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Mutation-Specific Pharmacology of the Long QT Syndrome

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Abstract The congenital long QT syndrome is a rare disease in which inherited mutations of genes coding for ion channel subunits, or channel interacting proteins, delay repolarization of the human ventricle and predispose mutation carriers to the risk of serious or fatal arrhythmias. Though a rare disorder, the long QT syndrome has provided invaluable insight from studies that have bridged clinical and pre-clinical (basic science) medicine. In this brief review, we summarize some of the key clinical and genetic characteristics of this disease and highlight novel findings about ion channel structure, function, and the causal relationship between channel dysfunction and human disease, that have come from investigations of this disorder.

Keywords Na^+ channel blocker · Lidocaine · Flecainide · Local anesthetic · Mutation · Channelopathies · Polymorphism · Structural determinants · Antiarrhythmic · Proarrhythmic · VGSC · TTX · Tonic block · Use-dependent block · $Na_V1.5$ · $Na_V1.1$ · SCN5A · SCN1A · Pharmacokinetics · Pharmacodynamics · Recovery from block · Singh–Vaughan Williams · Sicilian Gambit · CAST · CYP · Cytochrome enzymes · Long QT syndrome · Brugada syndrome · Conduction disorders · Isoform specificity · Molecular determinants

Abbreviations

LQTS	Long QT syndrome
QTc	Heart rate-corrected QT
RWS	Romano–Ward syndrome
β-ARs	β-Adrenergic receptors

1 Background

The common form of long QT syndrome (LQTS), Romano–Ward syndrome (RWS), is a heterogeneous, autosomal-dominant genetic disease caused by mutations of genes coding for ion channels expressed in the heart. These channels regulate cardiac rhythm by controlling electrical activity of the cardiac cycle. Dysfunction in channels expressed in ventricular (and presumably Purkinje fiber) cells delays cellular repolarization, causing the disease phenotype: prolonged QT intervals of the ECG. This channelopathy is clinically manifest by syncope and sudden death from ventricular arrhythmias, notably torsades de pointes (TdP) (Moss et al. 1991). Clinically, LQTS is identified by abnormal Q-T interval prolongation on the ECG. The QT prolongation reflects prolonged cellular action potentials and may arise from either a decrease in repolarizing cardiac membrane currents or an increase in depolarizing cardiac currents. These altered currents must occur late in the cardiac cycle to account for the prolonged Q-T interval. Most commonly, QT prolongation is produced by delayed repolarization due to reductions in either the rapidly or slowly activating delayed repolarizing cardiac potassium (K^+) currents, I_{Kr} or I_{Ks} (Sanguinetti and Spector 1997). Less commonly, QT prolongation results from prolonged depolarization due to a small persistent inward "leak" in cardiac sodium (Na^+) current I_{Na} (Bennett et al. 1995). Most recently, mutations in genes coding for important cardiac calcium channels, the so-called L-type calcium channels, have also been shown to dramatically prolong the Q-T interval and cause LQTS (Splawski et al. 2004).

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. The first family with LQTS was reported in 1957 by Jervell and Lange-Nielsen and was thought to be

an autosomal recessive disorder (Jervell and Lange-Nielsen 1957), but in 1997 it was shown to result from a double-dominant, homozygous mutation involving the KvLQT1 gene (Splawski et al. 1997a), now called the KCNQ1 gene. The more common autosomal dominant RWS was described in 1963–1964, and over 300 different mutations involving seven different genes (*LQT1–7*) have now been reported (Splawski et al. 2000). Most of the clinical information currently available regarding LQTS relates to the RWS. There is considerable variability in the clinical presentation of LQTS due to the different genotypes, different mutations, variable penetrance of the mutations, and possible genetic and environmental modifying factors. Clinical criteria have been developed to determine the probability of having LQTS, and genotype screening of suspect LQTS individuals and of family members from known LQTS families has progressively increased the number of subjects with genetically confirmed LQTS. The genes associated with LQTS have been numerically ordered by the chronology of their discovery (LQT1, LQT2, LQT3, ... LQT7), with 95% of the known mutations located in the first three of the seven identified LQTS genes. Current prophylactic and preventive therapy for LQTS to reduce the incidence of syncope and sudden death has involved left cervico-thoracic sympathetic ganglionectomy, β-blockers, pacemakers, implanted defibrillators, and gene/mutation-specific pharmacologic therapy (Moss 2003).

2
Arrhythmia Risk Factors Are Mutation/Gene-Specific

The discovery that distinct LQTS variants are associated with genes coding for different ion channel subunits has had a major impact on the diagnosis and analysis of LQTS patients. Critical evaluation of clinical data has revealed that there are distinct risk factors associated with the different LQTS genotypes, and that these must be taken into account during patient evaluation and diagnosis. The greatest difference in risk factors becomes apparent in comparing LQT3 syndrome patients (*SCN5A* mutations) and patients with LQT1 syndrome (*KCNQ1* mutations) or LQT2 syndrome (*hERG* mutations). The potential for understanding a mechanistic basis for arrhythmia risk was realized soon after the first genetic information relating mutations in genes coding for distinct ion channels became available, (Priori et al. 1997) but is still the focus of extensive clinical and basic investigation. In one such study, which focused on patients with KCNQ1 (LQT1), hERG (LQT2), and SCN5A (LQT3) mutations, a clear difference in arrhythmia risk emerged, and this difference appeared in a gene-specific manner. In the case of SCN5A mutation carriers (LQT3), risk of cardiac events was greatest during rest, (bradycardia) when sympathetic nerve activity is expected to be low. In contrast, cardiac events in LQT2 syndrome patients were associated with arousal and/or conditions in which patients were startled, whereas LQT1 syndrome patients were found to

be at greatest risk of experiencing cardiac events during exercise or conditions associated with elevated sympathetic nerve activity (Schwartz et al. 2001).

Additional evidence has continued to support the view that under conditions in which sympathetic nerve activity is likely to be high, such as during periods of exercise, patients harboring LQT1 mutations (Ackerman et al. 1999; Paavonen et al. 2001; Takenaka et al. 2003) are likely to experience dysfunctional regulation in cardiac electrical activity and hence an increased arrhythmia risk. The contrast between the role of adrenergic input and/or heart rate in the arrhythmia risk of LQT1 and LQT3 patients is clear and has raised the possibility of distinct therapeutic strategies in the management of patients with these LQTS variants. In fact β-blocker therapy has been shown to be most effective in preventing recurrence of cardiac events and lowering the death rate in LQT1 and LQT2 syndrome patients but is much less effective in the treatment of LQT3 syndrome patients (Moss et al. 2000; Priori 2004). β-Blocking drugs have minimal effects on the QTc interval but are associated with a significant reduction in cardiac events in LQTS patients, probably because these drugs modulate the stimulation of β-adrenergic receptors (β-ARs) and hence the regulation of downstream signaling targets during periods of elevated sympathetic nerve activity. Clinical data for genotyped patients continues to provide strong support for the hypothesis that the effectiveness of β-blocking drugs depends critically on the genetic basis of the disease with recent data providing evidence that there is still a high rate of cardiac events in LQT2 and LQT3 patients treated with β-blocking drugs (Priori et al. 2004). Consequently, even β-blockers do not provide absolute protection against fatal cardiac arrhythmias.

3
Mutation-Specific Pharmacology: Role of the Sodium Channel

The SCN5A gene encodes the α-subunit of the major cardiac voltage-gated sodium channel (George et al. 1995). Voltage-gated Na^+ channels are integral membrane proteins (Catterall 1995, 1996) that not only underlie excitation in excitable cells, but determine the vulnerability of the heart to dysfunctional rhythm by controlling the number of channels available to conduct inward Na^+ movement (Rivolta et al. 2001). Na^+ channels open in response to membrane depolarization, allowing a rapid selective influx of Na^+ which serves to further depolarize excitable cells and initiate multiple cellular signals (Catterall 2000). Within milliseconds of opening, Na^+ channels enter a non-conducting inactivated state (Stuhmer et al. 1989; Patton et al. 1992; West et al. 1992; McPhee et al. 1994, 1995, 1998; Kellenberger et al. 1997a,b). Channel inactivation is necessary to limit the duration of excitable cell depolarization. Therefore disruption of inactivation by inherited mutations, which delays cellular repolarization, is associated with a diverse range of human diseases including myotonias

(Yang et al. 1994), epilepsy and seizure disorders (Kearney et al. 2001; Lossin et al. 2002), autism (Weiss et al. 2003), and sudden cardiac death (Keating and Sanguinetti 2001; Kass and Moss 2003).

The Na^+ channel α-subunit, which forms the ion-conducting pore and contains channel gating components, consists of four homologous domains (I to IV; Sato et al. 2001). Each domain contains six α-helical transmembrane repeats (S1–S6), for which mutagenesis studies have revealed key functional roles (Catterall 2000). Voltage-dependent inactivation of Na^+ channels is a consequence of voltage-dependent activation (Aldrich et al. 1983), and inactivation is characterized by at least two distinguishable kinetic components: an initial rapid component (fast inactivation) and a slower component (slow inactivation). Within milliseconds of opening, Na^+ channels enter a non-conducting inactivated state as the inactivation gate, the cytoplasmic loop linking domains III and IV of the α-subunit, occludes the open pore (Stuhmer et al. 1989; Patton et al. 1992; West et al. 1992; McPhee et al. 1994, 1995, 1998; Kellenberger et al. 1996). Fast Na^+ channel inactivation is due to rapid block of the inner mouth of the channel pore by the cytoplasmic linker between domains III and IV that occurs within milliseconds of membrane depolarization (Vassilev et al. 1988; Stuhmer et al. 1989; Vassilev et al. 1989; West et al. 1992). Nuclear magnetic resonance (NMR) analysis of this inactivation linker (gate) in solution has revealed a rigid helical structure that is positioned such that it can block the pore, providing a structural explanation of the functional studies (Rohl et al. 1999) and a biological mechanism of inhibiting channel conduction.

The residues that form a hydrophobic triplet (IFM) in the III–IV linker are involved in inactivation gating (West et al. 1992). The IFM motif has been suggested to function as a 'latch' that holds the inactivation gate shut. Cysteine scanning of the residues I1485, F1486, and M1487 in the human cardiac Na^+ channel revealed that these amino acids contribute to stabilizing the fast-inactivation particle (Deschenes et al. 1999) in analogy to the brain Na^+ channel (Stuhmer et al. 1989; Sheets et al. 2000).

4
Na^+ Channel Block by Local Anesthetics Is Linked to Channel Inactivation

Blockade of voltage-dependent Na^+ channels has long been recognized as a potential therapeutic approach to the management of many cardiac arrhythmias, but with considerable risk of toxic side effects (Rosen et al. 1975). The discovery that mutant forms of Na^+ channels linked to inherited human cardiac arrhythmias might make distinct targets for Na^+ channel blocking drugs (An et al. 1996; Wang et al. 1997; Dumaine et al. 1996; Dumaine and Kirsch 1998; Nagatomo et al. 2000; Viswanathan et al. 2001) has stimulated reinvestigation of the molecular determinants of Na^+ channel blockade in the heart.

Voltage-dependent block of Na^+ channels by local anesthetics and related drugs has been well described within the framework of the modulated receptor hypothesis, which proposes that allosteric changes in a drug receptor occur when changes in voltage induce changes in channel conformation states (Hille 1977; Hondeghem and Katzung 1977). Extensive mutagenesis experiments have been performed with several different drugs in many sodium channel isoforms in an effort to define the molecular determinants of drug binding. While a clear consensus has not been reached regarding precisely where drug binds and there is certainly variability in drugs, isoforms, and how the data are interpreted, the current evidence strongly suggests that most drugs tested bind in the pore of the channel on the intracellular side of the selectivity filter.

Furthermore, mutagenesis studies by several groups find specific amino acid residues that contribute to drug binding on the S6 segment of domains I, III, and IV. The most dramatic effects on drug binding can be attributed primarily to two aromatic residues on DIV S6, a phenylalanine at position 11760 (F1760) and a tyrosine at position 1767 (Y1767) using $Na_V1.5$ numbering, that are conserved among sodium channel isoforms (Ragsdale et al. 1994 1996; Li et al. 1999; Weiser et al. 1999).

5
LQT-3 Mutations: A Common Phenotype Caused by a Range of Mutation-Induced Channel Function

Different LQT3 mutations can result in distinct functional changes in the activity of the sodium channel, but with similar degrees of QT prolongation and cardiac arrhythmias (Wang et al. 1995a,b). For example, the nine-base-pair deletion with loss of three amino acids (Δ-KPQ) in the linker between the third and fourth domains of the α-alpha unit of the sodium channel and three missense mutations in this gene (N1325S, R1623Q, and R1644H) all promote sustained and inappropriate sodium entry into the myocardial cell during the plateau phase of the action potential, resulting in prolonged ventricular repolarization and the LQTS phenotype. This mutation occurs in the cytoplasmic peptide that links two domains of the channel: domain III and domain IV (see Sect. 9), and, not surprisingly, alters the stability of inactivated, or nonconducting channels. In contrast, the functional consequences of the D1790G missense mutation on sodium channel gating are quite different. This mutation does not promote sustained inward sodium current, but rather causes a negative shift in steady-state inactivation with a similar LQTS phenotype (Abriel et al. 2000b). Despite these functional differences in channel activity, the phenotypical effect of the mutations is the same: QT prolongation. Interestingly, in vitro studies have shown that the D1790G mutation alters the response of the sodium channel to adrenergic stimulation, a finding that may have impli-

cations for triggers of this unique mutation (Tateyama et al. 2003). It should be noted that other mutations in the SCN5A gene can result in the Brugada syndrome and conduction system disorders without QT prolongation. At least one mutation (1795insD) has been shown to have a dual effect with inappropriate sodium entry at slow heart rates (LQTS ECG pattern) and reduced sodium entry at fast heart rates (Brugada ECG pattern; Veldkamp et al. 2000).

Mutation-specific pharmacologic therapy has been reported in two specific *SCN5A* mutations associated with LQTS. In 1995, Schwartz et al. reported that a single oral dose of the sodium-channel blocker mexiletine administered to seven LQT3 patients with the *Δ*KPQ deletion produced significant shortening of the QTc interval within 4 h (Schwartz et al. 1995). Similar QTc shortening in LQT3 patients with the *Δ*KPQ deletion has been reported with lidocaine and tocainide (Rosero et al. 1997). Preliminary clinical experience with flecainide revealed normalization of the QTc interval with low doses of this drug in patients with the *Δ*KPQ deletion (Windle et al. 2001). In 2000, Benhorin et al. reported the effectiveness of open-label oral flecainide in shortening the QTc in eight asymptomatic subjects with the D1790G mutation (Benhorin et al. 2000).

In the *SCN5A-Δ*KPQ deletion mutation, flecainide has high affinity for the sodium-channel protein and provides almost complete correction of the impaired inactivation (Nagatomo et al. 2000). A recent randomized, double-blind, placebo-controlled clinical trial in six male LQT3 subjects having the *Δ*KPQ deletion, with four 6-month alternating periods of low-dose flecainide (1.5 to 3.0 mg/kg/day) and placebo therapy (A.J. Moss, unpublished data). The average QTc values during placebo and flecainide therapies were 534 ms and 503 ms, respectively, with a change in QTc from baseline during 6-month flecainide therapy of −29 ms (95% confidence interval, −37 ms to −21 ms; $p<0.001$) at a mean flecainide blood level of 0.11±0.05 μg/ml. At this low flecainide blood level, there were minimal prolongations in P-R and QRS duration and no major adverse cardiac effects.

The *SCN5A*-D1790G mutation changes the sodium channel's interaction with flecainide. This mutation confers a high sensitivity to use-dependent block by flecainide, due in large part to the marked slowing of the repriming of the mutant channels in the presence of the drug (Abriel et al. 2000a). Flecainide tonic block is not affected by the D1790G mutation. These flecainide affects are different from those occurring with the *Δ*KPQ mutant channels, and may underlie the distinct efficacy of this drug in treating LQT3 patients harboring the D1790G mutation (Liu et al. 2002, 2003).

These flecainide findings in patients with the *Δ*KPQ and D1790G mutations provide encouraging evidence in support of mutation-specific pharmacologic therapy for two specific forms of the LQT3 disorder. Larger clinical trials with flecainide in patients with these two mutations are needed before this therapy can be recommended as safe and effective for patients with these genetic disorders.

6
Clinical Relevance of Mutations Within Different Regions of the Ion Channel: Structure/Function

The hERG gene encodes the ion channel involved in the rapid component of the delayed rectifier repolarization current (I_{Kr}), and mutations in this gene are responsible for the LQT2 form of LQTS (Nagatomo et al. 2000). Mutations in *hERG* are associated with diminution in the repolarizing I_{Kr} current with resultant prolongation of ventricular repolarization and lengthening of the Q-T interval. During the 1990s, it was appreciated that several drugs such as terfenadine and cisapride caused QT prolongation by reducing I_{Kr} current through the pore region of the hERG channel (Sanguinetti et al. 1996b). These findings raised the question whether mutations in the pore region of the hERG channel would be associated with a more virulent form of LQT2 than mutations in the non-pore region.

In a report from the International LQTS Registry, 44 different *hERG* mutations were identified in 201 subjects, with 14 mutations in 13 locations in the pore region (amino acid residues 550 through 650); (Moss et al. 2002; Fig. 1). Of the subjects, 35 had mutations in the pore region and 166 in non-pore regions. Using birth as the time origin with follow-up through age 40, subjects with pore mutations had more severe clinical manifestations of the genetic disor-

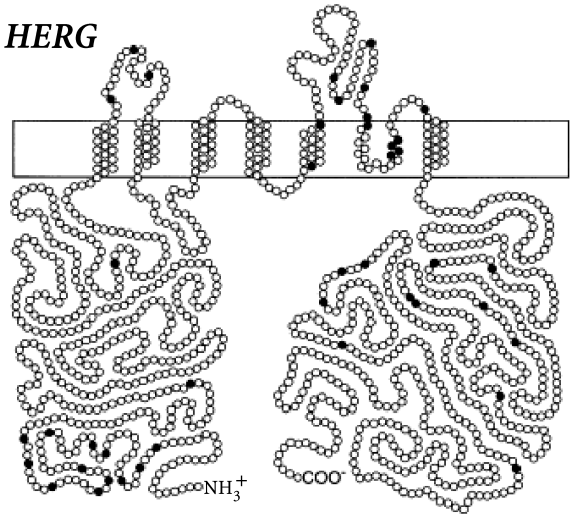

Fig. 1 Schematic representation of hERG potassium channel α-subunit involving the N-terminal portion (NH2), 6 membrane-spanning segments with the pore region extending from segments S5 to S6, and the C-terminus portion ($C00^{-}$). Mutation locations are indicated by *black dots*. Fourteen different mutations were located in 13 locations within the pore region. (Reprinted with permission from Moss et al. 2002)

der and experienced a higher frequency of arrhythmia-related cardiac events at an earlier age than did subjects with non-pore mutations. The cumulative probability of a first cardiac event before β-blockers were, initiated in subjects with pore mutations and non-pore mutations in the hERG channel are shown in Fig. 2, with a hazard ratio in the range of 11 ($p<0.0001$) at an adjusted QTc of 0.50 s. This study involved a limited number of different *hERG* mutations and only a small number of subjects with each mutation. Missense mutations made up 94% of the pore mutations, and thus it was not possible to evaluate risk by the mutation type within the pore region.

These findings indicate that mutations in different regions of the hERG potassium channel can be associated with different levels of risk for cardiac arrhythmias in LQT2. An important question is whether similar region-related risk phenomena exist in the other LQTS channels. Two studies evaluated the clinical risk of mutations located in different regions of the KCNQ1 (LQT1) gene and reported contradictory findings. Zareba et al. found no significant differences in clinical presentation, ECG parameters, and cardiac events among 294 LQT1 patients with *KCNQ1* mutations located in the pre-pore region including N-terminus (1–278), the pore region (279–354), and the post-pore

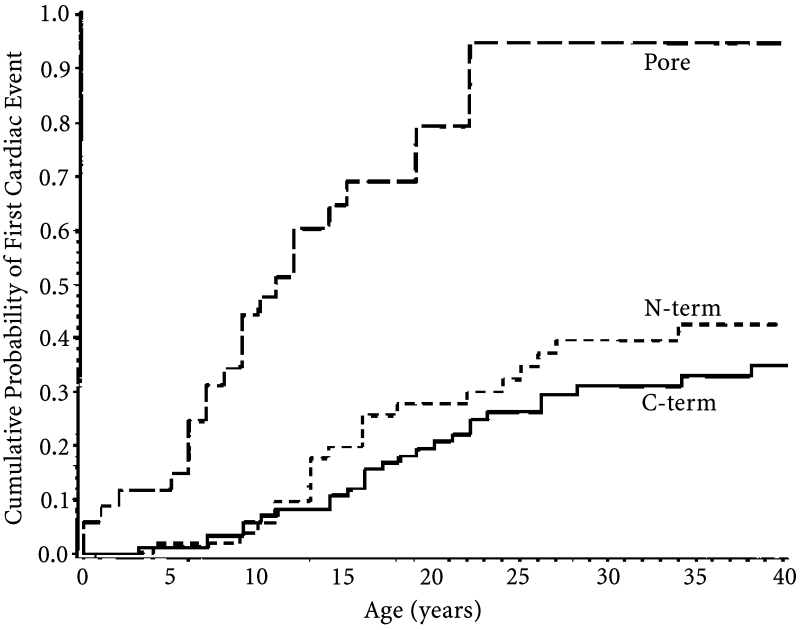

Fig. 2 Kaplan–Meier cumulative probability of first cardiac events from birth through age 40 years for subjects with mutations in pore ($n = 34$), N-terminus ($n = 54$), and C-terminus ($n = 91$) regions of the hERG channel. The curves are significantly different ($p < 0.0001$, log-rank), with the difference caused mainly by the high first-event rate in subjects with pore mutations. (Reprinted with permission from Moss et al. 2002)

region including C-terminus (>354) (Zareba et al. 2003). In contrast, Shimizu et al. studied 66 LQT1 patients and found that mutations in the transmembrane portion of *KCNQ1* were associated with a higher risk of LQTS-related cardiac events and had greater sensitivity to sympathetic stimulation than mutations located in the C-terminal region (Shimizu et al. 2004). These different findings in the two LQT1 studies may reflect, in part, population-related genetic heterogeneity, since the Zareba population was almost entirely Caucasian and the subjects in the Shimizu study were Japanese. Much larger homogeneous populations need to be studied to resolve this issue.

7
Basic Electrophysiology Revealed Through LQTS Studies

Though a rare congenital disorder, LQTS has provided a wealth of information about fundamental mechanisms underlying human cardiac electrophysiology that has come about because of true collaborative interactions between clinical and basic scientists. Our understanding of the mechanisms that control the critical plateau and repolarization phases of the human ventricular action potential has been raised to new levels through these studies which impact on the manner in which both potassium and sodium channels regulate this critical period of electrical activity.

8
Identification of Cardiac Delayed Rectifier Channels

It had been known since 1969 that potassium currents with unique kinetic and voltage-dependent properties were important to the cardiac action potential plateau (Noble and Tsien 1968; Noble and Tsien 1969). Because of the unique voltage-dependence, these currents were referred to as delayed rectifiers. In a pivotal study, Sanguinetti and Jurkiewicz used a pharmacological analysis to demonstrate two distinct components of the delayed rectifier potassium current in heart: I_{Kr} and I_{KS} (Sanguinetti and Jurkiewicz 1990). The I_{KS} component had previously been shown to be under control of the sympathetic nervous system, providing an increase in repolarization currents in the face of β-AR agonists in cellular models (Kass and Wiegers 1982), but the molecular identity and the relevance to human electrophysiology were not only not clear, but controversial. The clear clinical importance and the genetic basis of these potassium currents were revealed through LQTS investigations.

The first report linking potassium channel dysfunction to LQTS revealed the molecular identity of one of the delayed rectifier channels and confirmed the pharmacological evidence for independent channels underlying these currents (Sanguinetti et al. 1995). This report revealed that hERG encodes the α (pore

forming) subunit of the I_{Kr} channel and that the rectifying properties of this channel, identified previously by pharmacological dissection, were indigenous to the channel protein. Not only did this work provide the first clear evidence for a role of this channel in the congenital LQTS but also laid the baseline for future studies which would show that it is the hERG channel that underlies almost all cases of acquired LQTS (Sanguinetti et al. 1996a).

In 1996 it was discovered that LQTS variant 1 (LQT1) was caused by mutations in a gene (*KvLQT1*/*KCNQ1*) coding for an unusual potassium channel subunit that could be studied in heterologous expression systems (Wang et al. 1996) and the KvLQT1 gene product was found to be the α (pore forming) subunit of the I_{KS} channel (Barhanin et al. 1996; Sanguinetti et al. 1996b). Furthermore, these studies indicated that a previously reported, but as-yet poorly understood gene (*mink*) formed a key regulatory subunit of this important channel. Mutations in *mink* (later called *KCNE1*) have subsequently been linked to LQT5 (Splawski et al. 1997b). Now the molecular identity of the two cardiac delayed rectifiers had been established.

Clinical studies had provided convincing evidence linking sympathetic nerve activity and arrhythmia susceptibility in LQTS patients, particularly in patients harboring LQT1 mutations. These data and previous basic reports of the robust sensitivity of the slow delayed rectifier component, I_{KS}, to β-AR agonists (Kass and Wiegers 1982), motivated investigation of the molecular links between KCNQ1/KCNE1 channels to β-AR stimulation which revealed, for the first time, that the KCNQ1/KCNE1 channel is part of a macromolecular signaling complex in human heart (Marx et al. 2002). The channel complexes with an adaptor protein (AKAP 9 or yotiao) that in turn directly binds key enzymes in the β-AR signaling cascade [protein kinase A (PKA) and protein phosphatase 1 (PP1)]. Thus, the binding of yotiao to the KCNQ1 carboxy-terminus recruits signaling molecules to the channel to form a micro-signaling environment to control the phosphorylation state of the channel. When the channel is PKA phosphorylated, there is an increase in repolarizing (potassium channel) current, which provides a repolarization reserve to shorten action potentials. This must occur with the concomitant increase in heart rate, which is the fundamental response to sympathetic nerve stimulation, in order to preserve cardiac function during exercise. Mutations either in *KCNQ1* (Marx et al. 2002) or *KCNE1* (Kurokawa et al. 2003) can disrupt this regulation and create heterogeneity in the cellular response to β-AR stimulation, a novel mechanism that may contribute to the triggering of some arrhythmias in LQT1 and LQT5 (Kass et al. 2003). Importantly, disruption of the regulation of only the potassium channel by these mutations disrupts, at the cellular level, the coordinated response of one, but not all, channel/pump proteins that are regulated by PKA. Because many of the target proteins regulate cellular calcium homeostasis, it is entirely possible that the trigger underlying at least some forms of exercise-induced arrhythmias in LQT1 may be due to dysfunction in cellular calcium handling (Kass et al. 2003).

9
The Cardiac Sodium Channel and the Action Potential Plateau Phase

The report that mutations in *SCN5A*, the gene coding for the α-subunit of the major cardiac sodium channel, were associated with LQTS (Wang et al. 1995a) was surprising because this channel is associated most frequently with impulse conduction and hence the QRS but not the QT waveforms of the ECG. Sodium channels are voltage-gated channels that rapidly enter a non-conducting inactivated state during sustained depolarization such as the cardiac action potential plateau. Importantly, the first *SCN5A* mutation, the *Δ*KPQ mutation, physically disrupted a cytoplasmic peptide linker in the channel protein that, in basic biochemical and biophysical studies, had been shown to be a critical determinant of sodium channel inactivation: the inactivation gate (Stuhmer et al. 1989; Catterall 1995). This peptide links two domains (III and IV) of the channel and physically moves to occlude the channel pore upon depolarization. Once again, the combination of basic and clinical investigation has led to a clear understanding of the molecular basis of this key physiological parameter in human heart. Further, the demonstration that small changes in sodium channel inactivation such as those changes that occur in LQT3 mutations, can have life-threatening consequences confirms predictions made more than 50 years ago by Silvio Weidmann. Demonstrated that the cardiac action potential plateau was an exquisitely sensitive period of electrical activity that could adapt, with little energy expenditure, to small changes in ionic currents (Weidmann 1952).

Subsequent investigations of LQT3 mutations have revealed that not only is the domain III/IV intracellular linker key to inactivation and maintenance of the action potential plateau (and hence Q-T interval), but the channel carboxy terminal (C-T) domain is essential in this process also, and not only is disruption of the inactivation gate a mechanism by which LQT3 arrhythmias can be generated, but much more subtle changes in channel gating can also underlie these arrhythmias. For example, one LQT3 mutation (the I1768V mutation) speeds the recovery from inactivation in a voltage-dependent manner, and this leads to augmentation of depolarizing current during the repolarization phase of the action potential. The consequence is delayed repolarization, which underlies the clinical phenotype—prolonged QT (Clancy et al. 2003).

10
The Sodium Channel Inactivation Gate as a Molecular Complex

Recent work in which biochemical and functional experiments were combined directly addressed the question of whether or not the C-terminus may have a direct structural role in the control of channel inactivation, and, if so, how the C-T domain affects stabilization of the inactivated Na^+ channel. The con-

clusion from this work is that the cardiac sodium channel inactivation gate is a molecular complex, providing additional structural insight into the role of the carboxy-terminal domain in regulating channel activity. Experimental data support the view that the III–IV linker interacts directly with the carboxy terminal domain of the channel to stabilize inactivated channels (Motoike et al. 2004).

In these experiments, biochemical evidence was presented for direct physical interaction between the C-T domain of the channel and the III–IV linker inactivation gate. These biochemical data are remarkably consistent with a role of the C-terminal/III–IV linker in stabilization of the inactivated state. Further, using glutamate scaning of the III–IV linker peptide, a region on the linker was suggested to be the motif that coordinates III–IV linker/C-T interactions, and this motif was found to be distinct from the III–IV linker motif previously identified as the region that coordinates binding of the inactivation gate to the inner mouth of the channel pore. These data provided strong evidence that the inactivation gate of the voltage-dependent Na^+ channel is a molecular complex that consists of the III–IV linker and the C-terminal domain of the channel and that this interaction underlies the stabilization of the inactivated state by the C-T domain during prolonged depolarization. Uncoupling of this complex destabilizes inactivation and increases the likelihood of channel re-opening during prolonged depolarization.

11
Summary and Future Directions

Investigation into the molecular basis of inherited cardiac arrhythmias caused by mutations of the α-subunit of the principal cardiac sodium channel (Nav1.5) has led to an appreciation of the role of the carboxy terminal domain of the channel in regulating channel gating. Theoretical and experimental structural analysis of the channel C-T domain provides strong evidence for a highly structured region of the channel and that interactions between the C-T domain and the channel inactivation gate are necessary to control channel activity that directly affects action potential, and hence QT, duration in the heart. This structured region thus provides a novel target against which to develop drugs that have the potential to regulate the activity of this key cardiac ion channel, not by blocking the conduction pore, but by regulating, in an allosteric manner, channel gating. Investigations into the mechanisms underlying the clinical observations that LQT1 patients are at elevated arrhythmia risk during exercise have led to the unraveling of the molecular architecture of a critically important cardiac potassium channel and its interconnection to the sympathetic nervous system.

We have made considerable progress in understanding the importance of ion channel structure to human physiology since the first ion channel was cloned in 1982. We now have a better understanding of the molecular genetics, ion channel structures, and cellular electrophysiology that contribute to the

genesis of cardiac arrhythmias. Much of this improved insight has come directly from investigations of LQTS and other inherited arrhythmias and is being translated into more effective and more rational therapy for patients with electrical disorders of the cardiac rhythm. Much remains to be accomplished, and this will be done thorough continued collaboration of basic and clinical scientists in many ways based on the foundations laid by studies of LQTS.

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