The Long QT Syndrome: Prospects for Mutation-Specific Therapeutics

Robert S. Kass, Ph.D.

David Hosack Professor and Chairman

Department of Pharmacology

Columbia University College of Physicians & Surgeons

New York, NY 10032

Correspondence:

e-mail: rsk20@columbia.edu

Abstract:

The congenital Long QT Syndrome is a rare disorder in which mutation carriers are at risk for polymorphic ventricular tachycardia and/or sudden cardiac death. Discovery and analysis of gene muations associated with variants of this disorder have provided novel insight into mechanisms of cardiac arrhythmia and have raised the possibility of mutation-specific therapeutic intervention.

Background:

Long QT Syndrome is an infrequently occurring disorder in the general population, with a frequency estimated at about 1 in 5,000 people. Patients with LQTS are usually identified by QT prolongation on the ECG during clinical evaluation of unexplained syncope, as part of a family study when one family member has been identified with the syndrome, or in the investigation of patients with congenital neural deafness. Clinical criteria have been developed to determine the probability that a patient may have LQTS(Schwartz *et al.*, 1993). The two most important diagnostic features of LQTS are prolongation of the heart-rate corrected QT interval (QTc $\geq$0.46sec) and stress-induced syncope.

The syncope that occurs in this disorder is due to a transient, rapid, polymorphic ventricular tachycardia described as torsade de pointes (twisting of the points) that is associated with the underlying delayed ventricular repolarization manifest on the ECG as QTc prolongation. Sudden death occurs when a polymorphic ventricular tachycardia episode deteriorates into ventricular fibrillation. Syncope and sudden death are most frequent in childhood and adolescence. The risk of cardiac events is higher in males before puberty and higher in females during adulthood(Locati *et al.*, 1998;Schwartz *et al.*, 1993). The clinical course of patients with LQTS is quite variable, and it is influenced by the length of the QTc interval, gender, environmental factors, genotype, and therapy(Moss *et al.*, 1991).

Gene mutations and LQTS

The ventricular action potential of the human heart is distinct in that the temporal period separating excitation of ventricular cells from relaxation, or repolarization, is very long, typically on the order of 450 ms. This timing is crucial because as long as the ventricular tissue is depolarized it cannot be re-excited due to the unavailability of key voltage gated sodium channels which normally enter a non-conducting inactivated state during this period.

The duration of this depolarized state, often referred to as the plateau phase of the ventricular action potential, is not only cardio protective against premature excitation, but also is essential to maintaining the proper timing between diastolic filling and ejection intervals. It is the plateau phase of the ventricular action potential that thus determines the QT interval of the electrocardiogram. Over fifty years ago, Silvio Weidmann (Weidmann, 1951) discovered that this crucial plateau phase was maintained by a delicate balance of small ionic conductances, which though energetically favorable, predispose this period of the electrical cycle in the heart to disturbances that may be caused by otherwise harmlessly small changes in transmembrane ionic activity.

Before genetic information was used successfully to identify genes associated with the congenital long QT syndrome, various candidate ion channels (and thus channel coding genes) were reasonable suspects to consider as potentially underlying the clinical defect: delay in ventricular relaxation. Molecular genetic approaches removed at least some of this uncertainty by demonstrating clear association of mutations genes coding for ion channel subunits with LQTS (reviewed by (Sanguinetti, 2000)). This work has revealed that mutations in at least five genes coding for key cardiac potassium ion channels subunits that result in a loss or reduction of channel activity can cause variants of LQTS. Surprisingly, mutations in the principal (alpha) subunit of a key heart sodium channel (SCN5A) that, in general, result in a gain of channel function also cause yet another variant of LQTS (LQT-3). These discoveries have prompted at least three new areas of research that impact not only the diagnosis and management of LQTS, but also, our understanding of human cardiovascular physiology. These areas include (1) mutation-specific therapeutic strategies; (2) identification of mutation-specific risks of cardiac events; and (3) mechanistic insight into the role of altered channel function and regulation in the control of QT intervals in the heart.

Sodium channels and LQTS

Expression of ion channels in heterologous systems allows for investigation of inherited ion channel defects at the single protein and cellular level to directly identify the disease-associated alteration in ion channel function. Disease-linked mutations provide an opportunity to understand the mechanistic basis of human disease from altered molecular function to the clinical syndrome. Initial investigation of mutations in SCN5A, linked to variant 3 of the long QT syndrome (LQT-3), revealed striking mutation-induced defects in channel behavior that were consistent with the disease phenotype(Bennett *et al.*, 1995). Classically, Na^+ channel activation is associated with the spread of depolarization in the heart that underlies the QRS complex of the EKG. Here, however, defects in the Na^+ channel were found to be linked to delay in ventricular repolarization (prolongation of the QT interval). These initial experiments clearly showed a novel mechanism that could explain this unexpected result: the inherited mutation disrupted the "inactivation" process of the channel such that, during the plateau phase of the action potential, a small number of Na^+ channels do not inactivate (become non-conducting) but in fact re-open to provide a very small depolarizing current. Exactly as Weidmann had predicted (Weidmann, 1951), even though this late current is only a fraction of the total Na+ channel current responsible for the QRS complex, this small disease-associated perturbation in plateau current is responsible for prolonging the QT interval in mutation carriers and raising the risk of cardiac events (Clancy & Rudy, 1999). Thus, the molecular genetic analysis of long QT patients has led to a novel understanding of the importance of Na+ channel activity in controlling not only the QRS complex, but also, the duration of the ventricular electrical response, the QT interval. Recently, mutations in SCN1A, the gene coding for the human neuronal Na^+ channel α subunit associated with epilepsy, have been reported to cause similar defects in channel inactivation gating(Lossin *et al.*, 2002). As yet, the cellular consequences of such epilepsy mutations remain elusive, but it is very likely that mechanistic insights gained from investigation of cardiac defects are likely to have widespread implications.

Potassium channels, LQTS and sympathetic nerve activity

Investigation of the molecular basis of LQTS has led to fundamental insight into the molecular identity of key potassium channel subunits in the heart, notably the identity of the two key delayed rectifier currents I_{Kr} In particular, mutations in *KCNQ1*, which codes for the α subunit of the I_{KS} channel, cause LQT-1, and mutations in *KCNE1*, the gene coding for the auxiliary β subunit of the I_{Ks} channel, cause LQT-5 (Sanguinetti, 2000). It had been well-established in animal models that I_{Ks} is strongly regulated by sympathetic nervous system stimulation (SNS), such that SNS increases repolarizing current reserves and contributes to action potential shortening that occurs in parallel with the SNS-induced increases in heart rate(Kass & Wiegers, 1982). Further, clinical data indicate that carriers of mutations in either KCNQ1 or KCNE1 are at increased risk of experiencing a fatal cardiac arrhythmia in the face of elevated SNS activity (Schwartz *et al.*, 2001). Together these findings motivated investigation into molecular links between the SNS and regulation of KCNQ1/KCNE1 channels in the human heart.

The result of this work was the discovery that the KCNQ1/KCNE1 channel actually forms a macromolecular signaling complex: coupled to the carboxy terminal domain of the channel is an adaptor protein, yotiao, which in turn binds to the regulatory enzymes protein kinase A (PKA) and protein phosphatase 1 (PP1) (Marx *et al.*, 2002). Thus the channel, via the adapter protein, recruits enyzmes that can up (PKA) and down (PP1) regulate its activity by phospohorylation dephosphorylation of a serine in its amino terminal domain (Marx *et al.*, 2002). When this complex is disrupted, the channel is not properly regulated and there is imbalance in control of the ventricular action potential which leads to high risk of arrhythmia(Paavonen *et al.*, 2001;Piippo *et al.*, 2001). Because other targets of PKA such as the ryanodine receptor of the sarcoplasmic reticulum form independent macromolecular signaling complexes (Marx *et al.*, 2001)selective disruption of the KCNQ1/KCNE1 signaling complex by inherited mutations may disrupt a micro signaling domain restricted to one channel -- a potential novel mechanism that may mechanistically contribute to the genesis of cardiac arrhythmias(Kass *et al.*, 2003;Kurokawa *et al.*, 2003).

Genotype/Phenotype Correlation Diagnosis of the disease

The discovery that distinct LQTS variants were associated with genes coding for different ion channel subunits has had a major impact on the diagnosis and analysis of LQTS patients. It is clear that there are distinct risk factors associated with the different LQTS genotypes, and this must be clearly taken into account during patient evaluation and diagnosis. The greatest differences in risk factors appear when comparing LQT-3 (SCN5A mutations) patients with LQT-1 (KCNQ1 mutations) and LQT-2 (Herg mutations) patients. This was made clear in an extensive and collaborative study (Schwartz *et al.*, 2001). Risk of cardiac events was found to be greatest during rest, bradycardia, or certainly under conditions of low sympathetic nerve activity for LQT-3 patients. In contrast cardiac events in LQT-2 patients was associated with arousal and or conditions in which patients are startled, whereas LQT-1 patients were found to be at greatest risk of cardiac events during exercise, conditions of elevated heart rate and sympathetic nerve activity(Schwartz *et al.*, 2001). Clearly identifying the genotype is essential in diagnosis as risk factors are somewhat gene specific. Some groups have begun investigating the use of exercise or stress testing to estimate the likelihood of a genotype(Takenaka *et al.*, 2003). Additionally, a major effort has been made to link EKG waveform characteristics to genotype in order to estimate the genetic basis of familial LQTS in patient candidates(Moss *et al.*, 1995). However, ultimately, genotype identification by either direct sequencing or other technologies is necessary to unequivocally determine the genetic basis of the clinical defect.

Therapeutic strategy can be defined by genotype

One of the major contributions of the integrated approach of cellular, molecular, and genetic techniques in the investigation of the causes and treatment of congenital LQTS has been the emergence of mutation-specific strategies for disease therapy. Because the functional consequences of most of the sodium channel mutations that cause LQT-3 are subtle increases in channel activity during the action potential plateau, cellular experimental work

suggested that conventional sodium channel blockers such as mexiletine, tocainide, and lidocaine might prove useful in treating this LQTS variant (An *et al.*, 1996). This concept was recognized by many groups shortly after the first sodium channel mutation was discovered to be linked to LQTS(Priori *et al.*, 1996a;Priori *et al.*, 1996b). The basic concept proposed and later demonstrated using in vitro analysis of recombinant ion channels is that dysfunctional channel activity, caused by the gene mutations, might be more sensitive to conventional drug block, than wild type (normal) channel activity(Wang *et al.*, 1997;Kambouris *et al.*, 2000;Ono *et al.*, 2000;Nagatomo *et al.*, 2000)

(Abriel *et al.*, 2000). This has, in fact proven to be the case and important studies have shown that the predictions of in vitro cellular models have been born out both in animal models(Nuyens *et al.*, 2001), and, importantly in humans carrying LQT-3 mutations. (Benhorin *et al.*, 2000;Windle *et al.*, 2001). To date the sodium channel blocker flecainide have emerged with great promise as a therapeutic agent specific for LQT-3 patients, and evidence has been accumulating that it is the unique structure of this drug as well as its dependence on sodium channel reopening that confers its powerful therapeutic value(Clancy *et al.*, 2003;Liu *et al.*, 2003;Liu *et al.*, 2002).

Beta-blocker therapy is most effective in preventing recurrence of cardiac events and lowering the death rate in LQT-1 and LQT-2 patients, but is much less effective in LQT-3 patients (Moss *et al.*, 2000a;Schwartz *et al.*, 2001). Beta-blocking drugs are associated with a significant reduction in cardiac events in LQTS patients even though these drugs have minimal effect on the QTc interval (Moss *et al.*, 2000b). It is believed that the efficacy of β-blocker therapy is related to an attenuation of adrenergic-mediated trigger mechanisms in the disorder. Beta-blockers do not provide absolute protection against fatal cardiac arrhythmias. Recent studies indicate that the implanted cardioverter defibrillator is effective in preventing sudden cardiac death in LQTS patients (Zareba *et al.*, 2003). The combination of a β-blocker drugs and an implanted defibrillator is a safe and reliable form of therapy for managing high-risk LQTS patients. Particularly in the case of LQT-1 patients, β-blocker administration is

thought to reduce incidents of cellular imbalance in response to stimuation of β-adrenergic receptors (β-AR-s) which occurs when one target of β-AR stimulation, the KCNQ1/KCNE1 channel, is eliminated from the cellular response. The activity of this channel is normally greatly increased in response to sympathetic nerve stimulation (SNS) causing an increase in outward potassium channel current. This current increase may be thought of as a reserve of repolarizing current that must flow in order to shorten action potentials in the ventricles (QT intervals) in the face of increaed heart rate caused by SNS stimulation. When this reserve current is interrupted either by uncoupling the channel from the SNS pathway or by simply down regulating current through the channel by mutation, the result is failure to properly control the QT interval during SNS (exercise) and increasing electrical imbalance that favors arrhythmia. Blocking downstream targets of sympathetic nerve activity with beta blockers would be expected to be particularly effective for these forms of LQTS. On the other hand, if beta blockers are administered to LQT-3 mutation carriers, carriers who are at greater risk of serious arrhythmia during bradycardia, then instead of a therapeutic effect, these same drugs may be pro-arrhythmic in this patient group. Thus genotype, which underlies phenotype, can dictate the most promising therapeutic approach.

Summary

The Long QT syndrome has provided unique and valuable insight into the fundamental mechnisms of cardiac electrical activity. It has reaffirmed the delicate nature of the plateau phase of the cardiac action potential and clearly demonstrated that very small changes in the balance of ionic current during this phase of the action potential can cause severe alteration in the electrical response of the heart. New genes have been discovered by studying the molecular genetics of congenital LQTS and the association between genetic defect and clinical phenotype has provided the clearest picture yet of the roles of key ion channels in determination of QT intervals and the importance of QT regulation in cardiac physiology. The disease has offered further the clearest example of the importance of gaining genetic information about LQTS candidates because this information is not only

important for disease prognosis, but now clearly important, for tailoring therapy. Future investigations both at the clinical and molecular level will no doubt expand the number of specific targets for arrhythmia control, not only in this rare disease, but in many other congenital and acquired cardiac arrhythmias.

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