MOLECULAR AND GENETIC BASIS OF ELECTROPHYSIOLOGICAL DISEASE

The long QT syndrome

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Introduction

The long QT syndrome (LQTS) is a familiar disease\(^1\)\(^,\)^\(^2\) characterized by abnormally prolonged ventricular repolarization and a high incidence of malignant ventricular tachyarrhythmias, occurring mainly during physical or emotional stress.

Since its original description\(^3\)\(^,\)^\(^4\), it has become clear that no structural cardiac abnormalities are associated with LQTS, so patients have a morphologically intact heart. Rhythm disturbances were mainly the consequence of a ‘primary electrical disorder’.

With the aim of gaining a better understanding of the disease, Schwartz \textit{et al.} formulated a hypothesis based on clinical and experimental observations, and proposed that the phenotype was the consequence of an inherited abnormality of cardiac sympathetic innervation (Sympathetic Imbalance Hypothesis)\(^5\). It was with the advent of linkage analysis that the genetic studies confuted the theory and demonstrated that the predisposition to develop ventricular arrhythmias is the consequence of genetically determined alterations of cardiac ion channels.

The long QT syndrome is transmitted mainly as an autosomal dominant disease\(^3\)\(^,\)^\(^4\)\(^,\), the so-called Romano-Ward syndrome (R-W), that accounts for the majority of cases. The autosomal recessive form denominated Jervell and Lange-Nielsen syndrome (J-LN), is characterized by the coexistence of QT prolongation and congenital deafness\(^6\).

The long QT syndrome is associated with sudden cardiac death, therefore it is listed among the life-threatening diseases; what is rather unusual, however, is that when the proper diagnosis is established, the most severe complications can be prevented with the use of antiadrenergic treatments (drugs or surgical denervation). The ability of clinicians to recognize and treat the disease is extremely effective in preventing casualties in young patients. It is, therefore, of major relevance that clinical cardiologists, paediatricians, neurologists (who are frequently consulted initially when a young individual reports a syncopal episode) and sport physicians are able to recognize LQTS, or at least to suspect it and refer patients to specialized centres.

The following sections will briefly review clinical characteristics of LQTS and current knowledge about its genetic substrate.

Clinical presentation of LQTS

The two cardinal manifestations of LQTS are syncopal episodes and prolongation of repolarization, however, several additional features may help in establishing the diagnosis.

Syncopal episodes

Torsade de pointes (TdP) is considered to be the arrhythmia responsible for syncopal episodes. Occasionally, it degenerates into ventricular fibrillation (VF) and may lead to sudden death. Spontaneous termination of VF has been documented (Fig. 1).
Torsade de pointes may initiate without changes in heart rate and without specific sequences such as the short-long-short interval, even though a pause does often precede its onset\(^7\)–\(^9\).

The occurrence of syncopal episodes is typically associated with sudden increases in sympathetic activity, such as those occurring during intense emotion (particularly fright, but also anger) or physical activity (notably swimming)\(^10\). Loud noise (alarm clock, telephone, thunder) is the definitive trigger for some patients. Correlation between the genetic variants of the disease and the specific triggers associated with cardiac events has been established and will be discussed later in this paper. A higher incidence of syncope in correspondence with menses has been noted\(^10\), and an increase in the number of events has also been observed in the postpartum period\(^11\).

In sharp contrast with common knowledge, in some families cardiac arrest occurs almost exclusively either at rest or during sleep\(^12\)–\(^13\). As discussed in the following text, recent data suggest that the propensity for life-threatening arrhythmias under stress or at rest may be influenced by specific genetic mutations\(^14\).

When a young patient (child or teenager) seeks medical attention for the recurrence of syncopal events associated with convulsions, a neurologist is often involved initially to evaluate the possibility of epilepsy. If a syncopal episode caused by a ventricular tachyarrhythmia is prolonged, it may cause abnormalities on the EEG, so it is not infrequent that the patient is treated with antiepileptic agents ‘ex adjuvantibus’ with a diagnosis of an atypical form of epilepsy.

The present authors have recently observed a family in which the mother has been treated for 20 years with antiepileptic therapy and the daughter (pt5) for 7 years, until a recurrent syncopal episode brought the patients to a different hospital and diagnosis of LQTS was established. These patients have now been genotyped as affected by a mutation in the HERG gene (Fig. 2).

Other inappropriate diagnoses frequently attributed to LQTS patients are:

- ‘hysterical syncope’, frequently considered as the cause for fainting episodes in young ladies, and
- ‘affective spasms’ in children younger than 2 years of age, in whom temper tantrum and breath holding are considered the cause for the repeated syncope during stressful conditions such as being reprimanded by parents or relatives.

**Electrocardiographic aspects**

Over the years, it has become clear that in LQTS patients there is much more than a mere prolongation of ventricular repolarization. The T-wave has several morphologic patterns that are easily recognizable on the basis of clinical experience; they are difficult to quantify but very useful for diagnosis (Fig. 3).

**Figure 2** Pedigree of a LQT2 family. In this family, the mother (pt1) has been treated for 20 years with antiepileptic therapy and the daughter (pt5) for 7 years, until a recurrent syncopal episode brought the patients to a different hospital and diagnosis of LQTS was established. Results of the molecular screening are reported in the inset, showing an abnormal electrophoresis pattern in pt1 and 5. Polymerase chain reaction amplified DNA samples have been analysed by single strand conformational polymorphism. Subsequent DNA sequencing demonstrated the presence of single amino acid substitution in the HERG-encoded protein. *, gene carrier.

**QT prolongation**

Despite constant criticism, Bazett’s correction for heart rate continues to be used as a valid clinical tool\(^15\). Recent data on gender-related differences in the general population have suggested that QTc values up to 460 ms may be still normal among females\(^16\); on the contrary, values in excess of 440 ms are considered to be prolonged in males. The longer QT values present in normal women become evident only after puberty, but are absent at birth\(^17\), suggesting a role for hormonal changes. Kunchithapatham et al. recently studied 19 healthy pregnant women during pregnancy and after delivery, to assess the hormonal effect on the QTc interval\(^18\). The high concentrations of female hormones seen in pregnant women were not accompanied by an increase in the QTc and do not support a role for female hormones in explaining the observed gender difference in the QTc\(^18\).

The extent of QT prolongation is not strictly correlated with the likelihood of syncopal episodes, even though the occurrence of malignant arrhythmias is more frequent among patients with very marked prolongation (QTc>600 ms).

A major step forward in the comprehension of the disease was taken in 1980\(^19\) when Schwartz proposed that some patients may be affected by LQTS and still have a normal QT interval on the surface ECG. This consideration was initially supported by the identification of symptomatic family members with a normal
QTc, and was subsequently confirmed by the existence of gene carriers with a normal QTc. Data from the International Registry of LQTS indicated in 1989 that out of 503 family members with a QTc<440 ms, 50 (10%) had a cardiac arrest \[20\]. Similarly, the report by Garson et al. \[21\] on 287 LQTS patients indicated that 6% of them had a normal QTc. Finally, the present authors’ recent analysis of 566 genotyped patients revealed that 35 (6%) have a QTc<450 ms and, among them, 21 experienced syncope or cardiac arrest.

These data demonstrate that it is impossible to exclude the diagnosis of LQTS simply on the basis of a normal QTc. In the presence of clinical suspicion of LQTS, based on recurrent unexplained syncopal episodes accompanied by convulsions and cyanosis, or occurring in a family member of an LQTS proband, the evidence of a normal QT interval (especially if it is ‘borderline normal’), should not rule out the diagnosis.

T-wave morphology

In most LQTS patients, ventricular repolarization is not only prolonged, but is also altered in its morphology. Typically, the T-wave may be biphasic or notched, particularly in the precordial leads, suggesting regional differences in the time course of ventricular repolarization. Compared with healthy individuals of the same age and sex, the LQTS patients have biphasic or notched T-waves more frequently (62 vs 15%, \(P<0.001\)) \[22\]. When these patterns are present in young (<13 years) control subjects, they are usually limited to leads V2 and V3, whereas among LQTS patients they are usually visible from leads V2 to V5 and are often more pronounced in leads V3 and V4. The presence of these repolarization abnormalities is more frequent in those LQTS patients with cardiac events (81 vs 19%, \(P<0.005\)). Finally, and important for diagnosis, the appearance of notched T-waves in the recovery phase of exercise is markedly more frequent (85 vs 3%, \(P<0.0001\)) among patients than among healthy controls.

Which is the electrophysiological background of these notches? It has been hypothesized that T-wave notches may be related to early afterdepolarizations. This hypothesis is supported by the observation that patients with T-wave notches are at higher risk of cardiac arrhythmias \[23\].

**QT dispersion and QT complexity**

In 1986, De Ambroggi et al. \[24\] demonstrated, in a case-control study, the existence of an abnormal pattern of ventricular repolarization in LQTS patients, and identified two specific abnormalities in these patients:

- a larger than normal area of negative values in the anterior chest that can be interpreted as delayed repolarization of the anterior ventricular wall, and
- a complex multipeak distribution that suggests regional electrical disparities in the recovery process.

Inhomogeneous action potential duration and recovery of excitability in neighbouring myocardial areas represents a well-known arrhythmogenic substrate, being one of the conditions for the development of re-entrant circuits and sustained tachyarrhythmias \[25,26\]. Therefore, the possibility of an accurate detection of such a highly vulnerable substrate in the clinical setting could represent a considerable tool for the assessment of the risk of development of malignant ventricular tachyarrhythmias.

A simple approach to the evaluation of dispersion of repolarization and, therefore, of electrical instability, is
based on the 12-lead surface ECG[24], and quantifies the difference between the longest and the shortest QT interval (or QTc) measured in the 12 leads. QT dispersion is markedly prolonged among LQTS patients[27,29].

QT dispersion during therapy has prognostic implications: patients who remained free of syncope for more than 5 years after left cardiac sympathetic denervation or beta-blockers had a significantly lower QT dispersion than untreated subjects or non-responders to beta-blockade[28].

The persistence of excessive QT dispersion after institution of therapy with beta-blockers may identify patients likely to remain at high risk and, thus, suggest when it is necessary to proceed with left cardiac sympathetic denervation.

This study provided the first evidence that left cardiac sympathetic denervation enhances homogeneity of ventricular repolarization, and so increases ventricular fibrillation threshold (antifibrillatory effect).

QT dispersion is not the only easy-to-obtain index for the assessment of heterogeneous ventricular repolarization. Another interesting index is the principal component analysis (PCA), aimed at the quantification of the spatial components (vectors) of the T-wave, independently from its onset and offset.

Principal component anlaysis was initially applied to body surface mapping, but this is a quite a complex technique, requiring the use of multiple electrodes (60–150) applied to anterior and posterior chest[30].

The present authors developed a new algorithm for the calculation of PCA from 12-lead Holter recordings[31]. A set of eight values (eigenvectors) were identified which represent the magnitude of the different components of the entire repolarization process. The quantification of the relative contribution of these components provided an estimate of the complexity of repolarization.

Using this technique, a population of LQTS patients was evaluated and compared with healthy controls. The complexity of repolarization (CR), calculated every hour during Holter recordings and averaged (CR_{24 h}), was found to be significantly higher in the LQTS group[31]. In this study, CR_{24 h} showed 88% sensitivity and a 91% negative predictive value for the identification of LQTS patients. Interestingly, in LQTS patients, in addition to the increased CR_{24 h}, its variability was also significantly increased[31].

**T-wave alternans**

T-wave alternans is a beat-to-beat alternation in polarity or amplitude of the T-wave, that may be present at rest for brief moments but most commonly appears during emotional or physical stress and may precede TdP.

This electrocardiographic finding is a marker of major electrical instability and allows one to identify patients at particularly high risk (Fig. 4).

**Heart rate**

In 1975, Schwartz et al.[5] observed a lower than normal heart rate in most LQTS patients. This phenomenon is particularly striking in children and even in foetuses, and is a rather ubiquitous finding[12]. During exercise, most LQTS patients reach a heart rate level lower than that achieved by healthy controls matched by age and sex[33].

**Treatment**

A sudden increase in sympathetic activity[4], mostly mediated by the left cardiac sympathetic nerves, is the
trigger for most of the episodes of life-threatening arrhythmias in LQTS patients. Indeed, antiadrenergic therapies provide the greatest degree of protection. This concept was supported by a study published in 1985\textsuperscript{34} including 233 symptomatic patients. Data showed that the mortality at 15 years after the first syncope was 9% in the group treated by antiadrenergic therapy (beta-blockers and/or cardiac denervation), and more than 53% in the group not treated or treated by miscellaneous therapies not including beta-blockers. These data suggest that pharmacological and/or surgical antiadrenergic therapy radically modifies prognosis for symptomatic patients with LQTS. However, it is important to remember that they were not obtained in a prospective randomized trial. Despite the need for such a prospective trial advocated by all the investigators in the field, at the present time it must be considered unethical to have a control group not receiving beta-blockers; therefore, such a trial will have to be postponed until an adequate number of highly symptomatic patients have a defibrillator implanted, allowing a safe assessment of the role of antiadrenergic therapy in a rigorously designed trial.

**Beta-adrenergic blockade**

Beta-adrenergic blocking drugs are considered to be the treatment of choice in symptomatic LQTS patients, unless specific contraindications are present, and these agents are also prescribed prophylactically in some affected family members.

Propranolol is still the most widely used beta-blocker, because of its well-known tolerability in long-term therapy. The appropriate dose of propranolol is in the range of 2–3 mg kg\textsuperscript{-1} day\textsuperscript{-1}; large individual variability exists in the tolerability of beta-blockers. Since no strict information exists on the optimal dosage, dosage is adjusted to the maximum tolerated. Propranolol is contraindicated in patients with asthma and diabetes, and its short half-life makes necessary multiple daily administrations, that may reduce compliance. To improve compliance, nadolol (1 mg kg\textsuperscript{-1} day\textsuperscript{-1}) is used, as this allows single daily administration.

In patients who do not tolerate beta-blockers because of excessive bradycardia, and in patients in whom this therapy may have limited usefulness as they tend to have TdP at low heart rate, the combination of beta-blockers and cardiac pacing may be employed, or left cardiac sympathetic denervation is required.

**Left cardiac sympathetic denervation (LCSD)**

Left cardiac sympathetic denervation requires removal of the first four to five thoracic ganglia. This denervation is performed by an extrapleural approach which makes thoracotomy unnecessary. The average time for the complete operation is 35–40 min\textsuperscript{35}. Left cardiac sympathetic denervation produces impressive decreases ($P<0.0001$) in the number of patients with cardiac events (from 99 to 45%), in the number of cardiac events per patient (from 21 ± 31 to 1 ± 3), and in the number of patients with five or more cardiac events (from 65 to 9\%)\textsuperscript{35}.

Left cardiac sympathetic denervation prevents lethal arrhythmias of LQTS, not only by removing the trigger, but also by modifying the substrate. In fact, LCSD reduces QT dispersion, a marker of electrical instability\textsuperscript{28}.

Treatment in LQTS patients should always begin with beta-blockers, unless there are valid contraindications. However, if the patient continues to have syncope despite full-dose beta-blockade, LCSD could be performed and ICD implantation could be considered.

**Cardiac pacing**

Cardiac pacing is clearly indicated in LQTS patients with atioventricular block and whenever there is evidence of pause-dependent malignant arrhythmias\textsuperscript{39}. The first clear evidence of a benefit in using a pacemaker in some LQTS patients comes from the International Registry (unpubl. data). In 30 patients, a pacemaker was implanted after beta-blocker failure, without an associated increase in the dosage of beta-blockers. After a follow-up period of 2 years, a significant decrease in the number of patients with syncope was found.

Pacemakers should never be used as a sole therapy for LQTS. They should be regarded as an adjuvant to beta-blocking therapy in selected patients. In the International Registry, beta-blocker therapy was only withdrawn in 10 patients after pacemaker implantation, and three of them died suddenly within 2 years (unpubl. data).

In patients with pause-dependent TdP, the combination of a pacemaker and beta-blockers is not the only choice. Left cardiac sympathetic denervation should also be considered as this selective denervation does not significantly reduce heart rate.

**Implantable defibrillators**

The use of the defibrillator in LQTS is progressively increasing. There is no indication to use an ICD in asymptomatic patients because, as will be discussed later, there is incomplete manifestation of the disease. Therefore, in the authors’ opinion, prolongation of the QT interval is not a sufficiently strong predictor of cardiac arrest to justify an ICD implantation. Other patients that, in the authors’ practice, are not considered candidates for an ICD are individuals with a history of one or few pre-syncope or short-lasting syncopal episodes, especially if the nature of the events could be benign (vasovagal). It is obviously difficult to decide, based on the description of the event by the patient or by
the parents, the probability of the event being caused by a ventricular tachyarrhythmia; however, some criteria are adopted in clinical practice at least to estimate the probability of the occurrence of a tachyarrhythmia. If, for example, in an LQTS patient, very fast and/or irregular heart rhythm was detected by bystanders during the event, or if the patient was cyanotic and unconscious for a longer time than in a simple faint or if the patient had seizures or urinary or faecal incontinence, it would be considered more likely that a cardiac arrhythmia had occurred.

The ICD is considered to be an appropriate therapy, in addition to antiadrenergic therapy, in patients with documented TdP (fast rate, long-lasting episodes), or with TdP/VF that required cardioversion/resuscitation. Drawing the separation line between candidates for medical therapy and candidates for ICD is not easy; pros and cons should be carefully considered.

The arguments in support of a wider use of defibrillators are based on the urgency felt by many to minimize the risk of cardiac death in affected individuals; however, the price to be paid if a larger number of individuals is implanted will be reduced quality of life due to the device, in a large population of patients that may respond well to antiadrenergic therapy and therefore never experience an appropriate shock.

A problem in the use of an ICD in LQTS patients is that TdP are frequently self-terminating and do not lead to loss of consciousness, therefore these patients may experience inappropriate discharges. In addition, the present authors have also observed inappropriate discharges caused by double sensing on the T-wave, that may become morphologically aberrant and even exceed the QRS in amplitude. An inappropriate ICD discharge in a conscious patient leads to massive release of catecholamines which may precipitate further arrhythmias and produce a vicious circle.

In the case of implantation of the ICD in children and teenagers, it must be remembered that long-term follow-up in this patient group is not available, and furthermore they will have to live with the defibrillator for several decades.

Even in patients receiving an ICD, antiadrenergic therapy is implemented and maintained to reduce the occurrence of cardiac events and to prevent excessive heart rate increase (>190 bpm) that may trigger inappropriate shocks.

**Molecular genetics of LQTS**

Long QT syndrome is an inherited disease that only a few years ago was still called ‘idiopathic’ as the underlying causes were unknown. As for many other inherited diseases, the contribution of molecular techniques was necessary to unveil the mystery of LQTS. The most significant breakthrough occurred in 1991, when Keating et al. demonstrated tight linkage of LQTS to the Harvey RAS1 gene locus on the short arm of chromosome 11 (LQTI). This was followed by the finding that other LQTS families were linked to chromosomes 3 (LQT3) and 7 (LQT2) and by a report that linkage to chromosome 4 was also present in LQTS.

These early studies demonstrating genetic heterogeneity of LQTS paved the way to the identification of LQTS genes. Based on the clinical evidence that LQTS is an electrical disease, genes encoding ion channel proteins have been considered candidate genes for the disease. The successful identification of three LQTS genes conclusively proved the hypothesis, and since then LQTS has been included among the so-called ‘channelopathies’.

Expression studies have characterized the type of current conducted by each of these first three channels associated with LQTS. The gene for LQT2 is HERG, a potassium channel conducting I\(_\text{k}\) current. The gene for LQT3 is SCN5A, the cardiac sodium channel gene. The gene for LQT1 is KvLQT1, a component of the potassium channel conducting the I\(_\text{ks}\) current. This latter gene is also implicated in LQTS associated with congenital deafness.

Recently, another two LQTS genes have been identified on chromosome 21: KCNE1 or minK, the gene for LQT5; and KCNE2 or MiRP1, the gene for LQT6. The gene for LQT4 on chromosome 4 has not yet been identified.

The present authors have screened 200 probands for mutations in the five LQTS-related genes; a defect was identified in 46% of the patients, suggesting that approximately half of the patients have mutations on as yet unknown genes. The relative distribution of mutations among the known genes suggests that KvLQT1 accounts for 54% of the mutations identified, followed by HERG 35% and SCN5A 10%. KCNE1 and KCNE2 are very rare causes of LQTS (Priori, unpubl.).

**KvLQT1 and KCNE1**

Mutations in KvLQT1 cause LQT1, the most common genetic variant of LQTS; mutations in KCNE1 cause LQT5, a rare type of LQTS. The present authors screened 140 LQTS patients and identified only three KCNE1 mutations, thus suggesting a prevalence of less than 3% for LQT5. LQT1 and LQT5 may share common clinical features because the two gene products co-assemble to form the ion channel conducting the repolarizing cardiac current I\(_\text{ks}\). KvLQT1 and KCNE1 mutations prolong the QT interval, reducing this current.

Mutations in the KvLQT1 gene have been identified in families from all over the world. The reported mutations are mainly missense mutations, however, insertions, deletions and splice-donor errors have also been identified. Most of the families studied have their own specific mutation, but in some families, the alanine at position 246 is replaced by a valine or by a glutamic acid (KvLQT1 hot spot).
Expression studies have shown that mutations may result in non-functional proteins that do not co-assemble with the wild-type protein. In this case, the loss of function equates to a 50% reduction, because the wild-type allele is fully functional. However, other mutations result in poorly functional proteins that interfere in a ‘dominant negative’ fashion with the wild-type protein, and produce a loss of function greater than 50%. Thus, the more severe molecular defects, producing a non-functional protein, may result in a less severe electrophysiological defect.

Mutations in KvLQT1[42] and KCNE1[45,46] are also responsible for J-LN syndrome[47]; the recessive form is an extracellular a defect. A non-functional protein, may result in a less severe electrophysiological defect.

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Mutations in KvLQT1[42] and KCNE1[45,46] are also responsible for J-LN syndrome[47]; the recessive form is an extracellular a defect. A non-functional protein, may result in a less severe electrophysiological defect[50]. It is not yet known if the simultaneous presence of a heterozygous defect in KvLQT1 and in KCNE1 would result in the J-LN phenotype.

Interestingly, parents of J-LN patients are heterozygous carriers of KvLQT1 or KCNEDI mutations, therefore they are affected by the Romano Ward syndrome. Most of them have a normal QT interval and remain asymptomatic throughout life. These demonstrate that some ‘mild’ mutations may remain subclinical and only manifest when (by chance) a ‘double’ dose is inherited.

The spectra of severity of mutations is quite large and it creates an unsuspected range of clinical phenotypes. The present authors recently showed[57] that ‘mild’ mutations in KvLQT1 may be associated with a novel variant of the disease, called ‘recessive Romano Ward’ to account for the fact that heterozygous carriers show no cardiac and no auditory phenotype, while homozygous carriers manifest the cardiac phenotype but in the presence of fully conserved hearing function.

Other novel observations have emerged together with the identification of a growing number of patients with subclinical mutations. The evidence that mutation carriers may have a normal QT interval and be asymptomatic opens the inference that the prevalence of ion channel mutations in the general population may be higher than previously thought. Furthermore, mild mutations are expected to reduce the ‘repolarization reserve’[49], therefore, even in the absence of a prolongation of QT interval, the heart may be more susceptible to factors that further prolong ventricular repolarization. The present authors recently showed that drug-induced ‘Torsade de Pointes’ may be facilitated in patients with a subclinical LQTS mutation. The patient studied was a carrier of a KvLQT1 defect. Treatment with a low dose of cisapride (a prokinetic agent with IKr blocking properties) was sufficient to induce marked prolongation of QT interval and precipitate VF in this patient[59].

The mutations identified in HERG[41,43,60–63] are mainly missense mutations leading to changes in highly conserved amino acids. However, deletions, frame shifts and splice-donor errors have also been reported[43]. Point mutations have been identified in all four transmembrane segments, and expression studies have shown that functionally relevant reductions in IKr current are caused by minimal amino acid changes. The only ‘hot spot’ area described for HERG appears to be amino acid 561, where an A to V substitution has been described[41,54,64]. When the A561V mutant protein is expressed together with the wild-type protein, a dominant negative effect is produced, leading to a substantial reduction in the function of the channel[63].

In some patients, a reduction of IKr current is due to mutations in the cyclic-nucleotide-binding domain (NBD), located on the C-terminus of HERG[65]. These mutations appear to cause defective protein trafficking that results in the retention of mutant channels in the endoplasmic reticulum. This observation demonstrates that the NBD may play an important role in modulating HERG channel protein processing and trafficking.

The expression studies evaluated the effect on repolarization of different mutations[66], and found it was impossible to find a correlation between the severity of the clinical manifestations and the spectrum of HERG dysfunction as assessed in vitro[67]. This implies that other, still unknown, factors modulate the clinical phenotype, even of the same genotype.

**KCNEd2**

The gene for LQT6 is KCNEd2, a small integral membrane subunit that assembles with HERG to form the ion channel conducting the IKr current. Mutations in KCNEd2 are rare; Abbott et al.[68] screened 250 patients without mutations in KvLQT1, HERG, SCN5A and KCNEDI, and they identified three missense mutations and a rare polymorphism, thus suggesting a prevalence of 0.6% for LQT6. The present authors had a similar result; 200 LQTS patients were screened for mutations on KCNEd2 and a six base pair deletion (Delta156–161) was identified in one family (Priori, unpubl. data).

Channels formed with mutant MiRP1 subunits and HERG showed slower activation, faster deactivation and thereby a reduced potassium current[68].

MiRP1 is not the only subunit that assembles with HERG, Bianchi et al.[69] recently proved that minK is a cofactor in the expression of both IKr and IKr, and proposed that clinical manifestations of LQT5 may be complicated by differing effects of minK mutations on KvLQT1 and HERG.

**HERG**

Mutations in HERG cause LQT2. The gene product is the α-subunit of a potassium channel that carries the IKr current. HERG mutations prolong QT interval, reducing this current.

**SCN5A**

The gene for LQT3 is SCN5A, the voltage-gated cardiac sodium channel, responsible for the initial upstroke of
the action potential in the electrocardiogram. This channel protein contains four homologous domains (D1-DIV), each of which has six putative-spanning regions (S1–S6).

SCN5A was not an obvious candidate gene for LQTS because it seemed more logical that a defect in an outward current and not in an inward current would account for prolongation of the action potential and the QT interval. This is, in fact, the mechanism by which mutations on HERG, KvLQT1, KCNE1 or KCNE2 cause LQTS reduction or abolition of channel function. Mutations in SCN5A can cause LQTS mainly through a gain in function. Most of the mutations identified result in a small, sustained inward current which is likely to disrupt the normal balance between inward and outward currents during the plateau phase, and hence prolong cardiac action potential.

SCN5A is not only the gene for LQT3, but defects in this gene have also been identified in families with Brugada syndrome and Lev-Lenègre syndrome.[70,71]

Brugada syndrome is a recently described familial disease[72] characterized by right bundle branch block and ST-segment elevation in leads V1–V3, with a normal QT interval and no demonstrable structural heart disease. Patients with this disease are at high risk for VF and sudden death, typically occurring during sleep.[70,72]

The genetic basis of this disease was discovered last year by Chen et al.[73], who identified the first two mutations in SCN5A, located in the extracellular loops of DIII and DIV, respectively. Once expressed in Xenopus oocytes, these mutations showed a shift in the voltage dependence of steady-state inactivation towards more positive potentials and a faster recovery from inactivation.[73]

At that time (March 1998), there seemed to be a clear boundary between LQT3 and Brugada syndrome, because LQT3 mutations were located in the DIII-DIV intracellular linker responsible for inactivation of the sodium current, and Brugada mutations were located in extracellular linkers. LQT3 mutations would cause a delay in the inactivation leading to action potential duration prolongation, whereas Brugada mutations would cause a reduction of sodium current.

This boundary has become progressively less apparent. This year, many new mutations have been identified and they are located all over the gene without a clear distinction between the two syndromes. For instance, it has been demonstrated that a single amino acid substitution at residue 1623 (R1623Q) results in LQT3, while a similar point mutation at position 1620 (M1620T) results in Brugada syndrome.[73]. The present authors have identified a mutation in the same codon in a family with LQTS and in a family with Brugada syndrome (Priori, unpubl. data); the same amino acid was substituted with two different amino acids in the two families. These observations suggest that the nature of the substitution (i.e. which amino acid is substituted with what) is also likely to play a critical role in the production of a specific clinical phenotype.

Much more confusing is the recent detection in a family of a single mutation (1795insD) in the C-terminal domain, producing an overlapping phenotype.

When the genetic bases of LQTS were identified, molecular biologists and clinicians thought it would be possible to establish genotype-phenotype correlation in a relatively short time.

This correlation would be useful to distinguish severe and mild mutations, in order to guide the therapeutic approach on the basis of the predicted risk. Unfortunately, we are still a long way from this goal and, to understand better the diagnostic, functional and prognostic implications of the different mutations, the number of genotyped patients will need to be increased.

The following text will analyse the present impact of molecular biology in LQTS.

**Impact of molecular biology in LQTS**

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**Diagnosis**

In patients with a definite diagnosis of LQTS, the identification of the gene responsible for the disease may suggest modifications in the patient’s management. For instance, as explained later in this paper, a limitation of strenuous or competitive exercise is much more important in LQT1 than in LQT3 patients.[14]

When the diagnosis of LQTS is only suspected, the identification of a mutated LQTS gene makes the diagnosis certain; however, failure to identify a mutation does not rule out the diagnosis, because not all gene diseases are known.

Genetic testing is particularly useful in apparently asymptomatic relatives of a patient with LQTS, especially when the disease-causing mutation has previously been identified in the proband.

**Molecular genetics and risk stratification**

As explained previously, in LQTS the large heterogeneity of mutations within each disease-related gene has prevented the possibility of extrapolating mutation-specific prognostic information that could guide the therapeutic approach. On the other hand, gene-specific differences have been observed.
Data from the International Registry on 246 gene carriers (112 LQT1, 72 LQT2 and 62 LQT3) show that LQT1 and LQT2 gene carriers are at higher risk of becoming symptomatic and have a higher number of cardiac events than LQT3 gene carriers\[73\]. Although there are significant differences in the frequency of cardiac events among the three groups, the overall frequency of death related to LQTS is similar in each group. This means that the lethal nature of LQT3 is greater; 20% of all cardiac events are fatal in the LQT3 group compared with 4% in both LQT1 and LQT2 groups\[75\].

In another set of data based on more than 400 genotyped and symptomatic patients, a gene-related difference was observed in the age of the first event. J-LN patients are those with the earliest occurrence of cardiac events (78% by age 10 years) followed by LQT1 (63% by age 10 years), LQT2 (30% by age 10 years), and LQT3 (Schwartz et al., in preparation). Also, the risk of death in the first episode appears to be gene-related: 2% among LQT1, 4% among LQT2 and 12% among LQT3 patients.

*Molecular genetics and triggering events*

When heart rate increases, the physiological shortening of the QT interval is greater among LQT3 patients compared with that of the other genotypes, in agreement with experimental observations in isolated myocytes\[76\]. A reasonable inference is that LQT3 patients might be at lesser risk of syncope when heart rate increases for instance during physical exercise, and so, if that is true, beta-blockers should not be as useful in these patients as in the other genotypes.

To better understand the associations between triggering events and the various genotypes, almost 400 genotyped and symptomatic patients have been studied\[77\], and three main factors have been identified which are associated with syncope or cardiac arrest: exercise, emotion and events occurring during either sleep or at rest with and without arousal.

The most obvious difference is that between LQT1 and LQT3 patients:

- cardiac events at rest or during sleep: 3% of the LQT1 patients and 71% of the LQT3 patients
- cardiac events during exercise: 71% of the LQT1 patients and 12% of LQT3 patients.

The LQT2 patients show a pattern similar to that of the LQT3 patients. This can probably be attributed to the fact that both these groups have a normal Ik\(_s\) current.

These data support the observation made on the response to heart rate increases by LQT3 patients, and indicates that avoidance of physical exercise is not as important in LQT3 patients as in LQT1 patients, that are at risk almost exclusively when heart rate is significantly elevated, such as during exercise and emotion. This also suggests that in LQT1 patients, beta-blockers are particularly effective\[77\]. These clinical findings are in agreement with the experimental observation that when LQT1 is mimicked by the use of an Ik\(_s\) inhibitor, TdP is induced only in the presence of catecholamines\[78\].

Gene-based therapeutic approaches?

Finding the molecular basis of LQTS opened the possibility of attempting gene-specific therapy. The identification of the mutations on SCN5A and on HERG made it logical to hypothesize that interference with the Na\(^+\) inward current and enhancement of the repolarizing K\(^+\) currents might have been useful in LQTS patients with SCN5A and HERG mutations, respectively.

In fact, although both defects result in a prolonged QT interval, the cellular basis for the repolarization delay is distinctly different. In LQT3 patients, excess inward current maintains the plateau at a depolarized level, whereas in LQT2 patients, a reduction in outward current prevents the plateau from early termination. So in LQT3 patients, a Na\(^+\) channel blocker, such as mexiletine could be useful. By contrast in LQT2 patients, an increase in the extracellular concentration of potassium could enhance the repolarizing K\(^+\) currents.

In 1995, Schwartz et al. studied 11 LQTS patients (six LQT3 and seven LQT2) to test this hypothesis\[79\]. This study gave the first demonstration that mexiletine significantly shortens the QT interval among LQT3 patients, but not among LQT2 patients, and that LQT3 patients shorten their QT interval in response to an increased heart rate much more than LQT2 patients and controls, in agreement with experimental observations in isolated myocytes\[79\]. Therefore, in patients with SCN5A mutations, it is reasonable to test the potential efficacy of Na\(^+\) channel blockers, not just in shortening the QT interval, but above all in reducing life-threatening arrhythmias. Moreover, physical activity may not need to be restricted in LQT3 patients if an exercise stress test produces a significant shortening of the QT interval. Also, these patients may benefit from a pacemaker more than LQT1 and LQT2 patients.

As a counterpart of the findings with mexiletine in LQT3 patients\[79\], recent evidence suggested that an increase in the extracellular concentration of potassium may shorten the QT interval in LQT2 patients\[80\]. So in these patients, it could be logical to test various ways of increasing the extracellular K\(^+\) concentration; for instance, oral K\(^+\) supplements in combination with K\(^+\) sparing agents are worth testing.

These novel therapies are fascinating, but it is fundamental to remember that they are still experimental and no proof currently exists of their efficacy in reducing mortality. So, at present, beta-blockers remain the first-choice therapy.
LQTS: a common disease, mostly benign?

At the time when LQTS was initially described, only patients who presented with repeated syncopal episodes associated with prolonged loss of consciousness sought medical attention and were diagnosed as affected by LQTS. If these patients were left untreated, syncopal episodes would recur and eventually prove fatal in the majority of cases.

The ‘referral bias’ associated with the lack of diagnosis of individuals affected by more benign forms of LQTS has substantiated the common perception of LQTS as a severe disease associated with a high risk of sudden cardiac death in all affected individuals.

During the past few years, it has become progressively evident, particularly in those specialized centres where large numbers of LQTS patients are routinely treated, that in addition to the severe manifestations described, there are also numerous sporadic and familial cases with a very benign course.

In patients with a very mild form, the diagnosis of LQTS can be elusive, yet at least a minority of these patients may be still at risk of cardiac events. The present authors have recently identified two survivors of ‘idiopathic ventricular fibrillation’ who had a normal QT interval and were found to carry an LQTS related mutation (Priori, unpubl.). This supports the view that even patients with ‘normal’ repolarization, when carrying an ion channel defect, are at higher risk of sudden death.

Furthermore, the evidence that molecular diagnosis unmasks a high number of silent carriers of mutations among family members of probands[11], supports the hypothesis that these mutations are probably more common than previously anticipated. Why the very same defect is associated with an overt phenotype in some individuals and remains subclinical in others is currently unknown, and the search for modifiers that modulate the consequences of an ion channel mutation is one of the top priorities of research in the field.

What is relevant to clinicians is the fact that despite the several approaches in existence that can lead to a diagnosis of severe, mild and even subclinical forms of LQTS, the real missing information is the capability of determining, with a good level of accuracy, the risk in the individual patient.

In front of an ECG-based diagnosis in an asymptomatic child, there are no criteria, except maybe very weak ‘predictors’ such as family history and QT duration, to define the risk of the patient to develop malignant arrhythmias. The issue remains therefore, whether treatment with beta-blockers should be given even to those individuals that are asymptomatic or whether treatment should commence with the manifestation of the disease. In the latter case, the risk that in some (and this number is not known) individuals sudden death will be the first manifestation of the disease will have to be accepted.

References


