the disease gene are linked. The odds are expressed in logarithmic form called the LOD (logarithm of the odds) score. A limitation of linkage analysis is that large families are required to

Glossary of acronyms

LQT, Long QT

LQTS, Long QT syndrome

LQT1, Genetic defect on chromosome 11

LQT2, Genetic defect on chromosome 7

LQT3, Genetic defect on chromosome 3

SCN5A, Human cardiac sodium channel gene

HERG, Human eag-related gene

KVLQT1, Human potassium channel gene on chromosome 11p15.5

achieve significant LOD scores. Obviously this approach is not suitable for identifying genetic defects in the so-called "sporadic cases" in which a de novo mutation has occurred.

Molecular diagnosis of LQTS using linkage analysis requires that markers located on chromosomes 3, 4, 7, and 11 are evaluated in each family. In most cases linkage to one or more chromosome(s) can be positively excluded, but linkage to one of the chromosomes tested may not be established in all families. When linkage to all four chromosomes is excluded, the diagnosis of LQTS is still not ruled out, because not all LQTS forms are caused by genes located on chromosomes 3, 4, 7, and 11. Unfortunately we do not have a precise estimate of the percentage of LQTS cases caused by unidentified genetic defects. So although we know that the sensitivity of linkage analysis in LQTS is not 100%, we can make only an educated guess that the sensitivity is probably about 80%.

When linkage is established in a LQT kindred, the LQT gene located on that chromosome has to be carefully screened to identify the mutation responsible for the phenotype. This approach, called "mutational analysis", is also the elective method for studying sporadic cases and small families in which linkage cannot be established.

The search for specific mutations within a gene for LQTS is also limited by the diagnostic accuracy of the test. Because the complete genomic sequence is available only for the LQT3 gene, *SCN5A*,¹⁸ we are currently able to screen only about 60% of *HERG* (the LQT2 gene) and 50% of *KVLQT1* (the LQT1 gene) cases for mutations.

In practical terms this means that, with the support of dedicated molecular diagnostic facilities, genetic testing is worthwhile in all subjects with a diagnosis of LQTS, both familial and sporadic; but the cost of the approach must be weighed against the fact that a negative result, which is expected in 40–50% of cases, will not provide clinical information. Obviously a positive result will provide extremely valuable information.

When a mutation is found, all family members are screened and gene carriers identified; this allows the accuracy of clinical diagnosis to be confirmed and appropriate action to be taken. Asymptomatic gene carriers could be advised to avoid stressful conditions and competitive sport, and—depending on the malignancy of the disease in the family—antiadrenergic treatment can be started. Conversely family members identified as "non gene-carriers" can be assured that they can conduct a completely normal life.

Social impact of genetic testing

The impact of genetic information within a family is enormous and the practical and psychological consequences should not be underestimated.

Those identified as being in the line of transmission of the disease may feel guilty for having passed the "bad" genes; those who were clinically considered to be unlikely to be affected may be profoundly upset by the knowledge that they are a gene carrier; on the other hand, knowledge that not all their children are affected can be extremely positive and reassuring for the parents of sick children.

Because molecular diagnosis is relatively new its consequences in terms of discrimination against gene carriers are unknown. Gene carriers of diseases with high morbidity or mortality could be disadvantaged in their opportunities for employment and insurance. To limit this risk patients should be protected: access to genetic information should be strictly limited and release of results of molecular diagnostic restricted to the individuals who have been tested.

Conclusion

In the past five years major advances in the knowledge of the molecular basis of LQTS have greatly modified our understanding of the disease.

For many years the phenotype (prolonged QT interval and ventricular arrhythmias) has been regarded as the marker of a single disease. We now recognise that prolongation of ventricular repolarisation is the common phenotypic manifestation of different mutations on several genes: and each mutated gene should be regarded as the substrate for an individual disorder. The situation may be even more complex in that each mutation within a single gene may cause a disease with a specific clinical profile.

Molecular diagnosis of LQTS is now becoming available to practising cardiologists; however, the sensitivity and specificity of results must be carefully understood to avoid raising unrealistic expectations in patients. Finally the lack of information about the risk of severe arrhythmias developing in asymptomatic gene carriers limits our ability to define guidelines for their clinical management.

With the identification of three genes, we have only scratched the surface in the understanding of LQTS and it will take a large collaborative effort lasting several years before the clinical benefit of a molecular understanding of LQTS can be realised fully.

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