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Topical Review

Ion Channel Defects in Cardiac Arrhythmia

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Introduction

Voltage-gated ion channels initiate and conduct electrical activity in the heart. The channels open and close in response to changes in membrane potential, and allow ionic permeation across the plasmalemma. Because of the diversity of channels that participate in cardiac excitability and the complex relationships between gating and membrane potential, the functional roles of different ion channels have been difficult to determine. The idea that defective ion channels might be responsible for cardiac arrhythmia has received indirect support from the anti- or proarrhythmic actions of a host of drugs and toxins that are specific for Na^+, Ca^{2+} and K^+ channels. Recent advances in cloning and characterization of cardiac ion channel genes, together with identification of naturally occurring, disease-linked mutations have provided definitive evidence that certain types of arrhythmia may be caused by defects in the expression or function of ion channels. This review will describe some of the recent evidence and highlight areas of current interest.

Diverse Ion Channels Generate the Cardiac Action Potential

The relationships between cardiac ion channel genes, ionic currents recorded in voltage-clamped cells, the waveform of the action potential in unclamped cells, and the surface electrocardiogram (ECG) in vivo are summarized in Fig. 1. The initial upstroke of the action potential (phase 0, Fig. 1*B*) is driven by a brief surge of inward current through Na^+ channels that activate rapidly in response to pacemaker depolarization (*not illustrated*) and then close rapidly into an inactivated state even though the cell membrane remains partially depolarized throughout phases 1 and 2. The SCN5A gene encodes the major structural subunit of the cardiac Na^+ channel. Mutations in SCN5A have been linked to long-QT syndrome (LQTS) and to a form of idiopathic ventricular fibrillation known as Brugada syndrome.

Repolarization of the membrane to the diastolic level (phase 4) progresses in several steps (phase 1–3), each controlled by different channels. A transient outward current (I_{to1}) carried primarily by K^+ channels of the Kv4 subfamily controls the early repolarization phase 1. Although not genetically linked to disease, downregulation of Kv4.3 may contribute to action potential prolongation and arrhythmia in congestive heart failure (Kaab et al., 1998). Inward currents carried mainly by L-type Ca^{2+} channels but with a minor contribution from non-inactivated Na^+ channels control the phase 2 plateau. A linkage between Ca^{2+} channel defects and arrhythmia has not been established, but a reduction in the amplitude of $I_{\text{Ca-L}}$ may be responsible for action potential shortening in atrial fibrillation (Le Grand et al., 1994; Ouadid et al., 1995). Late repolarization (phase 3) is controlled by a variety of K^+ channels whose different functional properties produce characteristic ultra-rapid (I_{Kur}), rapid (I_{Kr}) and slow (I_{Ks}) components of the sustained outward current (I_K, delayed rectifying). Mutations of the genes that encode I_{Kr} and I_{Ks} channels have been linked to LQTS.

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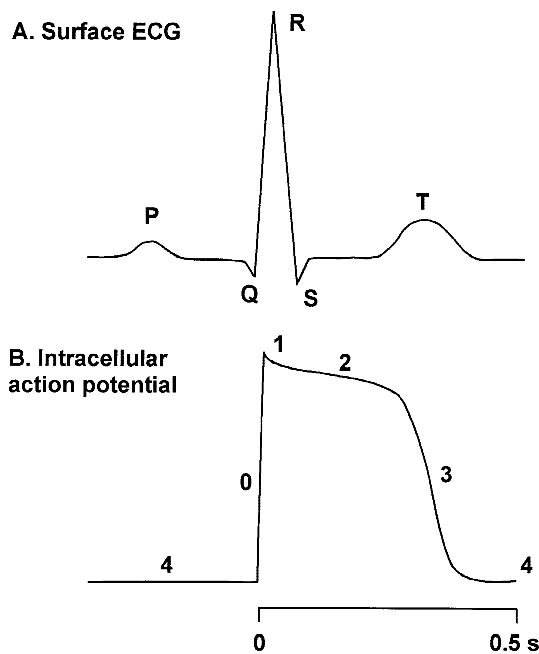

Fig. 1. Ionic basis of the cardiac action potential. Schematic diagrams of the surface electrocardiogram (*A*) and intracellular action potential (*B*) showing the temporal relationships between the two waveforms. In *A* the letters correspond to the standard ECG nomenclature. In *B* the phases are numbered according to standard classification described in Table 1 and text.

Ion Channel Defects are the Basis of Long-QT Syndrome

LQTS causes loss of consciousness and sudden death in otherwise healthy individuals during episodes of ventricular arrhythmia characterized by *Torsade de Pointes* (TdP, an unusual disruption of the ECG in which the QRS complex twists around the isoelectric axis) and ventricular fibrillation. Between episodes, LQTS patients show delays in repolarization such that the QTc interval (corrected for heart rate) of the surface ECG exceeds 0.45 sec (Keating et al., 1991*a*). Two forms of inherited LQTS are recognized: Jervell/Lange-Nielsen syndrome, associated with congenital deafness and transmitted in an autosomal recessive pattern; and Romano-Ward syndrome, without deafness and transmitted in an autosomal dominant pattern of inheritance. In addition, acquired LQTS, often occurring as a side effect of antiarrhythmic drugs that prolong the action potential or in association with hypokalemia, has similar characteristics and may involve the same ion channels (Roden, 1990).

Both the acquired and inherited forms appear to involve abnormal action potential repolarization and hence the possible involvement of K^+ and Na^+ channels. Delayed repolarization, caused by suppression of outward, repolarizing currents conducted by K^+ channels (Roden, 1993; Curran et al., 1995) or by potentiation of inward, depolarizing currents conducted by Na^+ channels (Boutjdir et al., 1994), allows a reactivation of Ca^{2+} channels (January & Riddle, 1988), and secondary triggered activity due to early afterdepolarizations (EAD; Zhou et al., 1992). The inherited form is caused by defects in ion channel structural genes that lead to abnormalities in channel expression or function. Whether susceptibility to acquired LQTS has a genetic component has not been clarified.

Recent genetic analysis has identified mutations in several different genes that present a similar clinical picture. Conversely, in some instances, mutations of the same genes can produce clinically distinct phenotypes depending on homo- or heterozygosity. The molecularly distinct inherited forms are denoted LQT1-LQT5 (Table 1), but the ionic basis and clinical manifestations can sometimes overlap. Both RWS and JLNS are caused by repolarization defects usually associated with different mutations in either of two K^+ channel genes (KCNQ1 and KCNE1). RWS is generally associated with heterozygous and JLNS with homozygous mutations of these genes. However, RWS also can be caused by heterozygous mutations in an unrelated K^+ channel gene (HERG) and a Na^+ channel gene (SCN5A).

The effects of mutations on channel function are broadly classified into loss-of-function and altered-function categories. Predictably, most of the Na^+ channel mutations are of the altered-function type, since increased amounts of persistent inward current rather than an overall reduction in Na^+ conductance would be required to delay repolarization. By contrast, decreased outward current through K^+ channels would be required to prolong repolarization. In principle this could result from loss-of-function mutations in which a reduction in conductance is proportional to the amount of nonfunctional protein (haplo-insufficiency) or through an alteration in gating that prevents mutant channels from opening normally in response to stimulation. The tetrameric structure of K^+ channels opens the possibility of more severe effects through dominant negative inhibition. In the heterozygous condition mutant subunits could be nonfunctional but retain the ability to assemble with normal α-subunits to produce nonfunctional, heteromeric channels. Thus, they could interfere with the functionality of the normal subunits and extend their inhibitory effect beyond the proportionality predicted by simple haplo-insufficiency. Dominant negative effects are often observed in LQTS mutations associated with K^+ channels but not for Na^+ channels that contain only a single α-subunit.

Defects in a Slow Delayed Rectifier K Channel Cause LQT1 and LQT5

Genetic linkage analysis in several families localized LQTS to a site on the short arm of chromosome 11

Table 1. Cardiac ion channels

Phase	Ionic currents		Major subunit	Gene	Chromosome	Disease
0 Upstroke	I_{Na}	Fast transient	hH1	SCN5A	3q21-q23	LQT3, IVF
	$I_{Ca\text{-}T}$	Transient T-type	α1H	CACNA1H	16p13.3	
1 Early repolarization	I_{to}	Transient outward K^+	Kv4.2/4.3	KCND2	?	
2 Plateau	$I_{Ca\text{-}L}$	L-type	α1C	CACNL1A1	12p13.3	
	$I_{Na/Ca}$	Na^+-Ca^{2+} exchange	NCX1	NCX1	2p22-p23	
3 Late repolarization	I_{Kur}	Delayed rectifier ultra-rapid	Kv1.5	KCNA5	12p13	
	I_{Kr}	Delayed retifier rapid	HERG	HERG	7q35-36	LQT2
	I_{Ks}	Delayed rectifier slow	Kvlqt1	KCNQ1	11p15.5	LQT1
			+minK	KCNE1	21q22.1	LQT5
4 Diastole	I_{K1}	Inward rectifier	Kir2.1	KCNJ2	?	

The ion channels responsible for the different phases of the action potential related to the genes that encode their major structural components and to congenital arrhythmia.

(11p15.5; Keating et al., 1991*a,b*). The LQT1 target (i.e., the first gene found to be associated with LQTS) was identified on the basis of mutations in affected members of 16 different LQT1 families (Wang et al., 1996*b*). The LQT1 gene was identified as a previously unknown cardiac K^+ channel gene (KvLQT1, also denoted KCNQ1) based on hydropathy analysis and sequence comparison to other known K^+ channels of the voltage-gated, six-transmembrane segment type (Kv channels) and the detection of RNA transcripts of the gene in heart tissue (Wang et al., 1996*b*). Isolation of a full length KvLQT1 (Sanguinetti et al., 1996*b*; Yang et al., 1997) and heterologous expression revealed a voltage-gated K^+ channel with gating properties unlike those of any known cardiac K^+ currents. Although it showed a general similarity to I_{Ks} (the slow component of the cardiac delayed rectifier current), including potentiation by cAMP and blockade by clofilium but not dofetilide (Yang et al., 1997), the ionic current produced by heterologous expression of KvLQT1 activated too rapidly and showed a greater degree of inactivation compared to I_{Ks} in cardiac myocytes. These results suggested that the I_{Ks} channel might be a heteromer assembled from pore-forming α-subunits and modulatory β-subunits. Co-expression of KvLQT1 with an auxiliary β-subunit, minK (also called IsK, encoded by KCNE1) recapitulated the functional characteristics of I_{Ks} including slow activation and absence of inactivation (Sanguinetti et al., 1996*b;* Barhanin et al., 1996, Yang et al., 1997). Moreover, coexpression of KvLQT1 and minK produced much larger macroscopic K^+ current than KvLQT1 alone (Sanguinetti et al., 1996*b*; Romey et al., 1997). Thus, the coassembly of polypeptides encoded by both KvLQT1 and KCNE1 genes provides the molecular basis of the I_{Ks} channel. Functional I_{Ks} channels are thought to contain four α-subunits by analogy with other Kv channels and the addition of one or more β-subunits, although not essential for ion conduction, modifies gating and conduction. Therefore, mutations in either KCNE1 or KvLQT1 can suppress I_{Ks}. In fact, several mutations in KCNE1, have been identified in a small group of LQTS families (Tyson et al., 1997). These mutations have been assigned to the LQT5 form of the disease although both LQT1 and LQT5 affect the same ionic current and have a similar clinical manifestation.

LQT1 mutations that produce truncated α-subunits, such as frame shift and splice donor mutations that cause premature termination (Table 2) in the transmembrane region, can have loss-of-function or dominant negative effects on normal subunits in heterozygous expression. Presently, about 35 LQT1 mutations have been published but the pathogenicity of less than half has been established by heterologous expression of the mutant channels. Nonetheless, the majority of mutations would be predicted to cause loss-of-function defects because of predicted truncations. When tested in heterologous systems, most of the mutant KvLQT1 α-subunits either by themselves (to mimic homozygous expression) or together with minK β-subunits, failed to generate K^+ currents. Coexpression of mutant and wild-type α-subunits (heterozygous expression) caused a marked decrease in the level of current when compared with equivalent amounts of wild type by itself. Moreover, the gating kinetics of the residual currents observed in heterozygous expression usually were indistinguishable from wild type, indicating a total loss-of-function in channels that incorporated mutant subunits (Chouabe et al., 1997; Wollnik et al., 1997; Shalaby et al., 1997).

Alternatively, mutations in either α- or β-subunits that produce nearly full length protein (particularly missense or deletions) can inhibit channel function through alterations in gating or conduction (e.g., decreased probability of opening or reduced single channel conductance). Relatively few LQT1 mutations fall into this altered-function category. In fact only one mutation (L273F) produced measurable K^+ current when expressed as a homotetramer. Its net effect in heteromeric expression with wild-type subunits was to reduce current

Table 2. KvLQT1 (LQT1) mutations

Location	Mutations
Transmembrane segments S1-S6	F167W/ΔG168, G269D, G325R, ΔF339, A341E, A341V, L342F, sp/A344, G345E, R366P
Intracellular loops	R174C, A178P/T, G189R, fs/R190, R190Q, V254M, L273F
Pore region	A300T, W305S, G306R, sp/V307, T312I, I313M, G314S, Y315S, D317N
C-terminus (intracellular)	fs/P447, fs/Q484, fs/Q544, R555C, T587M, R591H, fs/Q631, fs/P630

The codon numbering system is for the full-length human cardiac KvLQT1 clone (Yang et al., 1997). Missense mutations resulting in single substitutions are abbreviated using standard notation (wild-type amino acid residue followed by codon number and substituted residue). F168W/ΔG169 is a 3 base-pair deletion that results in both a substitution (F168W) and a deletion (ΔG169). Fs/R190, Fs/P447, Fs/Q484, Fs/Q544, Fs/Q632, Fs/P631 are insertion or deletions at the indicated codon that result in a frame shift and premature termination. Sp/V307 is a 3 base-pair deletion (ΔGGT) in the pore region that disrupts a splice-donor sequence in the pore region and causes premature termination. Sp/A344 is a single nucleotide substitution (g→a) that disrupts a splice-donor sequence in S6 and causes premature termination. ΔF339 is a 3 base-pair deletion that results in a deletion of Phe^{339} (Russell et al. 1996; Wang et al., 1996*b;* Donger et al., 1997; Neyroud et al., 1997; Tanaka et al. 1997; Ackerman et al., 1998; Kanters et al., 1998; Li et al., 1998; Priori et al. 1998; Saarinen et al., 1998; Neyroud et al., 1999).

levels, but the mechanism is unclear since gating and single channel conductance were not fully examined (Shalaby et al., 1997). Another mutation, R555C in the C-terminus, expressed current as a homotetramer but only when minK was present. Moreover, because activation required depolarizations 50 mV greater than in wild type (Chouabe et al., 1997), the mutant channels would be unlikely to open in the physiological range of potentials. The coexpression of wild-type and R555C produced channels with a 30 mV shift of the activation-voltage relationship. Thus, R555C is one of the few LQT1 mutations that has been reported to influence the gating properties of the KvLQT1 channel. The Arg^{555} residue is located near the end of the intracellular C-terminal tail in a region that has not previously been identified as a gating determinant. Furthermore, regulatory minK subunits that markedly alter KvLQT1 gating (Barhanin et al., 1996; Sanguinetti et al., 1996*b*), do not interact strongly with this region (Romey et al., 1997). Therefore, the C-terminus of KvLQT1 may play a novel role in regulating I_{Ks} gating.

Another KvLQT1 mutation that results in altered function, A300T, is unusual because it causes LQTS only in homozygous individuals. In *Xenopus* oocytes A300T when coexpressed with minK, produces currents levels reduced by 75% compared to wild type (Priori et al., 1998). The mechanism is unclear but cannot be attributed to altered voltage dependence of activation since a negative shift was observed. Reduced single channel is a possibility since Ala^{300} occupies a position in the pore-lining region. Compared to the loss of function produced by other mutations, the relatively mild effects of A300T are consistent with a recessive pattern of inheritance.

Several mutations in the auxiliary minK subunit, (LQT5), have been linked to both RWS and JLNS (Tyson et al., 1997; Splawski et al., 1997; Schulze-Bahr et al., 1997; Duggal et al., 1998). The minK protein, unlike its KvLQT1 partner, is a small polypeptide of 130 amino acid residues with only a single putative transmembrane segment (Takumi et al., 1988). It is encoded by the KCNE1 gene located on chromosome 21q22 (Chevillard et al., 1993). MinK exerts a regulatory influence through protein-protein interactions between the intracellular C-terminus of minK and the pore region of KvLQT1 (Romey et al., 1997) such that minK forms an integral part of the KvLQT1 pore (Wang et al., 1996*a*; Tai & Goldstein, 1998). Complementation of KvLQT1 pore structure by minK residues would be expected to have profound effects on both ion conduction and gating by analogy with other Kv channels (Debiasi et al., 1993; Heginbotham et al., 1994). Indeed, heterologous coexpression of KvLQT1 with minK has multiple effects including: a twofold increase in the amplitude of the macroscopic K^+ currents, an increase in single channel conductance, a slower time course of activation and complete removal of inactivation (Barhanin et al., 1996; Sanguinetti et al., 1996*b;* Pusch, 1998; Sesti & Goldstein, 1998; Tristani-Firouzi & Sanguinetti, 1998).

Although the regulatory effect of minK is complicated, mutations in KCNE1 would be likely to reduce the level of expression of I_{Ks} in the heart. For instance, a D76N mutation associated with both JLNS and RWS (Splawski et al., 1997; Schulze-Bahr et al., 1997; Duggal et al., 1998) and a S74L mutation associated with RWS (Splawski et al., 1997), both located in the cytoplasmic C-terminus, have been characterized in the *Xenopus* oocyte system (Splawski et al., 1997; Sesti & Goldstein, 1998). Co-injection of cRNA encoding the D76N minK mutant with KvLQT1 has been reported to produce either no expression of exogenous ionic current (Splawski et al., 1997) or channels with markedly reduced single channel conductance (Sesti & Goldstein, 1998). Both results are consistent with a strong dominant negative effect. To mimic the heterozygous condition, mixtures of D76N mutant, wild-type minK and KvLQT1 were co-injected (Splawski et al., 1997; Sesti & Goldstein, 1998). This combination expressed currents with reduced single channel amplitude and with gating kinetic parameters shifted in the depolarizing direction. Both effects would tend to reduce macroscopic currents. Co-injection of the S74L minK mutant with KvLQT1 had similar effects on both single channel conductance and

gating. These mutations suppress macroscopic I_{Ks} by changing both the gating and conductance properties of the channels without eliminating the ability of mutant minK subunits to coassemble with KvLQT1.

A double mutation resulting in the substitutions T59P and L60P has been identified in a JLNS family (Tyson et al., 1997). The functional effects of these mutations are not known but would be predicted to render minK nonfunctional and unable to coassemble with KvLQT1 on the basis of their location in the putative helical transmembrane domain and the helix-disruptive nature of the proline substitutions.

Defects in a Fast Delayed Rectifier K Channel Cause LQT2

LQTS was known to be genetically heterogeneous before additional loci were identified (Curran et al., 1993; Towbin et al., 1994). In 1995, Curran and colleagues showed linkage to chromosome 7q35-36 in six LQT families and identified several mutations in the HERG (human ether-a-go-go related) gene that encodes another cardiac K^+ channel α-subunit. This second form of congenital LQTS is denoted as LQT2.

HERG was originally cloned from a human hippocampal cDNA library (Warmke & Ganetzky, 1994) and identified as a Kv channel of the 6-transmembrane type by homology with the *Drosophila* gene (*eag*) that encodes a Kv channel (Bruggemann et al., 1993). HERG mRNA is expressed at high levels in the heart (Curran et al., 1995) and heterologous expression yields a K^+ current whose biophysical and pharmacological characteristics are very similar to I_{Kr}, the rapidly activating component of the delayed rectifier K^+ current in the heart (Trudeau et al., 1995; Sanguinetti et al., 1995). Like I_{Kr}, HERG-expressed currents are blocked by the class III antiarrhythmic agent, dofetilide (Spector et al., 1996*a*) and show an unusual current-voltage relationship typified by inward rectification (i.e., more efficient conduction of inward than of outward currents) due to rapid inactivation upon depolarization (Spector et al., 1996*b;* Smith et al., 1996). The structural basis of this form of rapid inactivation, however, is unlike that of the well-studied N-type inactivation of *Drosophila* Shaker K^+ channels in which the peptide residues in the amino terminus act as blocking particles that occlude the intracellular mouth of the pore (Hoshi et al., 1990). In fact, N-terminal deletion in HERG does not eliminate inactivation (Schonherr & Heinemann, 1996; Spector et al., 1996*b*). Instead, inactivation is highly sensitive to mutation of residues in the pore-lining region (Smith et al., 1996; Schonherr & Heinemann, 1996; Herzberg et al., 1998). Thus, LQT2 mutations, especially those in the pore that affect both gating and ion conduction, can drastically alter the fundamental current-voltage relationship of I_{Kr}.

Despite the qualitative similarities with I_{Kr}, HERG currents activate and deactivate at slower rates than I_{Kr} in native myocardial cells (Sanguinetti & Jurkiewicz, 1990; Yang et al., 1994). This has raised questions about the molecular identity of I_{Kr} and the possibility of alternate isoforms or auxiliary subunits. Since HERG was first isolated from brain, a search for cardiac-specific isoforms has been mounted. Two alternatively processed, cardiac-specific isoforms have been identified: a splice variant that has a much shorter intracellular amino terminus than HERG (London et al., 1997; Lees-Miller et al., 1997) and a splice variant with truncated intracellular carboxyl terminus (Kupershmidt et al., 1998). When coexpressed with HERG or by itself, the truncated N-terminus isoform displays faster deactivation gating kinetics (Lees-Miller et al., 1997; London et al., 1997). The C-terminus variant did not express current by itself but upon coexpression with full-length HERG produced currents with accelerated activation kinetics. In heterologous systems, coexpression produced currents that more closely approximated I_{Kr} and, therefore heteromers of full-length and truncated subunits may form the channels responsible for I_{Kr} in vivo. Thus far, functional analysis of LQT2 mutations has been performed only in homomeric, full-length HERG channels. Nonetheless, these results together with analysis of artificial terminal deletion constructs of HERG (Spector et al., 1996*a;* Schonherr & Heinemann, 1996) indicate important roles for both amino and carboxyl termini in the regulation of HERG gating.

In principle, LQT2 mutations that suppress the rise in potassium conductance mediated by I_{Kr} would be expected to slow phase 3 repolarization and prolong the QT interval. This could be achieved either by decreasing the number of functional channels or by impairing the gating mechanism that opens them. Phenotypic analysis of mutated channels expressed in heterologous systems has been used to characterize about half of the known LQT2 mutations. In nearly all cases (Table 3) expression of the mutant subunit by itself produced no detectable ionic current. But when coexpressed with wild-type subunits either suppression (dominant negative effect) or no effect on wild-type function was observed. Underlying mechanisms include defects in protein trafficking resulting in failure of the channels to reach the surface membrane (e.g., Y611H and V822M; Zhou et al., 1998), failure of mutant channels to coassemble with wild-type (e.g., fs/421, ΔI500-F508, Sanguinetti et al., 1996*a*), or increased levels of inactivation in heteromeric channels (e.g., A614V and V630L, Nakajima et al., 1998). In some cases, however, the biophysical defect is unclear. For instance, the N470D mutant subunit by itself expresses ionic current but when coinjected with wild type causes a modest reduction in wild-type expression without gross alteration of gating (Sanguinetti et al., 1996*a*). This mu-

Table 3. HERG (LQT2) mutations

Location	Mutations
Transmembrane segments S1-S6	Δbp1261, N470D, ΔI500-F508, A561V, A561I, G572C, N588D
Intracellular loops	T474I
Pore region	I593R, G601S, Y611H, V612L, A614V, G628S, N629D, N629S, V630A, V630L, N633S
Intracellular C-terminus	sp/intron 3, V822M

ΔBp1261 is a single base pair deletion that causes a frame shift and premature termination in the S1 segment. ΔI500-F508 is a deletion of nine amino acid residues in the S3 segment. Sp/intron 3 is a single base substitution that disrupts a splice-donor sequence in intron 3 and alters the C-terminus (Curran et al., 1995; Benson et al., 1996; Dausse et al., 1996; Nakajima et al., 1998; Satler et al., 1998; Splawski et al., 1998).

Table 4. SCN5A mutations (LQT3 and IVF)

Location	Mutations (disease)
Transmembrane S1-S6, repeats I-IV	sp/intron 7 (IVF), R1623Q (LQT3), R1644H (LQT3)
Pore region	fs/1398 (IVF)
Extracellular loops	R1232W+T1620M (IVF)
Intracellular loops	N1325S (LQT3), ΔK1505-Q1507 (LQT3)
Intracellular C-terminus	D1790G

Sp/intron 7 is a two base-pair insertion that disrupts a splice-donor site in intron 7 and predicts truncation of the polypeptide in the S2-S3 linker of repeat I. R1232W+T1620M is a double missense mutation. Fs/1398 is a single base-pair deletion in codon 1398 that causes a frameshift and premature termination in the pore region of repeat III. ΔK1505-Q1507 is a deletion of three residues in the intracellular linker between repeats III and IV. LQT3 is chromosome 3 linked long QT-syndrome and IVF is the Brugada variant of idiopathic ventricular fibrillation (Wang et al., 1995*a,b;* Yamagishi et al., 1997; Benhorin et al., 1997; Chen et al., 1998; Makita et al., 1998).

tation is unlikely to reduce single channel conductance given its location far from the pore region, but a subtle effect on gating that reduces the probability of channel opening is possible. A nearby mutation T474I is more problematic because of disagreements in published data. Nakajima et al. (1998) reported that when tested in *Xenopus* oocytes T474I did not express current whereas transfection of the same construct in HEK 293 cells was reported to express current with altered gating properties (Zhou et al., 1998). However, the alteration was in the form of a large hyperpolarizing shift in the voltage dependence of activation that would be expected to increase rather than suppress K^+ currents. Disagreements also exist in reports on the fs/421 mutation. This mutation produces a nonfunctional truncated polypeptide that has no effect when coinjected with wild type in *Xenopus* oocytes (Sanguinetti et al., 1996*a*), but produces a modest dominant negative effect when cotranfected with wild type in COS cells (Li et al., 1997). Whether these discrepancies are due to different expression systems remains to be determined.

Na Channel Defects Cause LQT3

An increase in action potential duration by prolonging the phase 2 plateau can be achieved through augmentation of inward currents carried by voltage-gated Ca^{2+} or Na^+ channels. Na^+ channel involvement derives from a late, slowly inactivating, tetrodotoxin-sensitive component of depolarizing current that helps maintain the phase 2 plateau (Kiyosue & Arita, 1989). Pharmacological agents that slow down Na^+ inactivation (e.g., anthopleurin A) mimic some aspects of LQTS (e.g., EADs and delayed repolarization) in ventricular muscle (Shimizu et al., 1979). To date no involvement of Ca^{2+} channel genes in LQTS has been found. But in a small number of families LQTS has been linked to SCN5A, the gene that encodes the cardiac Na^+ channel. This form of the disease is referred to as LQT3. In animal models, Na^+ channel blockade by tetrodotoxin at submaximal concentrations shortens the duration of the action potential (Attwell et al., 1979; Coraboeuf et al., 1979) by inhibiting a persistent inward current in Na^+ channels that do not completely inactivate (Table 4). Although small in magnitude, this persistent depolarizing current could have a pronounced effect on the action potential plateau since membrane input impedance is elevated during this phase.

In all cases thus far, LQT3 mutations have been shown to affect the inactivation gating mechanism. The most common biophysical defect is a marked increase in persistent current that, at the single channel level, appears in two forms: brief, dispersed reopenings that probably arise from decreased stability of the inactivated state (N1325S, R1644H, and ΔK1505-Q1507 mutants; Dumaine et al., 1996; Chandra et al., 1998), and prolonged bursts of openings that probably result from a modal switch in gating (ΔK1505-Q1507 mutant; Bennett et al., 1995). Wild-type channels can switch from a normal mode of gating in which brief openings are terminated by entry into an absorbing inactivated state, to an inactivation-deficient mode in which long openings and repetitive reopenings cluster to form long bursts. Mode switching is extremely infrequent in wild-type channels but increases markedly in the ΔK1505-Q1507 mutant (Bennett et al., 1995). The mechanism of mode switching and its regulation are unclear. A similar phenomenon has been observed in skeletal muscle Na^+ channels (SCN4a) where the absence of a β_1 subunit can slow the time course of inactivation and promote single channel bursting (Bennett et al., 1993). This is unlikely to be the mechanism for cardiac Na^+ channels where the β_1 subunit, although present in native tissue, has little effect on

macroscopic inactivation in heterologous expression (Makita et al., 1994). A brain Na^+ channel isoform, SCN8a has been found to generate persistent current in the presence of β_1 subunits (Smith et al., 1998) but its primary structure shows none of the amino acid substitutions corresponding to LQT3 mutations.

Two additional LQT3 mutations have been uncovered that have different biophysical actions. R1623Q was identified as a *de novo* mutation located in the S4 transmembrane segment of domain 4, associated with an especially severe form of LQTS (Yamagishi et al., 1997). Electrophysiological analysis revealed no persistent current, but rather a marked slowing of inactivation (Kambouris et al., 1998; Makita et al., 1998). The corresponding position, R1448, in the SCN4a skeletal muscle isoform, when mutated to Cys or His in some forms of paramyotonia, produces a similar phenotype (Chahine et al., 1994). Based on these and other results the D4/S4 region is believed to be a critical determinant of Na^+ channel inactivation.

Another LQT3 mutation is located in an intracellular C-terminal region not previously correlated with channel function. D1790G has been identified by genetic linkage to LQTS in a single family (Benhorin et al., 1997). Although this residue is highly conserved the mutation does not produce a distinctive phenotype when expressed in human embryonic kidney cells. However, coexpression with β_1 subunits produces a hyperpolarizing shift of the voltage-dependence of steady-state inactivation compared to coexpression of wild-type and β_1 subunits (An et al., 1998). The result is surprising in view of previous structure-function studies indicating that the α/β subunit interaction surface is located in extracellular domains (Makita et al., 1996; McCormick et al., 1998). Also, the relationship of this particular alteration in gating to prolongation of the action potential is unclear; a negative shift of inactivation would tend to suppress slow mode bursting (Dumaine & Kirsch, 1998). The influence of this mutation on cardiac excitation is therefore likely to be indirect.

A Form of Idiopathic Ventricular Fibrillation (IVF) Involves Na Channels

Spontaneous ventricular fibrillation in the presence of normal baseline QT intervals and in the absence of structural heart disease also may be associated with ion channel abnormalities. One form of IVF, Brugada syndrome (Brugada & Brugada, 1992), is characterized by an ECG pattern resembling right bundle branch block with elevated ST segment (sometimes referred to as a prominent "J-wave"; Bjerregaard et al., 1994) and a familial history of sudden cardiac death. In a canine model the J-wave ECG morphology is associated with heterogeneity in the expression of I_{to1} the K^+ current that regulates phase 1 action potential repolarization, in different cell layers of the ventricular myocardium. Notably blockade of I_{to1} by 4-aminopyridine reduces the heterogeneity and the amplitude of the J-wave (Yan & Antzelevitch, 1996), suggesting a K^+ channel abnormality. However, genetic linkage analysis of six Brugada syndrome families has identified three different Na^+ channel (SCN5A) mutations (Chen et al., 1998). Two of the mutations (Table 4) cause premature termination of the α-subunit either in transmembrane domains and would be expected to cause loss of function (Stühmer et al., 1989). A third case was linked to a double missense mutation that resulted in substitution of Trp and Met for Arg^{1232} and Thr^{1620}, respectively. Thr^{1620} is a highly conserved residue located in a region previously identified with regulation of Na^+ channel inactivation (Chahine et al., 1994) and, indeed the T1620M mutation caused a depolarizing shift in the voltage-dependence of inactivation and an acceleration of recovery from inactivation. The R1232W mutation appears to be a benign polymorphism (Chen et al., 1998). The arrhythmogenic potential of these changes may be related to the theory that antiarrhythmic class Ib drugs such as lidocaine act by producing the opposite effect, i.e., a negative shift in the availability versus membrane potential curve and a slowing of the time course of recovery. However, the correlation of this biophysical defect in the Na^+ channel with the ECG characteristics of IVF is obscure (Yan & Antzelevitch, 1996). Moreover, the relationship of the very different loss-of-function and altered-function mutations to the clinical pattern of Brugada syndrome remains to be established. The involvement of other ion channel genes would seem likely since some of the families were not linked to SCN5A.

Animal Models are Lacking

Although many of the mutations associated with LQTS have been characterized in heterologous expression systems, direct verification that mutant channels alter the excitability of cardiac myocytes or change the ECG in whole animals has been much more difficult to obtain. Transgenic mice have been used to overexpress dominant negative cardiac K^+ channel subunit fragments that selectively suppress expression of normal K^+ currents and therefore inhibit repolarization in a manner similar to that of naturally occurring LQT1 and LQT2 mutations in humans. Several K^+ channel subunits have been targeted using this approach including minK (LQT5), HERG (LQT2), Kv1.5 (a component of the ultra-rapid delayed rectifier, I_{Kur}, thus far not been implicated in LQTS), and Kv4.3 (a component of I_{to1} not implicated in LQTS). The results have been both intriguing, by providing new insights, and perplexing because of limitations of the mouse model.

Direct attempts to introduce LQT2 and LQT5 mutations in mouse heart have been successful in confirming dominant negative and loss-of-function mechanisms suspected from heterologous expression systems, but have not produced an accurate electrophysiological model of human LQTS. In part this is due to the markedly different characteristics of K^+ currents in mouse and human hearts. Because the basal heart rate in mice is at least five times faster than in humans, K^+ channels with very fast activation kinetics (I_{Kur} and I_{to1}) dominate the repolarization phase in mouse. Therefore genetic manipulation of the slower I_{Kr} and I_{Ks} channel components that play an important role in human disease, may have little effect on rhythmicity and ECG waveform in the mouse. This expectation has been fulfilled in several transgenic studies. For instance, Babij and colleagues (1998) overexpressed the HERG G638S mutation that is associated with LQT2 in humans. G638S exerts a strong dominant negative influence based on *Xenopus* oocyte expression (Sanguinetti et al., 1996*a*) and overexpression of G628S subunits in mouse heart eliminated I_{Kr} and prolonged the action potential in isolated myocytes at room temperature. But the transgenic animals showed no change on action potential duration in intact ventricular tissue at physiological temperature, and no QT interval prolongation in vivo (Babij et al., 1998). Similarly, ventricular repolarization was not affected in a minK (KCNE1) knockout mouse model (Charpentier et al., 1998) although inner ear defects similar to those associated with human Jervell-Lange-Nielsen syndrome were detected (Vetter et al., 1996). By contrast, dominant negative suppression of mouse genes that encode the α-subunits of K^+ channels associated with either I_{To} (Kv4.2/4.3, Barry et al., 1998) or I_{Kur} (Kv1.5, London et al., 1998), produced a marked QT interval prolongation that closely correlated with reductions in fast K^+ currents and protein subunit expression. These results clearly illustrate the power of transgenic models for analyzing the functional heterogeneity that underlie adaptation of cardiac K^+ currents to different physiological situations. Thus far no transgenic Na^+ channel manipulations that might provide more accurate models of LQT3 have been reported.

Conclusion

Genetic linkage analysis together with advances in ion channel structure-function analysis have opened new avenues for understanding the complex relationships between cardiac ionic currents and life-threatening arrhythmia. The advances have been particularly well developed in regard to repolarization abnormalities such as those associated with long QT syndrome and have highlighted the importance of HERG, KvLQT1, minK and SCN5A genes in this form of arrhythmia. However, a substantial gulf still exists between the molecular and the whole animal levels. A particularly difficult problem is the correlation of biophysical defects, that can be characterized in exquisite detail at the molecular level, with complex characteristics of arrhythmias observed at higher tissue and organ levels. Development of accurate animal models as well as more extensive correlations between genotype, molecular phenotype and clinical observations will be required to exploit these new avenues.

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