GENOTYPE AND SEVERITY OF LONG QT SYNDROME

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ABSTRACT:

Sudden cardiac death occurs in the United States with an incidence greater than 300,000 persons per year. The underlying cause of death is commonly considered to be due to primary or secondary arrhythmias. In cases in which no structural heart disease can be identified, the long QT syndromes (LQTS) are now commonly considered as likely causes. Multiple genes causing LQTS have been identified thus far, all encoding cardiac ion channels. These include two potassium channel α-subunits (KVLQT1, HERG), two potassium channel β-subunits (minK, MiRP1), and one sodium channel gene (SCN5A). The purpose of this review is to describe the current understanding of the molecular genetics of LQTS and the resultant phenotypes.

LQTS Disease Classification

The LQTS are diagnosed by surface electrocardiograms, clinical presentation, and family history (Schwartz et al., 1996; Priori et al., 1999a). These disorders of repolarization are characterized by the ECG abnormalities of prolongation of the QT interval corrected for heart rate (QTc), relative bradycardia, T wave abnormalities, and episodic ventricular tachyarrhythmias, particularly torsade de pointes (Fig. 1). The diagnosis usually relies on a QTc measurement of >460 to 480 ms using the formula QTc = \( \frac{QT}{RR} \) (Bazett, 1920) with associated T wave abnormalities. LQTS occurs either as an inherited disorder, sporadic disorder, or it may be acquired. In the latter case, acquired LQTS may be seen after the use of a variety of medications (i.e., antiarrhythmics, antihistamines, psychotropic drugs, antifungal drugs, macrolide antibiotics) or electrolyte abnormalities such as hypokalemia. The clinical presentation is similar in all forms of LQTS, however. Two inherited forms of LQTS with differing patterns of transmission have been described, and they include the Romano-Ward syndrome (RWS) and the Jervell and Lange-Nielsen syndrome (JLNS) (Schwartz et al., 1996; Priori et al., 1999a).

The Romano-Ward syndrome is the most common inherited form of LQTS and is transmitted as an autosomal dominant trait (Schwartz et al., 1996; Priori et al., 1999a); gene carriers are expected to be clinically affected (i.e., they have evidence of LQTS) and have a 50% likelihood of transmitting the disease-causing gene to their offspring. However, low penetration has been described, and therefore gene carriers may, in fact, have no clinical features of disease (Priori et al., 1999b). Individuals with RWS present with prolonged QT interval on ECG with the associated symptom complex of syncope, sudden death, and in some patients, seizures (Ratshin et al., 1971). Occasionally, other noncardiac abnormalities such as diabetes mellitus (Ewing et al., 1991), asthma (Weintraub et al., 1990), or syndactyly (Marks et al., 1995) may also be associated with QT prolongation. LQTS may also be involved in some cases of Sudden Infant Death syndrome (Schwartz et al., 1998; Towbin and Friedman, 1998). However, no other organ system is usually associated.

JLNS is an uncommon inherited form of LQTS. Classically, this disease has been described as having apparent autosomal recessive transmission (Schwartz et al., 1996; Priori et al., 1999a). These patients have the identical clinical presentation as those with RWS but also have associated sensorineural deafness (Schwartz et al., 1996; Priori et al., 1999a). Individuals with JLNS usually have longer QT intervals as compared with individuals with Romano-Ward syndrome and also have a more malignant course. Recently, this distinction has been blurred, as autosomal recessive cases of RWS have been described (Priori et al., 1998).

Mapping of LQTS Genes in Romano-Ward Syndrome

The first gene for autosomal dominant LQTS was mapped by Keating et al. (1991) to chromosome 11p15.5 (LQT1), followed shortly thereafter with the realization that LQTS locus heterogeneity existed (i.e., multiple genes cause LQTS) (Towbin et al., 1994). This was confirmed when Jiang et al. (1994) mapped the LQT2 and LQT3 genes to chromosome 7q35–36 and to chromosome 3p21–24, respectively. Schott et al. (1995) mapped the fourth LQTS locus to chromosome 4q25–27 (LQT4), while a fifth gene (minK) located on...
establishing on chromosome 21q22 (Vatta and Towbin, 2000) was shown to be \textit{LQT5}. More recently, a sixth gene, the \textit{minK}-related peptide 1 (\textit{MiRP1}), localized to 21q22 as well (Fig. 2), was identified (Vatta and Towbin, 2000). Several other families with autosomal dominant \textit{LQTS} are not linked to any known \textit{LQTS} loci, indicating the existence of additional \textit{LQTS}-causing genes.

**Gene Identification in Romano-Ward Syndrome**

\textit{LQT1: KVLQT1 (\textit{KCΝQ1})}. Positional cloning was used to identify the \textit{LQT1} gene on chromosome 11p15.5, the gene initially localized by Keating et al. (1991). This gene was found to be a novel potassium channel gene initially called \textit{KVLQT1} and later renamed \textit{KCΝQ1} (Wang et al., 1996). This potassium channel \(\alpha\)-subunit consists of 16 exons, spans approximately 400 kbp, and is widely expressed in human tissues including heart, kidney, lung, inner ear, placenta, and pancreas, but not in skeletal muscle, liver, or brain. Eleven different mutations (deletion and missense mutations) were initially identified, establishing \textit{KVLQT1 (\textit{KCΝQ1})} as \textit{LQT1}. To date, more than 100 families with mutations have been described, most with their own novel "private" mutations. However, there is at least one frequently mutated region (called a "hot-spot") of this gene (Wang et al., 1996; Li et al., 1998). This gene is now believed to be the most commonly mutated gene in \textit{LQTS}.

Analysis of the predicted amino acid sequence of the encoded protein suggests that it encodes a potassium channel \(\alpha\)-subunit with a conserved potassium-selective pore-signature sequence flanked by six membrane-spanning segments (Fig. 2). A putative voltage sensor is found in the fourth membrane-spanning domain (S4), and the selectively pore loop is between the fifth and sixth membrane-spanning domains (S5, S6). Electrophysiological characterization of the \textit{KVLQT1 (\textit{KCΝQ1})} protein in various heterologous systems confirmed this protein to be a voltage-gated potassium channel protein subunit, which requires a \(\beta\)-subunit to function properly (Barhanin et al., 1996; Sanguinetti et al., 1996). This \(\beta\)-subunit, which coassembles with \textit{KVLQT1 (\textit{KCΝQ1})}, is called \textit{minK} or \textit{KCNE1} and encodes a short protein with only 130 amino acids and only one transmembrane-spanning segment (Fig. 2). At the time of its initial identification, \textit{minK} did not have any sequence or structural homologies to any other cloned channels, but it is now known to be part of a protein family (Abbott et al., 1999). When \textit{minK} and \textit{KVLQT1} were coexpressed in either mammalian cell lines or \textit{Xenopus laevis} oocytes, a potassium current similar to the slowly activating potassium current (\(I_{Ks}\)) in cardiac myocytes (Fig. 3) was formed (Barhanin et al., 1996; Sanguinetti et al., 1996). The physical interaction between \textit{KVLQT1} and \textit{minK} was also confirmed by immunoprecipitation experiments (Barhanin et al., 1996).

Combination of normal and mutant \textit{KVLQT1 \(\alpha\)-subunits was found to form abnormal \(I_{Ks}\) channels; hence, \textit{LQTS}-associated mutations of \textit{KVLQT1 (\textit{KCΝQ1})} are believed to act predominantly through a dominant-negative mechanism (the mutant form of \textit{KVLQT1} interferes with the function of the normal wild-type form through a "poison pill"-type mechanism) or a loss-of-function mechanism (only the mutant form loses activity) (Wollnik et al., 1997; Vatta et al., 2000).

Since mutations in \textit{KVLQT1} were shown to cause \textit{LQTS (LQT1)}, mutations in \textit{minK} were sought since \textit{minK} plays an essential role in the development of \(I_{Ks}\) (see \textit{LQTS: minK}) (Splawski et al., 1997b).

\textit{LQT2: HERG}. Both \textit{LQT2} and \textit{LQT3} (described below) were identified by the positional candidate gene approach (Vatta et al., 2000). The candidate gene approach relies on a mechanistic hypothesis based on knowledge of the physiology of the disease of interest. Since \textit{LQTS} is considered a disorder of abnormal repolarization, genes encoding ion channels or proteins modulating channel function were considered candidates for \textit{LQTS}. After the initial localization of \textit{LQT2} to chromosome 7q35–36, candidate genes in this region including ion channels, modifiers of ion channels, and genes encoding elements of the sympathetic nervous system were analyzed. \textit{HERG} (human \textit{ether-a-go-go-related gene}), a cardiac potassium channel gene with six transmembrane segments (Fig. 2), which was originally cloned from a brain cDNA library and found to be expressed in neural-crest-derived neurons, microglia, a wide variety of tumor cell lines, and the heart, was one of the genes evaluated. Curran et al. (1995) demonstrated linkage of \textit{HERG} to the \textit{LQT2} locus on chromosome 7q35–36, and six \textit{LQTS}-associated mutations were identified in \textit{HERG}, including missense mutations, intragenic deletions, and a splicing mutation. Later, Schulze-Bahr et al. (1995) reported a single base pair deletion and a stop codon mutation in \textit{HERG}, confirming this gene to be a common cause of \textit{LQTS} when mutated. Currently, this gene is thought to be the second most common gene mutated in \textit{LQTS} (second to \textit{KVLQT1}), and mutations scattered throughout this entire gene have been seen. No hot spots have been recognized.

\textit{HERG} consists of 16 exons and spans 55 kbp of genomic sequence (Curran et al., 1995). The predicted topology of \textit{HERG} is shown in Fig. 2 and is similar to \textit{KVLQT1}. Unlike \textit{KVLQT1}, \textit{HERG} has extensive intracellular amino and carboxyl termini, with a region in the carboxyl-terminal domain having sequence similarity to nucleotide binding domains.

Electrophysiological and biophysical characterization of expressed \textit{HERG} in \textit{X. laevis} oocytes established that \textit{HERG} encodes the rapidly activating delayed rectifier potassium current \(I_{Ks}\) (Fig. 3) (Sanguinetti et al., 1995). Electrophysiological studies of \textit{LQTS}-associated muta-
tions showed that they act through either a loss-of-function or a dominant-negative mechanism (Sanguinetti et al., 1995; Vatta et al., 2000). In addition, protein trafficking abnormalities have been shown to occur (Furutani et al., 1999). This channel has been shown to coassemble with β-subunits for normal function, similar to that seen in I\(_{\text{Ks}}\). McDonald et al. (1997) initially suggested that the complexing of HERG with minK is needed to regulate the I\(_{\text{Ks}}\) potassium current. More recently, Abbott et al. (1999) identified MiRP1 as a β-subunit for HERG (see LQT6: MiRP1).

**LQT3: SCN5A.** The positional candidate gene approach was also used to establish that the gene responsible for chromosome 3-linked LQTS (LQT3) is the cardiac sodium channel gene SCN5A (Wang et al., 1995). SCN5A is highly expressed in human myocardium, but not in skeletal muscle, liver, or uterus. Recently, it was shown to be expressed in the brain. This gene encodes I\(_{\text{Na}}\), which is responsible for initiation of depolarization (Fig. 3). It consists of 28 exons that span 80 kbp and encodes a protein of 2016 amino acids with a putative structure that consists of four homologous domains (DI-DIV), each of which contains six membrane-spanning segments (S1–S6) similar to the structure of the potassium channel α-subunits (Fig. 2). Linkage studies with LQT3 families and SCN5A initially demonstrated linkage to the LQT3 locus on chromosome 3p21–24 (Wang et al., 1995). In addition, three mutations, one 9-bp intragenic deletion (ΔK\(_{1505}\)P\(_{1506}\)Q\(_{1507}\)) and two missense mutations (R\(_{1644}\)H and N\(_{1325}\)S), were identified in six LQTS families (Fig. 2). All three mutations were expressed in X. laevis oocytes, and all mutations generated a late phase of inactivation-resistant, mexiletine- and tetrodotoxin-sensitive whole-cell currents through multiple mechanisms (Bennett et al., 1995; Dumaine et al., 1996). Two of the three mutations showed dispersed reopening after the initial transient, but the other mutation showed both dispersed reopening and long-lasting bursts (Dumaine et al., 1996). These results suggested that SCN5A mutations act through a gain-of-function mechanism (the mutant channel functions normally, but with altered properties such as delayed inactivation) and that the mechanism of chromosome 3-linked LQTS is persistent noninactivated sodium current in the plateau phase of the action potential. Later, An et al. (1998) showed that not all mutations in SCN5A are associated with persistent current. Furthermore, Wei et al. (1999) identified a C-terminal SCN5A mutation, E1784K, which results in fast inactivation characterized by small, persistent current during long membrane depolarizations. These authors coexpressed the mutant with human sodium channel β1-subunits, which did not affect the persistent current, although shift in the voltage dependence of steady-state inactivation was seen. Neutralizing multiple negatively charged residues in the C terminus did not cause a more severe functional defect, suggesting that an allosteric effect, rather than a direct effect, in channel gating was responsible for channel dysfunction.

Another interesting finding was reported by Nagatomo et al. (1998), who found that ΔKPQ mutations have temperature-dependent activation and inactivation kinetics. At physiologic temperature, whole-cell patch clamp studies in HEK293 cells found faster inactivation and activation kinetics. In addition, faster decay was notable at voltages negative to \(-20\) mV, suggesting reduced voltage dependence of fast inactivation.

Furthermore, mutations in SCN5A were identified by our laboratory in patients with a significantly different clinical phenotype (Chen et al., 1998). Brugada syndrome, characterized by ST elevation in leads V1 to V3 with or without right bundle branch block and ventricular fibrillation (Brugada and Brugada, 1992) was found to occur from SCN5A mutations. At least one of these mutations (R1623Q) results in more rapid recovery from inactivation of the mutant channels or
loss of function. The specific mechanism causing the differences between LQTS and Brugada syndrome is not known. Interestingly, a very close mutation (T1623) results in classic LQTS (Kambouris et al., 1998).

**LQT5: minK.** minK (IsK, or KCNE1), was initially localized to chromosome 21 (21q22.1) and found to consist of three exons that span approximately 40 kbp. It encodes a short protein consisting of 130 amino acids and has only one transmembrane-spanning segment with small extracellular and intercellular regions (Fig. 2). When expressed in X. laevis oocytes, it produces potassium current that closely resembles the slowly activating delayed rectifier potassium current I\(\text{Kr}\) in cardiac cells (Barhanin et al., 1996; Sanguinetti et al., 1996). Initially, the minK clone could only be expressed in X. laevis oocytes and not in mammalian cell lines. However, with the cloning of KVLQT1 and coexpression of KVLQT1 and minK in both mammalian cell lines and X. laevis oocytes, it was recognized that minK alone cannot form a functional channel but induces the I\(\text{Kr}\) current by interacting with endogenous KVLQT1 protein in X. laevis oocytes and mammalian cells (Fig. 3). McDonald et al. (1997) showed that minK complexes with HERG to regulate the I\(\text{Kr}\) potassium current, as well. The importance of minK to the function of the I\(\text{Kr}\) was shown when Splawski et al. (1997b) identified mutations in two families with type 2 LQTS. In both cases, missense mutations (S74L, D76N) were identified that reduced I\(\text{Kr}\) by shifting the voltage dependence of activation and accelerating channel deactivation. Recently, minK was found to be a cofactor in the expression of both I\(\text{Kr}\) and I\(\text{Ks}\), and mutations in minK were shown to affect both channels. Trafficking abnormalities occur, as well (Bianchi et al., 1999). The functional consequences of these mutations included delayed cardiac repolarization and, hence, an increased risk of arrhythmias.

**LQT6: MiRP1.** MiRP1, the minK-related peptide 1, or KCNE2, is a novel potassium channel gene recently cloned and characterized by Abbott and colleagues (1999). MiRP1 is a 123-amino acid channel protein with a single predicted transmembrane segment similar to that described for minK. Chromosomal localization studies mapped this KCNE2 gene to chromosome 21q22.1 (Fig. 2) within 79 kbp of KCNE1 (minK) and arrayed in opposite orientation (Abbott et al., 1999). The open reading frames of these two genes share 34% identity, and both are contained in a single exon, suggesting that they are related through gene duplication and divergent evolution. This small integral membrane subunit protein assembles with HERG (LQT2) to alter its function, enabling full development of the I\(\text{Kr}\) current (Fig. 3). Abbott et al. (1999) identified three missense mutations associated with LQTS and ventricular fibrillation in KCNE2, and biophysical analysis demonstrated that these mutants form channels that open slowly and close rapidly, thus diminishing potassium currents. In one case (Q9E), the mutation led to acquired (drug-induced) torsade de pointes and ventricular fibrillation. However, none of the mutations caused classic LQTS.

Therefore, like minK, this channel protein acts as a β-subunit but, by itself, leads to ventricular arrhythmia risk when mutated. These similar channel proteins (i.e., minK and MiRP1) suggest that a family of channels exist that regulate ion channel α-subunits. The specific role of this subunit remains unclear and is currently being hotly debated.

**Genetics and Physiology of Autosomal Recessive LQTS (Jervell and Lange-Nielsen Syndrome).** Neyroud et al. (1997) reported the first molecular abnormality in patients with Jervell and Lange-Nielsen syndrome when they reported on two families in which three children were affected by JLNS and in whom a novel homozygous deletion-insertion mutation of KVLQT1 in three patients was found that resulted in premature termination at the C-terminal end of the KVLQT1 channel. This was confirmed when Splawski et al. (1997a) identified a homozygous insertion of a single nucleotide that caused a frameshift in the coding sequence after the second putative transmembrane domain (S2) of KVLQT1 in a family with JLNS. Together, these data strongly suggested that at least one form of JLNS is caused by homozygous mutations in KVLQT1. Others have confirmed this conclusion.

It is interesting that, in general, heterozygous mutations in KVLQT1 cause Romano-Ward syndrome (LQTS only), while homozygous mutations in KVLQT1 cause JLNS (LQTS and deafness). The likely explanation is as follows: although heterozygous KVLQT1 mutations act by a dominant-negative mechanism, some functional KVLQT1 potassium channels still exist in the stria vascularis of the inner ear. Therefore, congenital deafness is averted in patients with heterozygous KVLQT1 mutations. For patients with homozygous KVLQT1 mutations, no functional KVLQT1 potassium channels can be formed. It has been shown by in situ hybridization that KVLQT1 is expressed in the inner ear (Neyroud et al., 1997), suggesting that homozygous KVLQT1 mutations can cause the dysfunction of potassium secretion in the inner ear and lead to deafness. However, note that incomplete penetration exists and not all heterozygous or homozygous mutations follow this rule (Priori et al., 1998, 1999b).

Mutations in minK have been shown to result in JLNS syndrome, as well. Hence, abnormal I\(\text{Kr}\) current, whether it occurs due to homozygous mutations in KVLQT1 or minK, results in LQTS and deafness.

**Genotype-Phenotype Correlations.** Zareba et al. (1998) have recently shown that the mutated gene may result in a specific phenotype and may predict the outcome. For instance, they showed that mutations in LQT1 and LQT2 result in early symptoms (i.e., syncope), but the risk of sudden death was relatively low for any event. In contrast,
mutations in LQT3 resulted in a paucity of symptoms, but when symptoms occurred it was associated with a high likelihood of sudden death. Mutations in LQT1 and LQT2 appeared to be associated with stress-induced symptoms, including response to auditory triggers, while LQT3 appeared to be associated with sleep-associated symptoms. In addition, bradycardia and exercise-induced QT shortening have been seen in LQT3 patients (Schwartz et al., 1995). Coupled with the findings by Moss et al. (1995) that differences in the ECG patterns during the initiation of torsade de pointes have been seen in patients with asthma, other medications have been tried, such as mexiletine. When medical therapy fails, left sympathectomy or implantation of an automatic cardioverter defibrillator has been utilized.

Recently, genetic-based therapy has been described. Schwartz et al. (1995) showed that sodium channel blocking agents (i.e., mexiletine) shorten the QTc in patients with LQT3, while exogenous potassium supplementation or potassium channel openers have been shown to be potentially useful in patients with potassium channel defects. However, long-term potassium therapy with associated potassium-sparing agents has been unable to keep the serum potassium above 4 mmol/l due to renal potassium homeostasis. This suggests that potassium therapy may not be useful in the long-term. In addition, no definitive evidence that these approaches (i.e., sodium channel blockers, exogenous potassium, or potassium channel openers) improve survival has been published.

Animal Models of LQTS. Using an arterially perfused canine left ventricular wedge preparation developed pharmacologically, induced animal models of LQT1, LQT2, and LQT3 have been created. Using chromanol 293B, a specific IKs blocker, a model that mimics LQT1 was produced. In this model, IKs deficiency alone was not enough to induce torsade de pointes, but addition of β-adrenergic influence (i.e., isoproterenol) predisposed the myocardium to torsade by increasing transmural dispersion of repolarization. Addition of β-blocker or mexiletine reduced the ability to induce torsade, suggesting that these medications suppress the development of these arrhythmias.

Models for LQT2 and LQT3 were created by using d-sotalol (LQT2) or ATX-II (LQT3) in this wedge preparation. Both of these drugs preferentially prolong M cell action potential duration, with ATX-II (or ATX-II (IsK) proteins to form cardiac IKs potassium channel.

Conclusion

The long QT syndromes are genetically and clinically heterogeneous. The affected gene in any patient can lead to a wide spectrum of clinical outcomes depending on its specific mutation. These mutations, however, remain difficult to identify. However, once the genetic mutation is known, gene-specific therapy may be an option in the future.

References


