11

Cardiac Channelopathies: Disease at the Tip of a Patch Electrode

Brian P. Delisle
University of Kentucky
USA

1. Introduction

The ordered electrical excitation of the heart via the cardiac conduction system coordinates the efficient pumping of blood. Electrical impulses normally originate in the sinoatrial node and then propagate through the atria, atrioventricular node, and into the ventricles. The electrical activity of the heart can be measured non-invasively using an electrocardiogram (ECG). An ECG reflects the summed electrical activity of the individual contractile myocytes in the different regions of the heart over time. The first event is the "P wave" and corresponds to the depolarization of the atria, the next three waves "Q, R, and S" represent the progressive wave of depolarization through the ventricles, and the final wave of the cardiac cycle "T" corresponds to ventricular repolarization. The action potentials of contractile atrial and ventricular myoctes share several common characteristics: a stable resting or diastolic membrane potential (phase 4), a rapid depolarization (phase 0), an initial repolarization (phase 1), a plateau (phase 2), and a rapid repolarization to diastolic potential (phase 3). The different phases of the cardiac action potential reflect changes of the sarcolemma permeability to K^+, Na^+, and Ca^{2+}. Figure 1 shows the relationship between the ECG, a ventricular action potential, and several of the important ventricular K^+, Na^+, and Ca^{2+} currents (Nerbonne and Kass, 2005).

Arrhythmias are electrical disturbances that disrupt the normal initiation or propagation of the cardiac impulse. They cause abnormal impulse rates (bradycardia or tachycardia), block impulse propagation, or initiate the impulse to circle in a "reentry" loop. Atrial arrhythmias can result in the formation of blood clots and increase the risk of stroke, whereas ventricular arrhythmias can cause the inefficient pumping of blood, loss of consciousness, and sometimes death (Shah et al., 2006).

"Cardiac channelopathies" are caused by mutations in ion channel genes that encode the proteins that underlie the K^+, Na^+, or Ca^{2+} currents that shape the cardiac action potential (Figure 1). These mutations can cause syndromes that increase the risk for atrial and/or ventricular arrhythmias. Cardiac channelopathies typically cause a "gain- or loss-of-function" in one of these currents by altering channel synthesis, channel transport to and from the cell surface membrane (trafficking), channel gating, and/or single channel permeation (Figure 2) (Delisle et al., 2004). This chapter provides a description for several different cardiac syndromes that can be caused by channelopathies and the utilization of the patch-clamp technique to learn about functional phenotypes.

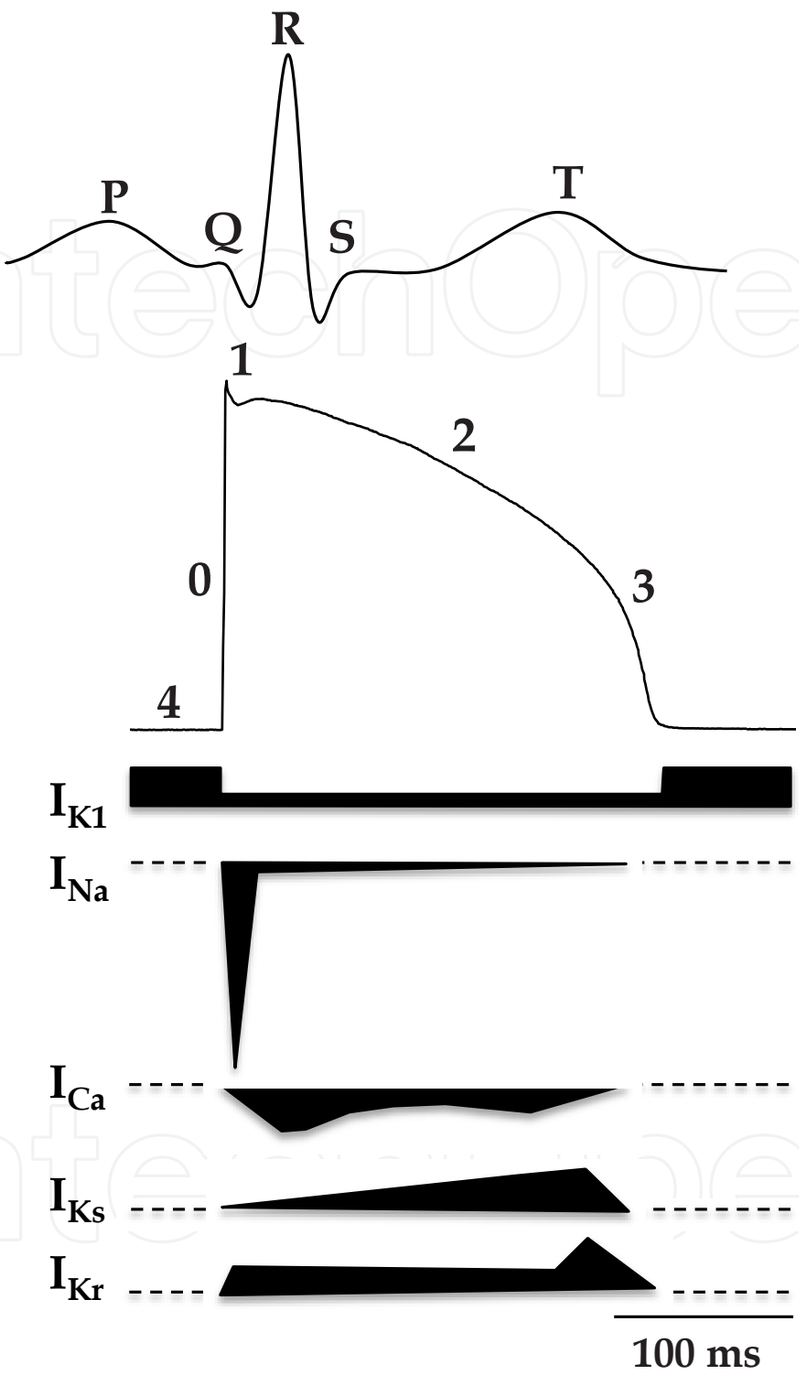


Fig. 1. The relationship between an ECG trace, a ventricular action potential, and important K^+, Na^+, and Ca^{2+} currents. The P, Q, R, S, T correspond to the different waves on the ECG, the 4, 0, 1, 2, 3 correspond to the different phases of the ventricular action potential. I_{K1} is the inwardly rectifying K^+ current, I_{Na} represents the Na^+ current, I_{Ca} represents the Ca^{2+} current, I_{Ks} is the slowly activating delayed rectifier K^+ current, and I_{Kr} is the rapidly activating delayed rectifier K^+ current.

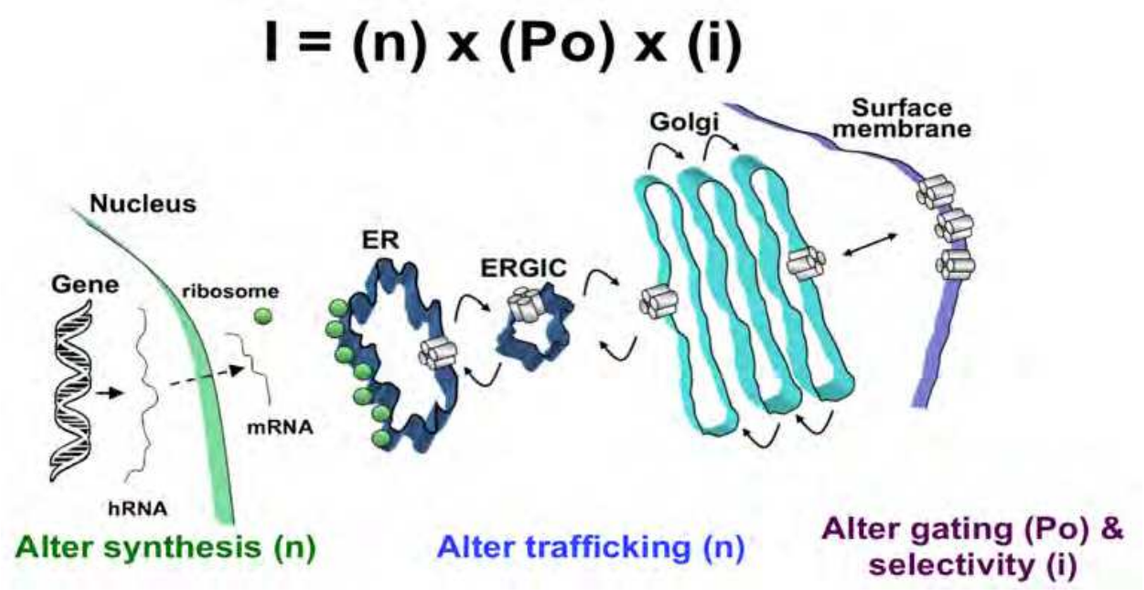


Fig. 2. A *cartoon* showing the synthesis, trafficking, and surface expression of ion channels. Macroscopic current (I) is a function of channel number at the cell surface (n), open probability (Po), and the amplitude of the single channel current (i). Cardiac channelopathy mutations can alter n by changing synthesis and trafficking, or they can alter channel function by changing gating and selectivity (hRNA = heterogeneous ribonucleic acid; mRNA = messenger ribonucleic acid; ER = Endoplasmic Reticulum; ERGIC = ER Golgi Intermediate Compartment).

2. Cardiac channelopathies

Hundreds of different mutations in over a dozen different genes that encode cardiac K^+, Na^+ and Ca^{2+} channels or their regulatory proteins are linked to several different congenital arrhythmia syndromes including Long QT syndrome, Short QT syndrome, Brugada Syndrome, and familial Atrial Fibrillation (Lehnart et al., 2007).

2.1 Long QT syndrome

Congenital Long QT syndrome (LQT) is typically characterized by an abnormally long corrected QT (QTc) interval on an ECG. It is one of the most common "monogenic" arrhythmia syndromes and occurs in ~1:2,500 healthy births (Lehnart et al., 2007; Crotti et al., 2008). LQT-linked mutations are postulated to cause a prolongation of the plateau phase of the ventricular action potential. This allows L-type Ca^{2+} channels to recover from inactivation and reopen, which leads to an early after depolarization. Early after depolarizations increase risk for polymorphic ventricular tachycardia (torsade de pointes), which can cause a loss of cardiac output, syncope, and sudden death. The majority of LQT-linked mutations follow a dominant inheritance pattern (Romano-Ward type of LQT), and it is linked to at least thirteen different genes (LQT1-LQT13; Table 1) (Delisle et al., 2004; Ruan et al., 2008); however, autosomal-recessive forms, Jervell and Lange-Nielsen syndrome (JLN) exist and are caused by homozygous or compound mutations in *KCNQ1* and *KCNE1*. Most LQT-linked mutations cause a prolongation of the ventricular action potential

duration by either decreasing cardiac K^+ currents, or increasing the "late" Na^+ or Ca^{2+} current. LQT1-3 account for 70-75% of all LQT cases, and the other 9 forms contribute an additional 5% (Kapa et al., 2009). The types of LQT mutations include splice, nonsense, frameshift, deletions, and missense, the latter type represents ~70% of the case-linked mutations.

Disease	Current	Chromosome	Defective gene (protein)	Frequency	Key reference
LQT1	I_{Ks}, ↓ amplitude	11p15.5	*KCNQ1* (Kv7.1)	~30-35%	Wang, et al., 1996
LQT2	I_{Kr}, ↓ amplitude	7q35-q36	*KCNH2, hERG* (Kv11.1)	~30-35%	Curran et al., 1995
LQT3	I_{Na}, ↑ late amplitude	3p22.2	*SCN5a* (Nav1.5)	~7-10%	Wang et al., 1995
LQT4	cell Ca^{2+}	4q25-4q26	*ANKB*	Rare	Schott et al., 1995, Mohler et al., 2003
LQT5	I_{Ks}, ↓amplitude	21q22.1-22.2	*KCNE1* (MiNK1)	Rare	Splawski et al., 1997
LQT6	I_{Kr}, ↓ amplitude	21q22.11	*KCNE2* (MiRP1)	Rare	Abbott et al., 1999
LQT7	I_{K1}, ↓ amplitude	17q23-17q24.2	*KCNJ2* (Kir2.1)	Rare	Tristani-Firouzi et al., 2002; Andelfinger et al., 2002.
LQT8	I_{Ca}, ↑ amplitude	12p13.33	*CACNA1C* (Cav1.2)	Rare	Splawski et al., 2005
LQT9	I_{Na}, ↑ late current	3p25	*CAV3* (caveolin 3)	Rare	Vatta et al., 2006
LQT10	I_{Na}, ↑ late current	11q23	*SCN4B*	Rare	Medeiros-Domingo et al., 2007
LQT11	I_{Ks}, ↓ amplitude	7q21-q22	*AKAP9* (Yotiao)	Rare	Chen et al., 2007
LQT12	I_{Na}, ↑ late amplitude	20q11.2	*SNTA1* (syntrophin-α1)	Rare	Ueda et al., 2008
LQT13	I_{KATP}, ↓ amplitude	11q24.3	*KCNJ5* (Kir3.4)	Rare	Yang et al., 2010
JLN1	I_{Ks}, ↓ amplitude	11p15.5	*KCNQ1* (Kv7.1)	Rare	Neyroud et al., 1997
JLN2	I_{Ks}, ↓ amplitude	21q22.1-22.2	*KCNE1* (MiNK1)	Rare	Schultze-Bahr et al., 1997

Table 1. Long QT syndrome disease types. Autosomal dominant forms (LQT1-12) include Romano Ward Syndrome (LQT1-6), Anderson Tawill Syndrome (LQT7), and Timothy Syndrome (LQT8). The autosomal recessive form is Jervell Lange-Neilson syndrome (JLN1-2) and is associated with deafness.

2.2 Short QT syndrome

Short QT syndrome (SQT) is characterized by a short QTc interval and can cause syncope, paroxysmal atrial fibrillation, and ventricular fibrillation (Lehnart et al., 2007; Patel et al.,

Disease	Current	Chromosome	Defective gene (protein)	Key reference
SQT1	I_{Kr}, ↑ amplitude	7q35-q36	*KCNH2, hERG* (Kv11.1)	Brugada et al., 2004
SQT2	I_{Ks}, ↑ amplitude	11p15.5	*KCNQ1* (Kv7.1)	Bellocq et al., 2004 Hong et al., 2005
SQT3	I_{K1}, ↑ amplitude	17q23-17q24.2	*KCNJ2* (Kir2.1)	Priori et al., 2005
SQT4	I_{Ca}, ↓ amplitude	12p13.33	*CACNA1C* (Cav1.2)	Antzelevitch et al., 2007
SQT5	I_{Ca}, ↓ amplitude	10p12.33-p12.31	*CACNB2* (Cavβ2)	Antzelevitch et al., 2007

Table 2. Short QT syndrome disease types.

2010). Similar to LQT, SQT follows a dominant inheritance pattern, but it is extremely rare and only a small number of families with missense mutations have been identified. SQT mutations are predicted to shorten the ventricular action potential duration by increasing cardiac K^+ currents or decreasing Ca^{2+} current.

2.3 Brugada syndrome

Brugada syndrome (BrS) is characterized by ST segment elevation, conduction abnormalities (prolonged PR or QRS segments), and an increased risk of ventricular fibrillation (Lehnart et al., 2007). The prevalence of Brugada syndrome is slightly more common than LQT at ~1:2,000. The ECG changes are not always obvious but they can be usually seen after the administration of drugs that block the cardiac Na^+ current. The majority of BrS patients have missense, nonsense, deletion, or insertion mutations in *SCN5A* that cause a reduction in Na^+ current (Table 3). There appears to be much more genetic heterogeneity associates with Brugada syndrome, because ~2/3^{rds} of the patients do not have a clear genotyped link. BrS-related cardiac events occur more often in adult men (this is likely because of gender differences in cardiac ion channel expression and hormone levels).

Disease	Current	Chromosome	Defective gene (protein)	Frequency	Key reference
BrS1	I_{Na}, ↓ amplitude	3p22.2	*ScN5a* (Nav1.5)	~10-30%	Chen, et al., 1998
BrS2	I_{Na}, ↓ amplitude	3p22.3	*GPDL1* (GPDL1)	Rare	London et al., 2007
					Van Norstrand et al., 2007
BrS3	I_{Ca}, ↓ amplitude	12p13.33	*CACNA1C* (Cav1.2)	Rare	Antzelevitch et al., 2007
BrS4	I_{Ca}, ↓ amplitude	10p12.33-p12.31	*CACNB2* (Cavβ2)	Rare	Antzelevitch et al., 2007
BrS5	I_{Na}, ↓ amplitude	19q13.12	*SCN1B* (Navβ1)	Rare	Watanabe et al., 2008
BrS6	I_{TO}, ↑ amplitude	11q13.4	*KCNE3* (MiRP2)	Rare	Delpon et al., 2008
BrS7	I_{Na}, ↓ amplitude	11q24.1	*SCN3B* (Navβ3)	Rare	Hu et al., 2009
BrS8	I_F, ↑ amplitude	15q24.1	*HCN4* (HCN4)	Rare	Ueda et al., 2009
BrS9	I_{Ca}, ↓ amplitude	7q21.11	*CACNA2D1* (α2ζ)	Rare	Burashnikov et al., 2010

Table 3. Brugada syndrome disease types.

2.4 Familial Atrial Fibrillation

The most common arrhythmia is Atrial Fibrillation (AF), and it is usually secondary to structural changes in the heart (i.e. organ heart disease, valvular disease, hypertensive heart disease, cardiac hypertrophy, etc.). In some instances AF in the absence of structural heat disease (lone AF) occurs, and it is typically caused by genetic factors. Mutations in ion channel genes linked to familial forms of AF have been identified, but these mutations in these genes only account for a small number of cases (Lehnart et al., 2007).

Disease	Current	Chromosome	Defective gene (protein)	Key reference
ATFB3	I_{Ks}, ↑amplitude	11p15.5	*KCNQ1* (Kv7.1)	Chen et al., 2003
ATFB4	I_{Ks}, ↑amplitude	21q22.11	*KCNE2* (MiRP1)	Yang et al., 2004
ATFB7	I_{Kur}, ↓ amplitude	12p13.32	*KCNA5* (Kv1.5)	Olson et al., 2006 Yang et al., 2009
ATFB9	I_{K1}, ↑ amplitude	17q24.3	*KCNJ2* (Kir2.1)	Xia et al., 2005
ATFB10	I_{Na}, ↑ or ↓ amplitude	3p22.2	*SCN5a* (Nav1.5)	Darbar et al., 2008 Ellinor et al., 2008 Makiyama et al., 2008 Benito et al., 2008
ATFB11	I_{CNX40}, ↓ amplitude	1q21.2	*GJA5* (connexin 40)	Gollob et al., 2006
ATFB12	I_{KATP}, ↓ ADP current	12p12.1	*ABCC9* (Sur2A)	Olson et al., 2007

Table 4. Familial Atrial Fibrillation syndrome disease types linked to channelopathies.

2.5 Summary

Many different types of cardiac arrhythmias are linked to channelopathies. Thus far, mutations in over 20 different genes that encode cardiac ion channel α-subunits, auxiliary channel subunits, or channel modulatory proteins are linked to one or more arrhythmia syndromes. This genetic heterogeneity has identified ion channel and macromolecular signaling complexes that are important for normal cardiac excitability and arrhythmia susceptibility. Many of these mutations are predicted to increase or decrease the macroscopic K^+, Na^+ or Ca^{2+} currents to increase arrhythmia susceptibility. This occurs by a number of different mechanisms, including alterations in protein synthesis, trafficking to/from the membrane, channel gating, and/or permeation.

3. Patch clamp techniques to study disease mechanisms

This section focuses on using the patch clamp technique to study disease mechanisms linked to the genetic variants in *KCNQ1* or *KCNH2*, which encode the voltage-gated K^+ channel α-subunits that underlie I_{Ks} (Kv7.1) and I_{Kr} (Kv11.1), respectively. Each Kv α-subunit has six transmembrane (TM) segments that form a voltage-sensor (TM1-TM4) and a pore domain (TM5-TM6) (Figure 3).

Mutations in these genes are the most common cause of "monogenic" arrhythmia syndromes (Lehnart et al., 2007; Kapa et al., 2009). *KCNQ1* and *KCHN2* mutations can cause a loss- or gain-of-function (Wang et al., 1996; Neyroud et al., 1997; Chen et al., 2003). Loss of function mutations can be splice-site, nonsense, or frame-shift and result in haploinsufficiency; however, the vast majority of *KCNQ1* and *KCNH2* mutations linked to arrhythmia syndromes are missense. In syndromes that follow a dominant inheritance pattern (i.e. LQT, SQT, familial atrial fibrillation, etc) these mutant α-subunits may co-assemble with wild type to generate functional phenotypes that cause a more severe phenotype (Figure 4).

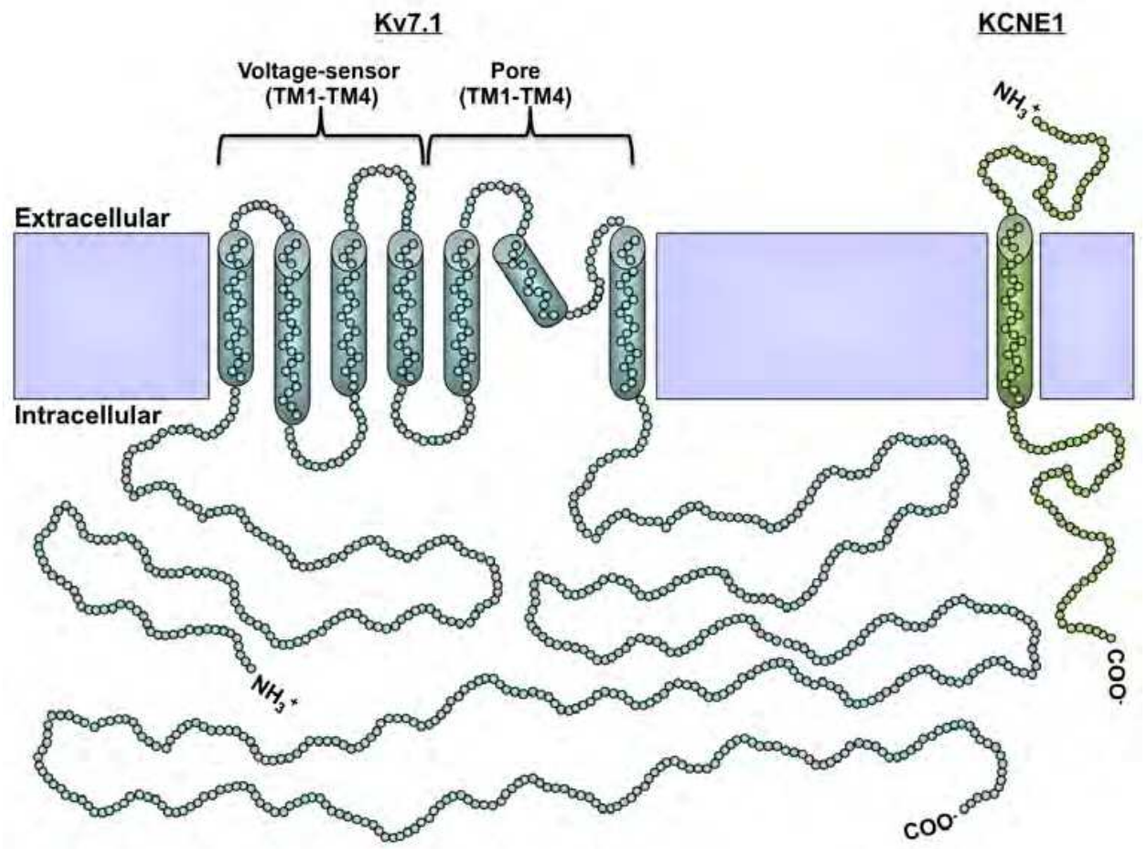


Fig. 3. A *cartoon* of a single Kv7.1 α-subunit and its putative auxiliary subunit KCNE1. The image shows a single Kv7.1 α-subunit and a single KCNE1 subunit. The membrane is represented by the purple rectangles, the individual amino acids are shown as small circles, and the secondary structures (α-helices) are shown as cylinders. The respective amino and carboxy termini are labeled NH_3^+ and COO^-, respectively. Heterolgous expression studies suggest that co-expression of Kv7.1 and KCNE1 are needed to generate macroscopic currents similar to native I_{Ks} in the heart (Barhanin et al., 1996; Sanguinetti et al., 1996).

3.1 Long QT type 1

Thus far, hundreds of different mutations in *KCNQ1* have been linked to LQT1. Kv7.1 tetramerizes to form the aqueous channel of the I_{Ks} in the heart (Kapa et al., 2009; http://www.fsm.it/cardmoc/). Mutations in *KCNQ1* account for ~30-35% of congenital LQT cases (Yuan et al., 2008; Kapa et al., 2009). Heterologous expression studies suggest that many LQT1 mutations throughout Kv7.1 cause a loss-of-function (Wollnik et al., 1997; Neyoud et al., 1998; Saarinen et al., 1998; Yamashita et al., 2001; Pippo et al., 2001; Deschênes et al., 2003; Yamaguchi et al., 2003; Thomas et al., 2005; Thomas et al., 2010).

LQT1 patients exhibit a wide-range of clinical phenotypes from asymptomatic to recurrent episodes of syncope and sudden cardiac arrest, and LQT1-related cardiac events are most often triggered by exercise (Priori et al., 2003; Napolitano et al., 2005; Schwartz et al., 2001; Priori et al., 2004; Ruan et al., 2008). Studies of multi-center LQT1 registries with hundreds

of patients suggest that the risk for LQT1 related cardiac events is related to the type/location of the LQT1 mutation (Zereba et al., 2003; Moss et al., 2007; Goldenberg et al.,

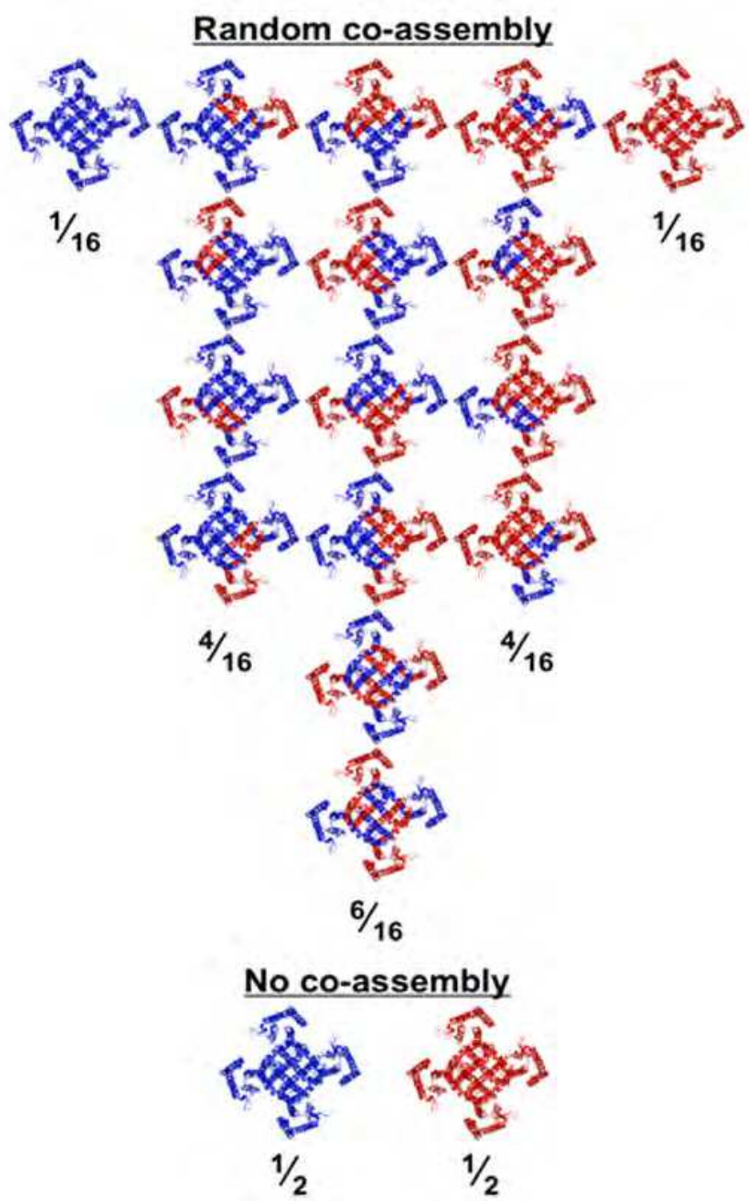


Fig. 4. A *cartoon* for two possible mechanisms of Kv α-subunit co-assembly. The images show Kv tetramers from the "extracellular" view. The first scenario assumes that WT (blue) and mutant (red) Kv α-subunits (blue) co-assemble both equally and randomly so only 1/16th of the total number of channels is all WT or mutant. The other 14/16^{ths} of the channels are a combination of WT and mutant Kv α-subunits with varying stoichometries. If the WT and mutant Kv α-subunits do not co-assemble, then half of the channels are all WT or mutant (January et al., 2000). The structural model for the Kv7.1 α-subunits used to generate the channels shown was developed by Smith and colleagues (2007).

2008; Jons et al., 2009). About 80% of LQT1 mutations are missense, and genetic testing for LQT1 is commercially available, but the diagnostic and prognostic value of a genotype positive test is limited because it does not distinguish between relatively benign or pathogenic variants (Kapa et al., 2009). Current treatments for LQT1 include life-style modification, β-blockers, implantable cardioverter defibrillators (ICDs), and/or left cardiac sympathetic denervation (Ruan et al., 2008). A major challenge for clinicians is to determine which therapeutic strategies are most appropriate for an individual. While many considerations need to be made, *in vitro* patch clamp studies of individual LQT1 mutations will provide a wealth of information, including identifying dominant negative functional phenotypes that may predict a more severe LQT1 clinical phenotype (Moss et al., 2007).

The most common way to determine if LQT1 mutations cause dominant negative suppression is to co-express WT and mutant channels in the heterologous expression systems. Initially a common heterologous expression system was *Xenopus laevis* oocytes. Indeed, the first studies that measured the functionality of LQT1-linked mutation utilized this system with the two-electrode voltage-clamp (Wang et al., 1999; Shalaby et al., 1997). An advantage to the *Xenopus* system is that investigators can control the amount of WT and mutant cRNA injected into cell. Unfortunately *Xenopus* oocytes express a Kv7 isoform that can make experiments with exogenously added Kv7.1/KCNE1 difficult to interpret (Lesage et al., 1993). Additionally, *Xenopus* oocytes are cultured at room temperature and the trafficking of many different LQT-linked mutations are temperature sensitive, which can also complicate the interpretation of the data (Zhou et al., 1999; Ficker et al., 2000; Anderson et al., 2006).

The whole-cell patch clamp technique of transiently expressing mammalian is an effective way to determine whether LQT1 mutations cause dominant negative effects. Mammalian cells can be transfected using chemical or electroporation techniques with Kv7.1 cDNA in plasmid expression vectors that contain WT-Kv7.1 or mutant Kv7.1 constructs. A disadvantage to this technique is that the uptake of the plasmid DNA is uncontrolled. Scenarios that further complicate this is that the cDNA for KCNE1 auxiliary channel subunits is also needed for transfection in order to generate native-like I_{Ks} (Barhanin et al., 1996; Sanguinetti et al., 1996). For co-expression studies that utilize multiple plasmids, WT-Kv7.1 and mutant cDNA plasmids are typically transfected in a 50:50 ratio (to mimic heterozygousity) using the total equivalent cDNA plasmid concentrations as in parallel experiments with WT or mutant only transfections. To favor the selection of cells that express multiple cDNA plasmids, cells are typically co-transfected with a fluorescent protein cDNA expression plasmid (i.e. green fluorescent proteins or GFP). The assumption is that cells expressing the fluorescent protein are likely to express the other plasmids as well.

Bartos and colleagues recently determined the effect of co-expressing WT-Kv7.1 and the LQT1-linked missense mutations E160K-Kv7.1 in Human Embryonic Kidney 293 (HEK293) (Bartos et al., 2011). This mutation has been previously characterized (Silva & Rudy, 2009; Wu D et al., 2010; Silva & Rudy, 2010;). The appropriate nucleotide change to generate the E160K-Kv7.1 mutations was engineered in the WT Kv7.1 (WT-Kv7.1) cDNA subcloned in pcDNA3 mammalian expression vector using the QuickChange Site Directed Mutagenesis

Kit (Stratagene, La Jolla, CA). HEK293 cells were transfected using Superfect (Qiagen Valencia, CA). Cells transfected with WT-Kv7.1 (3 µg) plasmid DNA expressed small currents that did not resemble I_{Ks}, and so to recapitulate I_{Ks}-like currents, cells were also transfected with KCNE1 (3 µg) plasmid DNA (total concentration was 6 µg). The final ratio of KCNE1 to Kv7.1 cDNA ratio is ~2:1. For co-expression studies (to mimic heterozygousity), they transfected KCNE1 (3 µg), WT-Kv7.1 (1.5 µg), and E160K-Kv7.1 (1.5 µg) plasmid DNA. Additionally, They transfected a third set of cells with KCNE1 (3 µg) and E160K-Kv7.1 (3 µg). Cells were also transfected with enhanced GFP cDNA subcloned in pKR5 expression plasmid (0.3 µg). The cells were cultured in Minimum Essential Media (with 10% Fetal Bovine Serum) at 37 ^{o}C in 5% CO_2 and analyzed 24-30 hrs after transfection. Functional analyses were done using standard whole cell patch clamp technique on GFP positive cells. An Axopatch-200B patch clamp amplifier (Axon Instruments, Union City, CA) was used to measure macroscopic currents and cell capacitance. The uncompensated pipette resistances were 1-2 MΩ and series resistance was compensated between up to 95%. pCLAMP 10 software (Axon Instruments, Union City, CA) was used to acquire current signals and Origin (7.0, Microcal, Northhampton, MA) was used for performing data analyses. The external solution contained (in mM) 137 NaCl, 4 KCl, 1.8 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES (pH 7.4 with NaOH), and an internal pipette solution contained (in mM) 130 KCl, 1 MgCl2, 5 EDTA, 5 MgATP, 10 HEPES (pH 7.2 with KOH). Figure 5 shows representative families of whole-cell currents measured from cells expressing KCNE1 with WT-Kv7.1, WT- and E160K-Kv7.1, E160K-Kv7.1. The currents were measured by applying "step" pulses from -80 mV to 70 mV in 10 mV increments for 5 s, followed by a "tail" pulse to -50 mV for 5 s. The intersweep holding potential and duration was -80 mV and 10 s, respectively. Cells expressing WT-Kv7.1 exhibited large macroscopic currents that resembled native I_{Ks}. Cells expressing WT- and E160K-Kv7.1 showed currents with similar characteristics as I_{Ks}, but the amplitudes were smaller than cells expressing WT-Kv7.1. Similar to that previously reported, cells expressing E160K-Kv7.1 did not generate hardly any current at all (Silva et al., 2009; Wu et al., 2010; Silva et al., 2010; Bartos et al., 2010). The mean peak tail current

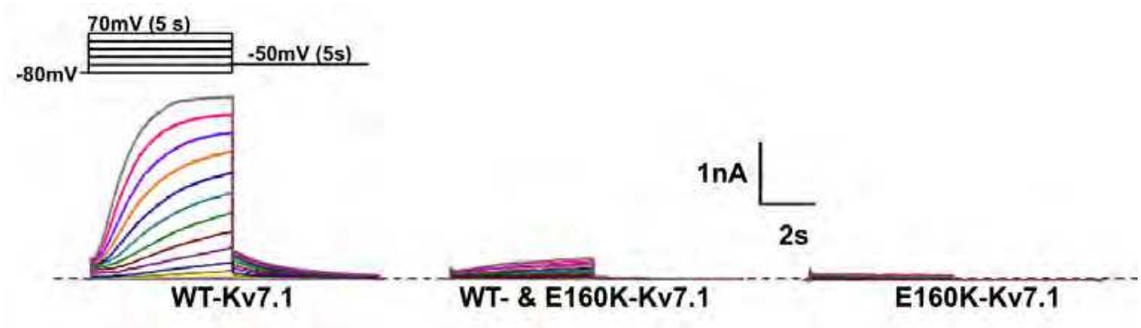


Fig. 5. The LQT1-linked mutation E160K-Kv7.1 causes dominant negative suppression of WT-Kv7.1. Representative families of whole-cell currents recorded from HEK293 cells expressing KCNE1 and WT-Kv7.1 , WT- & E160K-Kv7.1, or E160K-Kv7.1. The corresponding voltage protocol used to measure the currents is also shown. Several mechanistic and computational studies suggest that E160K-Kv7.1 causes a loss of function by alter gating (Silva et al., 2009; Wu D et al., 2010; Silva et al., 2010; Bartos et al, 2011).

data for cells expressing WT-Kv7.1 or WT- and E160K-Kv7.1 were described using the Boltzmann function:

$$I = (I_{MIN}\text{-}I_{MAX})/(1+e(V\text{-}V_{½})/k) + I_{MAX} \quad (1)$$

where I_{MIN} is the minimally activated current; I_{MAX} is the maximally activated current; $V_{½}$ is the mid-point potential for half maximal activation; and *k* is the slope factor. Compared to cells expressing WT-Kv7.1, cells expressing WT- and E160K-Kv7.1 did not alter I_{MIN}, $V_{½}$, or *k*, but it did reduce I_{MAX} by more than half that of cells expressing WT-Kv7.1. These data suggest that E160K-Kv7.1 causes dominant negative suppression of WT-Kv7.1. Additionally, this mutation is associated with a relatively severe LQT1-related clinical phenotype (Silvia et al., 2009).

3.2 Long QT type 2

Similar to LQT1, LQT2 is linked to hundred of different *KCNH2* mutations (Kapa et al., 2009; http://www.fsm.it/cardmoc/); mutations in *KCNH2* account for ~30-35% of congenital LQT cases (Yuan et al., 2008; Kapa et al., 2009); and heterologous expression studies suggest that many LQT2 mutations throughout the Kv11.1 α-subunit cause a loss-of-function (Sanguinetti et al., 1996; Zhou et al., 1998; Zhou et al., 1999; Chen et al., 1999; Furutani et al., 1999; Nakajima et al., 1999; Lees-Miller et al., 2000; Huang et al., 2001; Paulussen et al., 2002; Hayashi et al., 2002; Yang et al., 2002; Johnson et al., 2003; Hayashi et al., 2004; Rossenbacker

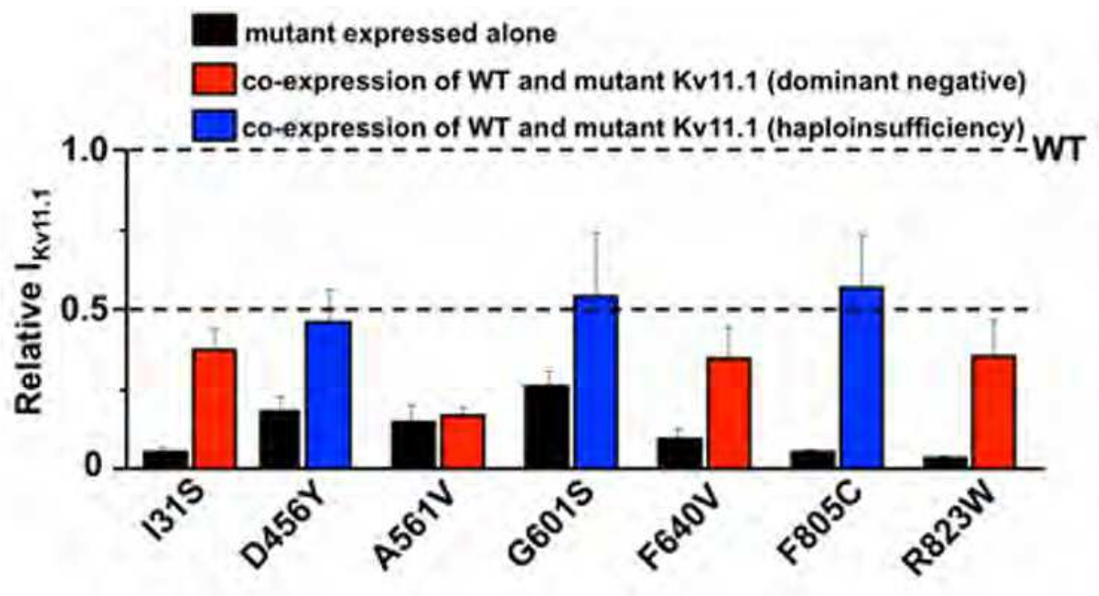


Fig. 6. Trafficking-deficient LQT2-linked mutations generate a spectrum of functional phenotypes. Maximally activated currents were measured from cells expressing WT-Kv11.1, mutant Kv11.1, or WT and mutant Kv11.1 (Modified from Anderson et al., 2006). The relative amplitudes compared to WT-Kv11.1 are shown. Mutations expressed alone all reduced maximally activated current by > 0.5 (black). Co-expression of WT and Kv11.1 mutations suggested that are caused dominant negative effects and reduced maximally activated current by >0.5 (red), whereas others resulted in haploinsufficiency and reduced maximally activated current by ~0.5 (blue).

et al., 2005; Anderson et al., 2006; Gong et al., 2007). Studies suggest that approximately 90% of LQT2 missense mutations cause a loss-of-function preventing the trafficking of the mutant Kv11.1 to the cell surface membrane (Anderson et al., 2006). These "trafficking-deficient" mutations can cause dominant negative effects by inhibiting the trafficking of WT-Kv11.1 (Ficker et al., 2000). Using similar techniques as described above co-expressing WT-Kv7.1 and LQT1 mutations, Anderson and colleagues (2006) co-expressed WT-Kv11.1 with different trafficking-deficient LQT2 mutations in HEK293 cells. Cells were depolarized to 50 mV for 5 seconds, and the maximally activated peak tail $I_{Kv11.1}$ was measured at a test pulse to −120 mV for 3 seconds. Figure 6 shows the relative mean peak amplitude of tail current measured from cells expressing only the trafficking-deficient LQT2 mutations or co-expressing WT-Kv11.1. The data suggest some trafficking-deficient LQT2 mutations can cause dominant negative effects whereas others cause haploinsufficiency.

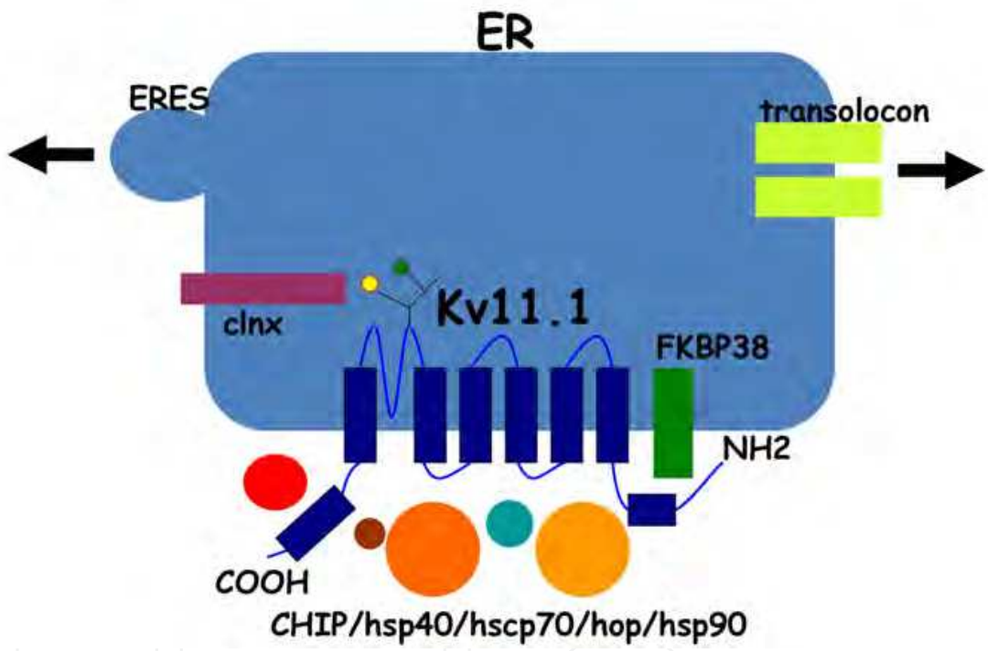


Fig. 7. A *cartoon* showing the different chaperones that regulate the trafficking of Kv11.1 in the secretory pathway. In the ER, several different chaperones and co-chaperones, including calnexin (clns), CHIP, heat shock proteins 40, 70 ,90 (hsp40, hscp70, hsp90), hop, and FKBP38 associate with Kv11.1. Misfolded Kv11.1 is prevented from trafficking into the secretory pathway in Endoplasmic Reticulum Exit Sites (ERES) and is degraded in the transolocan/proteasomal pathway.

Trafficking-deficient LQT2 channels are retained in the Endoplasmic Reticulum (ER). Several studies suggest that the mutations cause "misofolding" of Kv11.1 and cellular quality control mechanisms prevent its export into the secretory pathway (Ficker 2003; Delisle et al., 2005, Gong et al, 2005; Gong et al., 2006; Walker et al., 2007; Walker et al., 2010; Li et al., 2010). Indeed, many different molecular chaperone proteins that regulate the trafficking of WT-Kv11.1 and LQT2 mutations have been identified (Figure 7). Misfolded Kv11.1 undergoes degradation through the proteasome pathway. To understand how

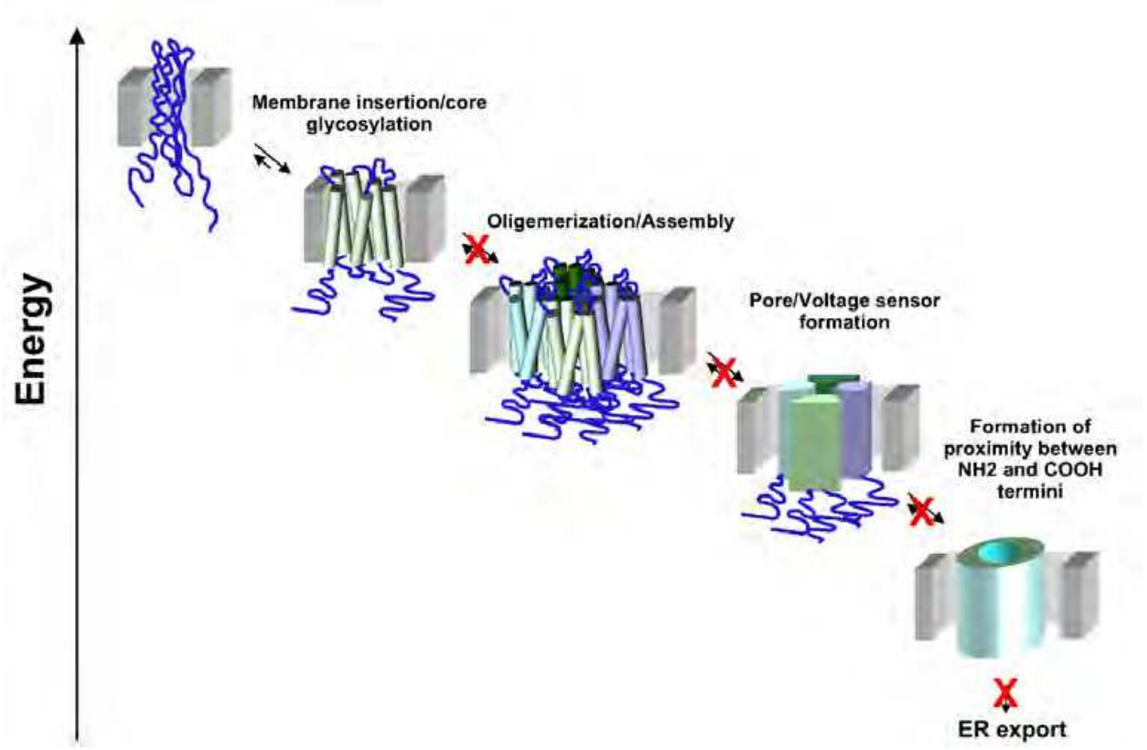


Fig. 8. A *cartoon* showing how some trafficking-deficient LQT2 mutations may disrupt distinct steps in Kv11.1 biogenesis. Trafficking-deficient LQT2 mutations may cause haploinsufficiency by preventing the oligomerization or co-assembly of WT- and mutant Kv11.1 α-subunits. Alternatively, mutant Kv11.1 α-subunits may cause dominant negative effects by co-assembling with WT-Kv11.1 and disrupt pore/voltage sensor formation or formation of proximity between the amino (NH2) or carboxy (COOH) termini. The cartoon shows the different stages of biogenesis, from the formation of secondary structures (cylinders) to the a folded channel (large cylinder with a circle).

trafficking-deficient LQT2 mutations may cause dominant negative or haploinsufficiency, the steps that underlie the assembly and folding of Kv channel proteins during biogenesis need to be understood. Schulteis and colleagues (1998) proposed the following sequence of events during Shaker K^+ channel biogenesis in the ER: 1) membrane insertion and core-glycosylation, 2) assembly of Shaker α and β subunit channel proteins, 3) formation of the pore and voltage sensor, and 4) formation of proximity between adjacent amino and carboxy termini. Assuming a similar sequence of events for Kv11.1 (Figure 8), then trafficking-deficient LQT2 mutations may be capable of disrupting different steps in Kv11.1 channel biogenesis to cause either dominant negative or haploinsufficiency. LQT2 mutations that do not co-assemble do not cause dominant negative suppression of WT-Kv11.1 channel proteins (Ficker et al., 2002) and this would suggest that some trafficking-deficient LQT2 mutations disrupt Kv11.1 biogenesis before the co-assembly step(s). Dominant negative trafficking-deficient LQT2 disrupt Kv11.1 biogenesis steps after co-assembly. The latter group may disrupt Kv11.1 biogenesis steps such as the folding of the voltage sensor/pore, or the formation of proximity between adjacent amino and carboxy termini.

3.3 Summary

The most common cardiac channelopathies are caused by mutations in *KCNQ1* (Kv7.1) and *KCNH2* (Kv11.1) that follow a dominant inheritance pattern and cause LQT1 and LQT2, respectively. The vast majority of these mutations are missense, and they can co-assemble with WT α-subunits to cause effects that range from less than or more than (dominant negative) haploinsufficiency. *In vitro* techniques that utilize the whole cell patch clamp can identify dominant negative mutations that suppress channel function and/or trafficking. We conclude that the patch clamp technique is useful for identifying particularly pathogenic LQT1 and LQT2 mutations.

4. Conclusions

Cardiac channelopathies have informed us about the channels and proteins that are important for atrial and ventricular excitability. Correlations between *in vitro* and clinical data suggest that patch clamp technique can provide prognostic information about individual mutations. We expect advancements in genotyping and high throughput molecular, tissue culture, and patch clamp techniques may enable clinicians to assess the risk of individual patient mutations identified through genotyping using *in vitro* patch clamp assays.

5. Acknowledgements

To Jennifer L. Smith, Daniel C. Bartos, Don E. Burgess, and Allison Reloj for their work in preparation of this chapter, and the National Heart Lung and Blood Institute grant R01 HL087039.

6. References

Abbott. G.W., Sesti, F., Splawski, I., Buck, M.E., Lehmann, M.H., Timothy, K.W., Keating, M.T., & Goldstein, S.A. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell.* 1999;97:175-187.

Andelfinger, G., Tapper, A.R., Welch, R.C., Vanoye, C.G., George, A.L. Jr, & Benson, D.W.KCNJ2 mutation results in Andersen syndrome with sex-specific cardiac and skeletal muscle phenotypes. *Am J Hum Genet.* 2002;71:663-668.

Anderson, C.L., Delisle, B.P., Anson, B.D., Kilby, J.A., Will, M.L., Tester, D.J., Gong, Q., Zhou, Z., Ackerman, M.J., & January, C.T. Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. *Circulation.* 2006;113:365-373.

Antzelevitch, C., Pollevick, G. D., Cordeiro, J. M., Casis, O., Sanguinetti, M. C., Aizawa, Y., Guerchicoff, A., Pfeiffer, R., Oliva, A., Wollnik, B., Gelber, P., & Bonaros, E. P., Jr., and 11 others. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation.* 2007;115: 442-449.

Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., & Romey, G. K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. *Nature.*1996;384:78-80.

Bartos, D.C., Duchatelet, S., Burgess, D.E., Klug, D., Denjoy, I., Peat, R., Lupoglazoff, J.M., Fressart, V., Berthet, M., Ackerman, M.J., January, C.T., Guicheney, P., & Delisle, B.P. R231C mutation in KCNQ1 causes long QT syndrome type 1 and familial atrial fibrillation. *Heart Rhythm.* 2011;8:48-55.

Bellocq, C., van Ginneken, A.C., Bezzina, C.R., Alders, M., Escande, D., Mannens, M.M., Baró, I., & Wilde, A.A. Mutation in the KCNQ1 gene leading to the short QT-interval syndrome. *Circulation.* 2004;109:2394-2397.

Benito, B., Brugada, R., Perich, R.M., Lizotte, E., Cinca, J., Mont, L., Berruezo, A, Tolosana, J.M., Freixa, X., Brugada, P., & Brugada, J. A mutation in the sodium channel is responsible for the association of long QT syndrome and familial atrial fibrillation. *Heart Rhythm.* 2008;5:1434-1440.

Brugada, R., Hong, K., Dumaine, R., Cordeiro, J., Gaita, F., Borggrefe, M., Menendez, T.M., Brugada, J., Pollevick, G.D., Wolpert, C., Burashnikov, E., Matsuo, K., Wu, Y.S., Guerchicoff, A., Bianchi, F., Giustetto, C., Schimpf, R., Brugada, P., & Antzelevitch, C. Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation.* 2004;109:30-35.

Burashnikov, E., Pfeiffer, R., Barajas-Martinez, H., Delpón, E., Hu, D., Desai, M., Borggrefe, M., Häissaguerre, M., Kanter, R., Pollevick, G.D., Guerchicoff, A., Laiño, R., Marieb, M., Nademanee, K., Nam, G.B., Robles, R., Schimpf, R., Stapleton, D.D., Viskin, S., Winters, S., Wolpert, C., Zimmern, S., Veltmann, C., & Antzelevitch, C. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. *Heart Rhythm.* 2010;7:1872-1882.

Chen, J., Zou A., Splawski, I., Keating, M.T., & Sanguinetti, M.C. Long QT syndrome-associated mutations in the Per-Arnt-Sim (PAS) domain of HERG potassium channels accelerate channel deactivation. *J Biol Chem.* 1999;274:10113-10118.

Chen, L., Marquardt, M.L., Tester, D.J., Sampson, K.J., Ackerman, M.J., & Kass, R.S. Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc Natl Acad Sci U S A.* 2007;104:20990-20995.

Chen, Q., Kirsch, G. E., Zhang, D., Brugada, R., Brugada, J., Brugada, P., Potenza, D., Moya, A., Borggrefe, M., Breithardt, G., Ortiz-Lopez, R., Wang, Z., Antzelevitch, C., O'Brien, R. E., Schulze-Bahr, E., Keating, M. T., Towbin, J. A., & Wang, Q. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature.* 1998;392: 293-295.

Chen, Y.-H., Xu, S.-J., Bendahhou, S., Wang, X.-L., Wang, Y., Xu, W.-Y., Jin, H.-W., Sun, H., Su, X.-Y., Zhuang, Q.-N., Yang, Y.-Q., Li, Y.-B., Liu, Y., Xu, H.-J., Li, X.-F., Ma, N., Mou, C.-P., Chen, Z., Barhanin, J., & Huang, W. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science.* 2003; 299: 251-254.

Crotti, L., Celano, G., Dagradi, F., & Schwartz, P.J. Congenital long QT syndrome. *Orphanet J Rare Dis.* 2008;3:18.

Curran, M.E., Splawski, I., Timothy, K.W., Vincent, G.M., Green, E.D., & Keating, M.T. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell.* 1995;80:795-803.

Darbar, D., Kannankeril, P. J., Donahue, B. S., Kucera, G., Stubblefield, T., Haines, J. L., George, A. L., Jr., & Roden, D. M. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. *Circulation.* 2008;117: 1927-1935.

Deschênes, D., Acharfi, S., Pouliot, V., Hegele, R., Krahn, A., Daleau, P., & Chahine, M. Biophysical characteristics of a new mutation on the KCNQ1 potassium channel (L251P) causing long QT syndrome. *Can J Physiol Pharmacol.* 2003;81:129-134.

Delisle, B.P., Anson, B.D., Rajamani, S., & January, C.T. Biology of cardiac arrhythmias: ion channel protein trafficking. *Circ Res.* 2004;94:1418-1428.

Delisle, B.P., Slind, J.K., Kilby, J.A., Anderson, C.L., Anson, B.D., Balijepalli, R.C., Tester, D.J., Ackerman, M.J., Kamp, T.J., & January, C.T..Intragenic suppression of trafficking-defective KCNH2 channels associated with long QT syndrome. *Mol Pharmacol.* 2005;68:233-240.

Delpon, E., Cordeiro, J. M., Nunez, L., Thomsen, P. E. B., Guerchicoff, A., Pollevick, G. D., Wu, Y., Kanters, J. K., Larsen, C. T., Burashnikov, E., Christiansen, M., & Antzelevitch, C. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. *Circ. Arrhythmia Electrophysiol.* 2008;1: 209-218.

Ellinor, P. T., Nam, E. G., Shea, M. A., Milan, D. J., Ruskin, J. N., & MacRae, C. A. Cardiac sodium channel mutation in atrial fibrillation. Heart Rhythm 2008;5: 99-105.

Ficker, E., Dennis, A.T., Obejero-Paz, C.A., Castaldo, P., Taglialatela, M., & Brown, A.M. Retention in the endoplasmic reticulum as a mechanism of dominant-negative current suppression in human long QT syndrome. *J Mol Cell Cardiol.* 2000;32:2327-2337.

Ficker, E., Dennis, A.T., Wang, L., & Brown, A.M. Role of the cytosolic chaperones Hsp70 and Hsp90 in maturation of the cardiac potassium channel HERG. *Circ Res*. 2003;92:e87-e100.

Ficker, E., Thomas, D., Viswanathan, P.C., Dennis, A.T., Priori, S.G., Napolitano, C., Memmi, M., Wible, B.A., Kaufman, E.S., Iyengar, S., Schwartz, P.J., Rudy, Y., & Brown, A.M. Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. *Am J Physiol Heart Circ Physiol.* 2000;279:H1748-H1756.

Furutani. M., Trudeau, M.C., Hagiwara, N., Seki, A., Gong, Q., Zhou, Z., Imamura, S., Nagashima, H., Kasanuki, H., Takao, A., Momma, K., January, C.T., Robertson, G.A., & Matsuoka, R. Novel mechanism associated with an inherited cardiac arrhythmia: defective protein trafficking by the mutant HERG (G601S) potassium channel. *Circulation.* 1999;99:2290-2294.

Goldenberg, I. & Moss, A.J. Long QT syndrome. *J Am Coll Cardiol.* 2008;51:2291-3000.

Gollob, M. H., Jones, D. L., Krahn, A. D., Danis, L., Gong, X.-Q., Shao, Q., Liu, X., Veinot, J. P., Tang, A. S. L., Stewart, A. F. R., Tesson, F., Klein, G. J., Yee, R., Skanes, A. C., Guiraudon, G. M., Ebihara, L., & Bai, D. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. *New Eng. J. Med.* 2006; 354: 2677-2688.

Gong, Q., Jones, M.A., & Zhou, Z. Mechanisms of pharmacological rescue of trafficking-defective hERG mutant channels in human long QT syndrome. *J Biol Chem.* 2006;281(7):4069-4074.

Gong Q, Keeney DR, Molinari M, Zhou Z. Degradation of trafficking-defective long QT syndrome type II mutant channels by the ubiquitin-proteasome pathway. *J Biol Chem.* 2005;280:19419-19425.

Gong, Q., Zhang, L., Vincent, G.M., Horne, B.D., & Zhou, Z. Nonsense mutations in hERG cause a decrease in mutant mRNA transcripts by nonsense-mediated mRNA decay in human long-QT syndrome. *Circulation.* 2007;116:17-24.

Hayashi, K., Shimizu, M., Ino, H., Yamaguchi, M., Mabuchi, H., Hoshi, N., & Higashida, H. Characterization of a novel missense mutation E637K in the pore-S6 loop of HERG in a patient with long QT syndrome. *Cardiovasc Res.* 2002;54:67-76.

Hayashi, K., Shimizu, M., Ino, H., Yamaguchi, M., Terai, H., Hoshi, N., Higashida, H., Terashima, N., Uno, Y., Kanaya, H., & Mabuchi, H. Probucol aggravates long QT syndrome associated with a novel missense mutation M124T in the N-terminus of HERG. *Clin Sci (Lond).* 2004;107:175-182.

Hong, K., Piper, D.R., Diaz-Valdecantos, A., Brugada, J., Oliva, A., Burashnikov, E., Santos-de-Soto, J., Grueso-Montero, J., Diaz-Enfante, E., Brugada, P., Sachse, F., & Sanguinetti, M.C., Brugada R. De novo KCNQ1 mutation responsible for atrial fibrillation and short QT syndrome in utero. *Cardiovasc Res.* 2005;68(3):433-440.

Hu, D., Barajas-Martinez, H., Burashnikov, E., Springer, M., Wu, Y., Varro, A., Pfeiffer, R., Koopmann, T. T., Cordeiro, J. M., Guerchicoff, A., Pollevick, G. D., & Antzelevitch, C. A mutation in the beta-3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. *Circ. Cardiovasc. Genet.* 2009;2: 270-278.

Huang, F.D., Chen, J., Lin, M., Keating, M.T., & Sanguinetti, M.C. Long-QT syndrome-associated missense mutations in the pore helix of the HERG potassium channel. *Circulation.* 2001;104:1071-1075.

January, C.T., Gong, Q., & Zhou, Z. Long QT syndrome: cellular basis and arrhythmia mechanism in LQT2. *J Cardiovasc Electrophysiol.* 2000;11:1413-1418

Johnson, W.H. Jr, Yang, P., Yang, T., Lau, Y.R., Mostella, B.A., Wolff, D.J., Roden, D.M., & Benson, D.W. Clinical, genetic, and biophysical characterization of a homozygous HERG mutation causing severe neonatal long QT syndrome. *Pediatr Res.* 2003;53:744-748.

Jons, C., Moss, A.J., Lopes, C.M., McNitt, S., Zareba, W., Goldenberg, I., Qi, M., Wilde, A.A., Shimizu, W., Kanters, J.K., Towbin, J.A., Ackerman, M.J., & Robinson, J.L. Mutations in conserved amino acids in the KCNQ1 channel and risk of cardiac events in type-1 long-QT syndrome. *J Cardiovasc Electrophysiol.* 2009;20:859-865.

Kapa, S., Tester, D.J., Salisbury, B.A., Harris-Kerr, C., Pungliya, M.S., Alders, M., Wilde, A.A., & Ackerman, M.J. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation.* 2009;120:1752-1760.

Lees-Miller, J.P., Duan, Y., Teng, G.Q., Thorstad, K., & Duff, H.J.. Novel gain-of-function mechanism in K(+) channel-related long-QT syndrome: altered gating and selectivity in the HERG1 N629D mutant. *Circ Res.* 2000 ;86:507-513.

Lehnart, S.E., Ackerman, M.J., Benson, D.W., Jr, Brugada, R., Clancy, C.E., Donahue, J.K., George, A.L. Jr, Grant, A.O., Groft, S.C., January, C.T., Lathrop, D.A., Lederer, W.J., Makielski, J.C., Mohler, P.J., Moss, A., Nerbonne, J.M., Olson, T.M., Przywara, D.A., Towbin, J.A., Wang, L.H., & Marks, A.R. Inherited arrhythmias: a National Heart, Lung, and Blood Institute and Office of Rare Diseases workshop consensus report about the diagnosis, phenotyping, molecular mechanisms, and therapeutic approaches for primary cardiomyopathies of gene mutations affecting ion channel function. *Circulation.* 2007;116:2325-2345.

Lesage, F., Attali, B., Lakey, J., Honoré, E., Romey, G., Faurobert, E., Lazdunski, M., & Barhanin, J. Are Xenopus oocytes unique in displaying functional IsK channel heterologous expression? *Receptors Channels.* 1993;1(2):143-152.

Li, P., Ninomiya H, Kurata, Y., Kato, M., Miake, J., Yamamoto, Y., Igawa, O,. Nakai, A., Higaki, K., Toyoda, F., Wu, J., Horie, M,. Matsuura, H., Yoshida, A., Shirayoshi, Y., Hiraoka, M., & Hisatome, I. Reciprocal control of hERG stability by Hsp70 and Hsc70 with implication for restoration of LQT2 mutant stability. *Circ Res.* 2011;108:458-468.

London, B., Michalec, M., Mehdi, H., Zhu, X., Kerchner, L., Sanyal, S., Viswanathan, P. C., Pfahnl, A. E., Shang, L. L., Madhusudanan, M., Baty, C. J., Lagana, S., Aleong, R., Gutmann, R., Ackerman, M. J., McNamara, D. M., Weiss, R., & Dudley, S. C., Jr. Mutation in glycerol-3-phosphate dehydrogenase 1-like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. *Circulation* 2007;116: 2260-2268.

Makiyama, T., Akao, M., Shizuta, S., Doi, T., Nishiyama, K., Oka, Y., Ohno, S., Nishio, Y., Tsuji, K., Itoh, H., Kimura, T., Kita, T., & Horie, M. A novel SCN5A gain-of-function mutation M1875T associated with familial atrial fibrillation. *J Am Coll Cardiol.* 2008;52:1326-1334.

Medeiros-Domingo, A., Kaku, T., Tester, D.J., Iturralde-Torres, P., Itty, A., Ye, B., Valdivia, C., Ueda, K., Canizales-Quinteros, S., Tusié-Luna, M.T., Makielski, J.C., & Ackerman, M.J. SCN4B-encoded sodium channel beta4 subunit in congenital long-QT syndrome. *Circulation.* 2007;116:134-142.

Mohler, P.J., Schott, J.J., Gramolini, A.O., Dilly, K.W., Guatimosim, S., duBell, W.H., Song, L.S., Haurogné, K., Kyndt, F., Ali, M.E., Rogers, T.B., Lederer, W.J., Escande, D., Le Marec, H., & Bennett, V. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature.* 2003;421:634-639.

Moss, A.J., Shimizu, W., Wilde, A.A., Towbin, J.A., Zareba, W., Robinson, J.L., Qi, M., Vincent, G.M., Ackerman, M.J., Kaufman, E.S., Hofman, N., Seth, R., Kamakura, S., Miyamoto, Y., Goldenberg, I., Andrews, M.L., & McNitt, S. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation.* 2007;115:2481-2489.

Nakajima, T., Furukawa, T., Hirano, Y., Tanaka, T., Sakurada, H., Takahashi, T., Nagai, R., Itoh, T., Katayama, Y., Nakamura, Y., & Hiraoka, M. Voltage-shift of the current activation in HERG S4 mutation (R534C) in LQT2. *Cardiovasc Res.* 1999;44:283-293.

Napolitano, C., Priori, S.G., Schwartz, P.J., Bloise, R., Ronchetti, E., Nastoli, J., Bottelli, G., Cerrone, M., & Leonardi, S. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA.* 2005;294:2975-2980.

Nerbonne, J.M. & Kass, R.S. Molecular physiology of cardiac repolarization. *Physiol Rev.* 2005;85:1205-1253.

Neyroud, N., Denjoy, I., Donger, C., Gary, F., Villain, E., Leenhardt, A., Benali, K., Schwartz, K., Coumel, P., & Guicheney, P. Heterozygous mutation in the pore of potassium channel gene KvLQT1 causes an apparently normal phenotype in long QT syndrome. *Eur J Hum Genet.* 1998;6:129-133.

Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Fauré, S., Gary, F., Coumel, P., Petit, C., Schwartz, K., & Guicheney, P. A novel mutation in the potassium channel gene KVLQT1 causes the Jervell and Lange-Nielsen cardioauditory syndrome. *Nat Genet.* 1997;15:186-189.

Olson, T. M., Alekseev, A. E., Liu, X. K., Park, S., Zingman, L. V., Bienengraeber, M., Sattiraju, S., Ballew, J. D., Jahangir, A., & Terzic, A. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. *Hum. Molec. Genet.* 2006;15: 2185-2191.

Olson, T. M., Alekseev, A. E., Moreau, C., Liu, X. K., Zingman, L. V., Miki, T., Seino, S., Asirvatham, S. J., Jahangir, A., & Terzic, A. K(ATP) channel mutation confers risk for vein of Marshall adrenergic atrial fibrillation. *Nat. Clin. Pract. Cardiovasc. Med.* 2007;4: 110-116.

Paulussen, A., Raes, A., Matthijs, G., Snyders, D.J., Cohen, N., & Aerssens, J. A novel mutation (T65P) in the PAS domain of the human potassium channel HERG results in the long QT syndrome by trafficking deficiency. *J Biol Chem.* 2002;277:48610-48616. Epub 2002 Sep 26.

Piippo, K., Swan, H., Pasternack, M., Chapman, H., Paavonen, K., Viitasalo, M., Toivonen, L., & Kontula, K. A founder mutation of the potassium channel KCNQ1 in long QT syndrome: implications for estimation of disease prevalence and molecular diagnostics. *J Am Coll Cardiol.* 2001;37:562-568.

Priori, S.G., Napolitano, C., Schwartz, P.J., Grillo, M., Bloise, R., Ronchetti, E., Moncalvo, C., Tulipani, C., Veia, A., Bottelli, G., & Nastoli, J. Association of long QT syndrome loci and cardiac events among patients treated with beta-blockers. *JAMA.* 2004;292:1341-1344.

Priori, S.G., Pandit, S.V., Rivolta, I., Berenfeld, O., Ronchetti, E., Dhamoon, A., Napolitano, C., Anumonwo, J., di Barletta, M.R., Gudapakkam, S., Bosi, G., Stramba-Badiale, M., & Jalife, J. A novel form of short QT syndrome (SQT3) is caused by a mutation in the KCNJ2 gene. *Circ Res.* 2005;96:800-807.

Priori, S.G., Schwartz, P.J., Napolitano, C., Bloise, R., Ronchetti, E., Grillo, M., Vicentini, A., Spazzolini, C., Nastoli, J., Bottelli, G., Folli, R., & Cappelletti, D. Risk stratification in the long-QT syndrome. *N Engl J Med.* 2003;348:1866-1874.

Rossenbacker, T., Mubagwa, K., Jongbloed, R.J., Vereecke, J., Devriendt, K., Gewillig, M., Carmeliet, E., Collen, D., Heidbüchel, H., & Carmeliet, P. Novel mutation in the Per-Arnt-Sim domain of KCNH2 causes a malignant form of long-QT syndrome. *Circulation.* 2005;111:961-968.

Ruan, Y., Liu, N., Napolitano, C., & Priori, S.G. Therapeutic strategies for long-QT syndrome: does the molecular substrate matter? *Circ Arrhythm Electrophysiol.* 2008;1:290-297.

Saarinen, K., Swan, H., Kainulainen, K., Toivonen, L., Viitasalo, M., & Kontula, K. Molecular genetics of the long QT syndrome: two novel mutations of the KVLQT1 gene and phenotypic expression of the mutant gene in a large kindred. *Hum Mutat.* 1998;11:158-165.

Sanguinetti, M.C., Curran, M.E., Spector, P.S., & Keating, M.T. Spectrum of HERG K+-channel dysfunction in an inherited cardiac arrhythmia *Proc Natl Acad Sci U S A.* 1996;93:2208-2212.

Sanguinetti, M.C., Curran, M.E., Zou, A., Shen, J., Spector, P.S., Atkinson, D.L., & Keating, M.T. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature.*1996;384:80-83.

Schott, J.J., Charpentier, F., Peltier, S., Foley, P., Drouin, E., Bouhour, J.B., Donnelly, P., Vergnaud, G., Bachner, L., Moisan, J.P., Le Marec, H., & Pascal, O. Mapping of a

gene for long QT syndrome to chromosome 4q25-27. *Am J Hum Genet.* 1995;57:1114-1122.

Schulteis, C.T., Nagaya, N., & Papazian, D.M. Subunit folding and assembly steps are interspersed during Shaker potassium channel biogenesis. *J Biol Chem.* 1998;273:26210-26217.

Schulze-Bahr, E., Haverkamp, W., Wedekind, H., Rubie, C., Hördt, M., Borggrefe, M., Assmann, G., Breithardt, G., & Funke, H. Autosomal recessive long-QT syndrome (Jervell Lange-Nielsen syndrome) is genetically heterogeneous. *Hum Genet.* 1997;100:573-576.

Schwartz, P.J., Priori, S.G., Spazzolini, C., Moss, A.J., Vincent, G.M., Napolitano, C., Denjoy, I., Guicheney, P., Breithardt, G., Keating, M.T., Towbin, J.A., Beggs, A.H., Brink, P., Wilde, A.A., Toivonen, L., Zareba, W., Robinson, J.L., Timothy, K.W., Corfield, V., Wattanasirichaigoon, D., Corbett, C., Haverkamp, W., Schulze-Bahr, E., Lehmann, M.H., Schwartz, K., Coumel, P., & Bloise, R. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation.* 2001;103:89-95.

Shah, M., Akar, F.G., & Tomaselli, G.F. Molecular basis of arrhythmias. *Circulation.* 2005;112:2517-2529.

Shalaby, F.Y., Levesque, P.C., Yang, W.P., Little, W.A., Conder, M.L., Jenkins-West, T., & Blanar, M.A. Dominant-negative KvLQT1 mutations underlie the LQT1 form of long QT syndrome. *Circulation.* 1997;96:1733-1736.

Silva, J.R., Pan, H., Wu, D., Nekouzadeh, A., Decker, K.F., Cui, J., Baker, N.A., Sept, D., & Rudy, Y. A multiscale model linking ion-channel molecular dynamics and electrostatics to the cardiac action potential. *Proc Natl Acad Sci U S A.* 2009;106:11102-11106.

Silva, J.R. & Rudy, Y. Multi-scale electrophysiology modeling: from atom to organ. *J Gen Physiol.* 2010;135:575-581

Smith, J.L., McBride, C.M., Nataraj, P.S., Bartos, D.C., January, C.T., & Delisle, B.P. Trafficking-deficient hERG K^+ channels linked to long QT syndrome are regulated by a microtubule-dependent quality control compartment in the ER. *Am J Physiol Cell Physiol.* 2011;301:C75-C85.

Smith, J.A., Vanoye, C.G., George, A.L. Jr, Meiler, J., & Sanders, C.R.. Structural models for the KCNQ1 voltage-gated potassium channel. *Biochemistry.* 2007;46:14141-14152.

Splawski, I., Timothy, K.W., Decher, N., Kumar, P., Sachse, F.B., Beggs, A.H., Sanguinetti, M.C., & Keating, M.T. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci U S A.* 2005;102:8089-8096.

Splawski, I., Tristani-Firouzi, M., Lehmann, M.H., Sanguinetti, M.C., & Keating, M..T. Mutations in the hminK gene cause long QT syndrome and suppress IKs function. *Nat Genet.* 1997;17:338-340.

Thomas, D., Khalil, M., Alter, M., Schweizer, P.A., Karle, C.A., Wimmer, A.B., Licka, M., Katus, H.A., Koenen, M., Ulmer, H.E., & Zehelein, J. Biophysical characterization of KCNQ1 P320 mutations linked to long QT syndrome 1. *J Mol Cell Cardiol.* 2010;48:230-237.

Thomas, D., Wimmer, A.B., Karle, C.A., Licka, M., Alter, M., Khalil, M., Ulmer, H.E., Kathöfer, S., Kiehn, J., Katus, H.A., Schoels, W., Koenen, M., & Zehelein, J.

Dominant-negative I(Ks) suppression by KCNQ1-deltaF339 potassium channels linked to Romano-Ward syndrome. *Cardiovasc Res.* 2005;67:487-497.

Tristani-Firouzi M, Jensen JL, Donaldson MR, Sansone V, Meola G, Hahn A, Bendahhou S, Kwiecinski H, Fidzianska A, Plaster N, Fu YH, Ptacek LJ, Tawil R.Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome). *J Clin Invest.* 2002;110:381-388.

Ueda, K., Hirano, Y., Higashiuesato, Y., Aizawa, Y., Hayashi, T., Inagaki, N., Tana, T., Ohya, Y., Takishita, S., Muratani, H., Hiraoka, & M., Kimura, A. Role of HCN4 channel in preventing ventricular arrhythmia. *J. Hum. Genet.* 2009;54: 115-121, 2009.

Ueda, K., Valdivia C, Medeiros-Domingo, A., Tester, D.J., Vatta, M., Farrugia, G., Ackerman, M.J., & Makielski, J.C. Syntrophin mutation associated with long QT syndrome through activation of the nNOS-SCN5A macromolecular complex. *Proc Natl Acad Sci U S A.* 2008;105:9355-9360.

Van Norstrand, D. W., Valdivia, C. R., Tester, D. J., Ueda, K., London, B., Makielski, J. C., & Ackerman, M. J. Molecular and functional characterization of novel glycerol-3-phosphate dehydrogenase 1-like gene (GPD1-L) mutations in sudden infant death syndrome. *Circulation*. 2007;116: 2253-2259, 2007.

Vatta, M., Ackerman, M.J., Ye, B., Makielski, J.C., Ughanze, E.E., Taylor, E.W., Tester, D.J., Balijepalli, R.C, Foell, J.D., Li, Z., Kamp, T.J., & Towbin, J.A. Mutant caveolin-3 induces persistent late sodium current and is associated with long-QT syndrome. *Circulation.* 2006;114:2104-2112.

Walker, V.E., Atanasiu, R., Lam, H., & Shrier, A. Co-chaperone FKBP38 promotes HERG trafficking. *J Biol Chem.* 2007 A;282:23509-23016.

Walker, V.E., Wong, M.J., Atanasiu, R., Hantouche, C., Young, J.C., & Shrier, A. Hsp40 chaperones promote degradation of the HERG potassium channel. *J Biol Chem.* 2010;285:3319-3329.

Wang, Q., Curran, M.E., Splawski, I., Burn, T.C., Millholland, J.M., VanRaay, T.J., Shen, J., Timothy, K.W., Vincent, G.M., de Jager, T., Schwartz, P.J., Toubin, J.A., Moss, A.J., Atkinson, D.L., Landes, G.M., Connors, T.D., & Keating, M.T. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet.* 1996;12:17-23.

Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J.L., Moss, A.J., Towbin, J.A., & Keating, M.T. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell.* 1995;80:805-811.

Wang, Z., Tristani-Firouzi, M., Xu, Q., Lin, M., Keating, M.T., & Sanguinetti, M.C. Functional effects of mutations in KvLQT1 that cause long QT syndrome. J Cardiovasc Electrophysiol. 1999 Jun;10(6):817-26.

Watanabe, H., Koopmann, T. T., Le Scouarnec, S., Yang, T., Ingram, C. R., Schott, J.-J., Demolombe, S., Probst, V., Anselme, F., Escande, D., Wiesfeld, A. C. P., Pfeufer, A., Kaab, S., Wichmann, H.-E., Hasdemir, C., Aizawa, Y., Wilde, A. A. M., Roden, D. M., & Bezzina, C. R. Sodium channel beta-1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J. Clin. Invest.* 118: 2260-2268, 2008.

Wollnik, B., Schroeder, B.C., Kubisch, C., Esperer, H.D., Wieacker, P., & Jentsch, T.J. Pathophysiological mechanisms of dominant and recessive KVLQT1 K+ channel mutations found in inherited cardiac arrhythmias. Hum Mol Genet. 1997;6:1943-

1949.Xia, M., Jin, Q., Bendahhou, S., He, Y., Larroque, M.-M., Chen, Y., Zhou, Q., Yang, Y., Liu, Y., Liu, B., Zhu, Q., Zhou, Y., et al. A Kir2.1 gain-of-function mutation underlies familial atrial fibrillation. *Biochem. Biophys. Res. Commun*. 2005;332: 1012-1019.

Wu, D., Delaloye, K., Zaydman, M.A., Nekouzadeh, A., Rudy, Y., & Cui, J. State-dependent electrostatic interactions of S4 arginines with E1 in S2 during Kv7.1 activation. *J Gen Physiol*. 2010;135:595-606.

Yamaguchi, M., Shimizu, M., Ino, H., Terai, H., Hayashi, K., Mabuchi, H., Hoshi, N., & Higashida, H. Clinical and electrophysiological characterization of a novel mutation (F193L) in the KCNQ1 gene associated with long QT syndrome. *Clin Sci (Lond)*. 2003;104:377-382.

Yamashita, F., Horie, M., Kubota, T., Yoshida, H., Yumoto, Y., Kobori, A., Ninomiya, T., Kono, Y., Haruna, T., Tsuji, K., Washizuka, T., Takano, M., Otani, H., Sasayama, S., & Aizawa, Y. Characterization and subcellular localization of KCNQ1 with a heterozygous mutation in the C terminus. *J Mol Cell Cardiol.* 2001;33:197-207.

Yang, P., Kanki, H., Drolet, B., Yang, T., Wei, J., Viswanathan, P.C., Hohnloser, S.H., Shimizu, W., Schwartz, P.J,. Stanton, M., Murray, K.T., Norris, K., George, A.L., & Jr, Roden, D,M,. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointe. *Circulation.* 2002;105:1943-2948s.

Yang, Y., Li, J., Lin, X., Yang, Y., Hong, K., Wang, L., Liu, J., Li, L., Yan, D., Liang, D., Xiao, J., Jin, H., Wu, J., Zhang, Y., & Chen, Y.-H. Novel KCNA5 loss-of-function mutations responsible for atrial fibrillation. *J. Hum. Genet.* 2009;54: 277-283.

Yang, Y., Xia, M., Jin, Q., Bendahhou, S., Shi, J., Chen, Y., Liang, B., Lin, J., Liu, Y., Liu, B., Zhou, Q., Zhang, D., & 11 others. Identification of a KCNE2 gain-of-function mutation in patients with familial atrial fibrillation. *Am. J. Hum. Genet.* 2004;75: 899-905.

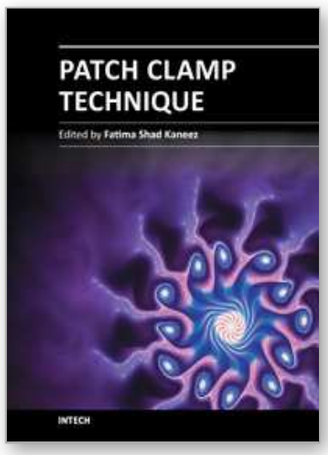
Yang, Y., Yang, Y., Liang, B., Liu, J., Li, J., Grunnet, M., Olesen, S.P., Rasmussen, H.B., Ellinor, P.T., Gao, L., Lin, X., Li, L., Wang, L., Xiao, J., Liu, Y., Liu, Y., Zhang, S., Liang, D., Peng, L., Jespersen, T., & Chen, Y.H. Identification of a Kir3.4 mutation in congenital long QT syndrome. *Am J Hum Genet*. 2010;86:872-880.

Zareba, W., Moss, A.J,. Sheu, G., Kaufman, E.S., Priori, S., Vincent, G.M., Towbin, J.A., Benhorin, J., Schwartz, P.J., Napolitano, C., Hall, W.J., Keating, M.T., Qi, M., Robinson, J.L., & Andrews, M.L. Location of mutation in the KCNQ1 and phenotypic presentation of long QT syndrome. *J Cardiovasc Electrophysiol.* 2003;14:1149-1153.

Zhou, Z., Gong, Q., Epstein, M.L., & January, C.T. HERG channel dysfunction in human long QT syndrome. Intracellular transport and functional defects. *J Biol Chem.* 1998;273:21061-21066.

Zhou, Z., Gong, Q., & January, C.T. Correction of defective protein trafficking of a mutant HERG potassium channel in human long QT syndrome. Pharmacological and temperature effects. *J Biol Chem*. 1999;274:31123-31126.

http://www.fsm.it/cardmoc/



Patch Clamp Technique
Edited by Prof. Fatima Shad Kaneez

ISBN 978-953-51-0406-3
Hard cover, 356 pages
Publisher InTech
Published online 23, March, 2012
Published in print edition March, 2012

This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen