Molecular and Cellular Mechanisms of Cardiac Arrhythmias

Review

Mark T. Keating*‡ and Michael C. Sanguinetti†‡
*Howard Hughes Medical Institute
Department of Cell Biology
Harvard Medical School
Department of Cardiology
Children's Hospital
Boston, Massachusetts 02115
†Department of Medicine
Division of Cardiology
Eccles Program in Human Molecular Biology
    and Genetics
University of Utah Health Sciences Center
Salt Lake City, Utah 84112

Introduction

The heart is a pump (Figure 1). Through coordinated contraction of the atria, blood is pumped into the ventricles. The ventricles, which do most of the work of the heart, contract synchronously to pump blood to the rest of the body. The right ventricle pumps deoxygenated blood to the lungs for gas exchange. The left ventricle, the most forceful chamber of the heart, pumps blood to the rest of the organs, including the brain, kidneys, liver, skeletal muscle, and other vital organs. Through this pumping action, the left ventricle maintains systemic blood pressure at approximately 120 over 80 millimeters of mercury. The brain is particularly sensitive to blood pressure and flow. If the coordinated contraction of ventricles is stopped for only a few seconds, blood pressure drops and consciousness is lost. This sudden loss of consciousness is called syncope. If the mechanical activity of the heart is lost for more than a few minutes, permanent brain damage and death ensue.

The mechanical activity of the heart is controlled by electrical impulses (Figure 1). These impulses are intrinsic to the heart but are also modulated by neuronal activity through the autonomic nervous system. Specialized cells in the right atrium known as the sino-atrial node act as the pacemaker, spontaneously firing ~70 times per minute at rest and up to about 200 times per minute during rigorous exercise. This impulse is conveyed to all atrial myocytes, leading to coordinated depolarization and contraction of the atria. On a surface electrocardiogram (ECG) atrial depolarization can be visualized as a P wave. The electrical activity of the pacemaker is also conveyed to specialized cells that connect the atria and the ventricles, known as the atrioventricular node. Here, the electrical activity is delayed for about 20 milliseconds, giving the atria time to pump blood into the ventricles. Then, the electrical activity is conveyed to specialized fibers known as bundle branches, leading to rapid depolarization of all ventricular myocytes and coordinated contraction of the ventricles. Depolarization of the ventricles can be visualized on the ECG as the QRS complex. Ventricular myocytes

then slowly repolarize, which is denoted as the T wave on the ECG, leading to cardiac relaxation and completion of one cardiac cycle.

The rhythmic activity of the human heart is clearly apparent by ultrasound after only five weeks of gestation. It is remarkable to think how flawlessly the heart performs in the course of one's life, beating ~70 times per minute over a course of 75 to 90 years. One's heart must beat, therefore, ~36 million times a year, a remarkable feat considering that there is virtually no tolerance for failure.

Cardiac Arrhythmias and Sudden Death

Although most hearts beat with remarkable fidelity and resilience, under certain circumstances the rhythm of the heart can fail. This is known as a cardiac arrhythmia. When the heartbeat is too slow (bradyarrhythmia or bradycardia) blood pressure cannot be maintained, leading to loss of consciousness and death. Bradyarrhythmias often result from disease or death of pacemaker and other specialized conducting cells and can be effectively treated with artificial, electronic pacemakers. Similarly, if the heart rhythm is too rapid (tachyarrhythmia or tachycardia), blood pressure cannot be maintained, leading to syncope and sudden death. The most dangerous tachyarrhythmias are focused in the ventricles and are known as ventricular tachycardia, torsades de pointes ventricular tachycardia, and ventricular fibrillation (Figure 1). Torsades de pointes means twisting around the point, an allusion to the alternating axis of the QRS complex around the isoelectric line of the ECG during this arrhythmia (Lazzara, 1997) (Figure 1).

Cardiac arrhythmias are a leading cause of morbidity and mortality. More than 300,000 individuals in the United States die suddenly every year, and in most cases it is assumed that the underlying cause of sudden death is ventricular tachyarrhythmia (Kannel et al., 1987; Willich et al., 1987). Despite their importance, until recently the understanding of the molecular mechanisms underlying life-threatening ventricular tachyarrhythmias was poor. The ability to predict, prevent, and treat these disorders remains a major scientific and medical challenge.

An example of an individual with cardiac arrhythmia may provide insight into these disorders (Splawski et al., 1997a). A 25-year-old woman was seen for routine evaluation by her obstetrician. She was healthy and in her 35th week of gestation in an apparently uncomplicated pregnancy. During examination the physician noted a bradyarrhythmia in the fetus. The fetus's heart rate was only 70–80 beats per minute, approximately half the normal rate. Ultrasonic examination, however, revealed normal fetal development, and the pregnancy was allowed to come to term. The child was born without complication at 39 weeks gestation.

During the first feeding, the child experienced distress and turned blue. She was rushed to the neonatal intensive care unit, where a series of tests were performed. All of these tests were normal except for the ECG, which
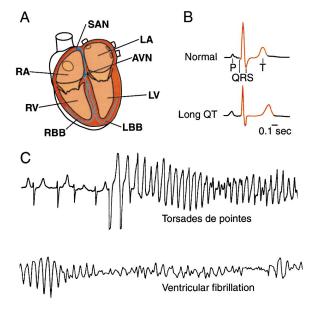
‡E-mail: mkeating@genetics.med.harvard.edu

Figure 1. The Heart Is an Electrically Activated Pump

(A) A schematic representation of a four-chambered heart including specialized cells that act as a pacemaker (the sino-atrial node, SAN) and conducting tissues such as the atrio-ventricular node (AVN) and the right and left bundle branches (RBB, LBB). RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle. (B) Normal electrocardiogram (ECG) and an ECG showing QT interval prolongation. Note that voltage is on the Y axis and time is on the X axis. The P wave represents atrial depolarization, QRS complex represents ventricular depolarization and the T wave represents ventricular repolarization. A normal QTc interval is ~0.40 s. QTc interval prolongation results from abnormal cardiac repolarization. (C) ECG showing normal sinus rhythm converting to torsades de pointes ventricular tachycardia and ventricular fibrillation, a life-threatening cardiac arrhythmia.

showed bradycardia with a heart rate of 82 beats per minute and a prolonged QTc interval of ~0.60 s. The rate-corrected QT (QTc) interval is a temporal measure of ventricular repolarization and is normally about 0.40 s. Because of these findings, a pediatric cardiologist was consulted, who ordered audiographic evaluation. These tests showed severe bilateral hearing loss and a tentative diagnosis of Jervell and Lange-Nielsen syndrome was made. This disorder, a form of long QT syndrome, is a familial cardiac arrhythmia associated with congenital deafness. It was previously believed to be inherited as an autosomal recessive trait. The child was placed on beta-blockers, drugs that reduce the autonomic nervous activity of the heart, and observed for several days without complication. She was sent home after 10 days with a monitor. Because Jervell and Lange-Nielsen syndrome was thought to be purely recessive, no clinical evaluation of the child's parents or other family members was performed. Several months after the birth of the child, her overstressed mother was awakened by an alarm clock, stood up, had a cardiac arrest, and died. She had previously enjoyed excellent health, and had no history of syncope.

Long QT Syndrome, a Familial Cardiac Arrhythmia
Long QT syndrome is a group of disorders that is usually characterized by a prolonged QT interval on the ECG

(Moss, 1995) (Figure 1). It can be associated with syncope and sudden death due to episodic cardiac arrhythmias, particularly torsades de pointes ventricular tachycardia and ventricular fibrillation (Vincent et al., 1992; Schwartz et al., 1993). Most individuals with long QT syndrome have no other symptoms or signs of disease, and arrhythmias are relatively rare except in severe cases. As noted above, however, some cases of long QT syndrome are associated with congenital deafness and this disorder has also been associated with syndactyly, an abnormal webbing of fingers or toes (Marks et al., 1995).

The long QT syndromes can be divided on clinical grounds into two main types: familial and acquired. There are at least two familial forms of long QT syndrome. One, which was described above, was believed to be inherited as an autosomal recessive trait and associated with congenital deafness, the Jervell and Lange-Nielsen syndrome (Jervell and Lange-Nielsen, 1957). A second, more common familial form is inherited as an autosomal dominant trait with no other phenotypic abnormalities. This form, which is sometimes referred to as the Romano Ward syndrome (Romano et al., 1963; Ward, 1964), is usually associated with less arrhythmia risk than the autosomal recessive form. The most common form of long QT syndrome is acquired. There are many different causes of acquired long QT syndrome, including heart diseases such as cardiomyopathy and cardiac ischemia, bradycardia, and metabolic abnormalities like reduced serum potassium concentration (Roden et al., 1996). Treatment with many medications, including certain antibiotics, antihistamines, and antiarrhythmics is the most common cause of acquired long QT syndrome (Roden, 1998).

When molecular studies of long QT syndrome were initiated, there were two main theories invoked to explain the pathogenesis of this disorder. One was the autonomic imbalance hypothesis. This theory was based on studies showing that manipulation of the autonomic nervous system in dogs could lead to QT prolongation and cardiac arrhythmias (Abildskov, 1991). A second hypothesis, the cardiac ion channel hypothesis, suggested that inherited or acquired dysfunction of cardiac ion channels could lead to this disorder. While it is clear that the autonomic nervous system plays a secondary role in many cardiac arrhythmias, the cardiac ion channel hypothesis has proved to be the primary mechanism of arrhythmia susceptibility in studies reported to date (Figure 2).

Mutations in Cardiac Ion Channel Genes Cause Arrhythmia Susceptibility
To define genes that contribute to arrhythmia susceptibility, familial forms of this disorder were examined, particularly long QT syndrome. Over the last six years, six arrhythmia susceptibility genes were discovered: *KVLQT1*, *HERG*, *SCN5A*, *minK*, *MiRP1*, *RyR2* (Table 1) (Curran et al., 1995; Wang et al., 1995a, 1995b, 1996; Splawski et al., 1997a, 1997b; Chen et al., 1998; Abbott et al., 1999; Priori et al., 2000). *KVLQT1* was discovered using positional cloning. *HERG*, *SCN5A*, and *RyR2* were identified using the positional cloning–candidate gene approach, and *minK* and *MiRP1* were discovered using a
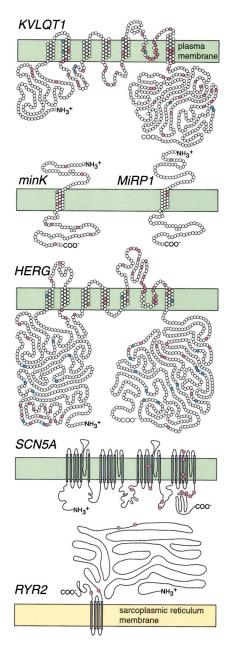
Figure 2. Ion Channel Mutations Cause Cardiac Arrhythmias

Schematic representation of ion channels encoded by arrhythmia susceptibility genes including KVLQT1 potassium channel α subunits, HERG potassium channel α subunits, SCN5A sodium channel α subunits, minK potassium channel β subunits, MiRP1 potassium channel β subunits, and RyR2 ryanodine receptor/calcium release channel. Missense mutations are indicated by red dots, and the location of frameshifts or intragenic deletions are indicated by blue dots.

candidate gene approach. A seventh long QT syndrome locus was mapped to chromosome 4, but this gene has not yet been identified (Schott et al., 1995). Additional locus heterogeneity was described and several additional arrhythmia genes await discovery (Jiang et al., 1994). These studies provided the first molecular insight into the pathogenesis of cardiac arrhythmias.

As noted in the case report described above, Jervell and Lange-Nielsen syndrome was previously believed to be an autosomal recessive disorder. Phenotypic evaluation of this family, however, revealed a more complicated picture (Splawski et al., 1997a). Many members of the family, including the proband's father, had subtle prolongation of the QT interval with normal hearing. Some individuals gave a history of one or two syncopal episodes, but no cases of sudden death were noted. Furthermore, pedigree analyses revealed that the proband resulted from a consanguineous marriage in that her parents were second cousins. This led to the hypothesis that homozygous mutations of an autosomal dominant long QT syndrome gene might cause Jervell and Lange-Nielsen syndrome. This proved to be the case, and it is now clear that homozygous mutations of either KVLQT1 or minK can cause this disorder (Neyroud et al., 1997; Splawski et al., 1997b; Duggal et al., 1998). The molecular genetics also helped to define the clinical picture. One aspect of Jervell and Lange-Nielsen syndrome, congenital deafness, is inherited as an autosomal recessive trait in that heterozygous carriers have no obvious hearing deficit. However, arrhythmia susceptibility is inherited as a semidominant trait. That is, heterozygotes and homozygotes both have arrhythmia susceptibility, but the risk of arrhythmia in homozygotes is much greater. Homozygous mutations of HERG have also been reported (Hoorntje et al., 1999). This condition also causes severe arrhythmia susceptibility, but is not associated with other phenotypic abnormalities.

Autosomal dominant long QT syndrome genes became candidates for involvement in other familial arrhythmia susceptibility syndromes. Although the familial occurrence of virtually every arrhythmia has been reported, in most cases, the mode of inheritance is unclear. Familial ventricular fibrillation, by contrast, can be inherited as a clear, autosomal dominant trait. As in long QT syndrome, people with familial ventricular fibrillation often appear healthy (Martini, 1989). Electrocardiographic evaluation of these individuals shows no evidence of QT interval prolongation. In some cases, subtle prolongation of the QRS complex can be demonstrated. A distinct electrocardiographic feature of elevation of the ST segment has been described in some individuals and referred to as the Brugada syndrome (Brugada et al., 1997). Some forms of familial ventricular fibrillation have also been associated with conduction abnormalities. In all cases, these individuals are at increased risk for episodic ventricular fibrillation, a particularly lethal arrhythmia. When ventricular fibrillation occurs, there is no cardiac output, and permanent brain damage and death ensue unless the arrhythmia is controlled. As noted above, SCN5A mutations can cause arrhythmia susceptibility in certain familial forms of long QT syndrome (Table 1). Recent molecular genetic studies have demonstrated that SCN5A mutations can also cause familial ventricular fibrillation (Chen et al., 1998). Thus, SCN5A mutations can cause several different forms of arrhythmia susceptibility.

The genetic basis for a third familial cardiac arrhythmia, catecholaminergic ventricular tachycardia, has recently become apparent. This disorder is characterized by syncope and sudden death in otherwise healthy young individuals due to episodic ventricular tachycardia. Recent studies demonstrate that this disorder is

Table 1. Molecular and Cellular Mechanisms of Cardiac Arrhythmias

Disease[a]	Inheritance	Gene[b] (alternate name)	Protein	Mechanism
LQT	autosomal dominant	KVLQT1 (KCNQ1)	$I_{Ks}K^+$ channel α subunit	repolarization abnormality
		HERG (KCNH2)	$I_{Kr}K^+$ channel α subunit	repolarization abnormality
		SCN5A	$I_{Na}K^+$ channel α subunit	repolarization abnormality
		LQT4	?	?
		minK (KCNE1)	$I_{Ks}K^+$ channel β subunit	repolarization abnormality
		MiRP1 (KCNE2)	$I_{Kr}K^+$ channel β subunit	repolarization abnormality
		LQT7	?	?
	autosomal recessive (with deafness)	KVLQT1	$I_{Ks}K^+$ channel α subunit	repolarization abnormality
		minK	$I_{Ks}K^+$ channel β subunit	repolarization abnormality
	syndactyly associated	?	?	?
	acquired (drug induced)	HERG	$I_{Kr}K^+$ channel α subunit	repolarization abnormality
		MiRP1	$I_{Kr}K^+$ channel β subunit	repolarization abnormality
IVF	autosomal dominant	SCN5A	$I_{Na}Na^+$ channel α subunit	conduction abnormality
		IVF2	?	?
CVT	autosomal dominant	RyR2	Ryanodine receptor/ Ca^{2+} release channel	calcium overload

[a] LQT = long QT syndrome; IVF = familial idiopathic ventricular fibrillation; CVT = catecholaminergic ventricular tachycardia.
[b] LQT4, LQT7, IVF2: genes not identified.

caused by mutations in *RyR2*, the ryanodine receptor gene (Priori et al., 2000).

In summary, the genetic basis of arrhythmia susceptibility has begun to emerge. Six arrhythmia genes have been identified to date: *SCN5A*, *KVLQT1*, *minK*, *HERG*, *MiRP1*, *RyR2*. *SCN5A* mutations can cause both long QT syndrome and familial ventricular fibrillation. *KVLQT1*, *minK*, *HERG*, and *MiRP1* mutations have been implicated in long QT syndrome. *RyR2* mutations cause catecholaminergic ventricular tachycardia. In general, arrhythmia susceptibility is more severe in homozygotes than in heterozygotes. Although some familial forms of arrhythmia susceptibility are associated with additional obvious phenotypic abnormalities (e.g., congenital neural deafness in Jervell and Lange-Nielsen syndrome), most of these individuals appear grossly normal and go undetected until their first arrhythmia strikes.

Ion Channels and the Cardiac Action Potential
Like other excitable cells, including neurons, skeletal muscle, and smooth muscle, cardiac myocyte excitability results from action potentials. The cardiac myocyte action potential, however, is distinctive in its duration, which is much longer at ~300 ms. By contrast, the action potentials of neurons and skeletal muscle last a few milliseconds. The cardiac action potential consists of five phases, numbered 0–4 (Figure 3). Phase 0 represents depolarization of the myocyte. This phase is initiated by the rapid opening (activation) of voltage-gated sodium channels. Depolarization of all ventricular myocytes is measurable as the QRS complex on the surface ECG. Phase 1 of the cardiac action potential occurs immediately after the peak of depolarization and is recognized as a partial repolarization of the membrane. This small repolarizing effect is due to the closure (inactivation) of cardiac sodium channels, and activation of transient outward potassium current. Phase 2 of the action potential is the plateau phase. The relatively long duration of this phase is unique to ventricular and Purkinje fiber myocytes. The plateau is generated primarily by slowly decreasing inward calcium currents through

L-type calcium channels and gradually increasing outward current through several types of potassium channels. The total amount of current during the plateau phase of the cardiac action potential is small. As a consequence, relatively small changes in ion current during this phase can have a major impact on action potential duration. At this point in the cardiac cycle the ECG has returned to baseline. Phase 3 represents myocellular repolarization, an effect mediated by outward potassium currents. Physiologic and pharmacologic studies have defined two main repolarizing potassium currents, I_{Kr} and I_{Ks}, that sum to terminate the plateau phase and initiate final repolarization (Sanguinetti and Jurkiewicz, 1990). Other currents such as the plateau delayed rectifier K^+ current (I_{Kp}) and the inward rectifier K^+ current (I_{K1}) also contribute to repolarization. I_{Kr} is the rapidly activating delayed rectifier potassium current that is specifically blocked by methanesulfonanilide drugs. When I_{Kr} current is blocked by these drugs, I_{Ks}, the slowly activating delayed rectifier potassium current, remains. The repolarization phase correlates with the T wave on surface ECG. Phase 4 is the final phase of the action potential and signals a return of membrane potential to its baseline near −85 mV. This phase represents ventricular relaxation or diastole and is indicated on the ECG as a return to baseline. Thus, the coordinated opening and closing of ion channels mediates the cardiac action potential. Duration of the QT interval on surface ECG is related to the length of ventricular action potentials.

Sodium Channel Dysfunction Can Cause Several Different Types of Arrhythmia
Investigators had previously demonstrated that *SCN5A* encodes the α subunits of sodium channels that are responsible for initiating cardiac action potentials (Gellens et al., 1992). This gene is located on chromosome 3p21-p24 and encodes a protein with a predicted topology of four major domains, DI through DIV (Figure 2). Each of these domains is believed to have a structure similar to a voltage-gated potassium channel with six membrane-spanning domains (S1–S6) and pore do-
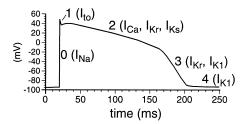
Figure 3. The Cardiac Action Potential Is Mediated by Ion Currents

Schematic representation of a cardiac action potential with voltage on the Y axis and time on the X axis. Note that the cardiac action potential duration is quite long, for example, 200 milliseconds for the ventricular myocyte shown here. Inward sodium current mediates the rapid phase 0 depolarization. Transient outward K^+ currents (I_{to}) mediate rapid phase 1 repolarization. The L-type inward calcium current contributes to the long plateau duration (phase 2). Many outward potassium currents are responsible for repolarization of the cardiac action potential (phase 2 and 3), including I_{Kr}, I_{Ks}, and I_{K1}. Thus, the coordinate opening and closing of cardiac ion channels is responsible for cardiac excitability.

mains located between S5 and S6. The α subunit can form functional channels, but accessory β subunits have also been identified that alter some biophysical properties of the channel. Mutational analyses have revealed 14 distinct mutations of *SCN5A* associated with long QT syndrome (Splawski et al., 2000).

Based on the location of these mutations and the physiology of the disease, it was hypothesized that gain-of-function mutations in *SCN5A* would cause long QT syndrome. Normally, cardiac sodium channels open briefly in response to membrane depolarization. The channel is then inactivated and remains closed for the remainder of the action potential. Sodium channel inactivation is mediated by an intracellular domain located between DIII and DIV. This domain is referred to as the inactivation gate and is thought to physically block the inner mouth of the channel pore. Several *SCN5A* mutations associated with long QT syndrome were identified in this region. Physiologic characterization of one of these mutants (Δ KPQ) led to the discovery that the mutations destabilized the inactivation gate (Bennett et al., 1995). Activation of these mutant sodium channels is normal and the rate of inactivation appears slightly faster than normal, but mutant channels can also reopen during the plateau phase of the action potential. The net effect is a small, maintained depolarizing current that is modeled to be present during the plateau phase of the action potential (Bennett et al., 1995; Dumaine et al., 1996). This lengthens action potential duration. Other long QT syndrome-associated mutations of *SCN5A* had slightly different effects on mutant channels at the single channel level, but all led to maintained depolarizing currents and action potential prolongation, setting up a substrate for arrhythmia.

SCN5A mutations also cause familial ventricular fibrillation (Table 1). In some cases, these mutations are clearly loss-of-function. For example, a nonsense mutation of *SCN5A* has been associated with ventricular fibrillation in an otherwise healthy individual who has no obvious electrocardiographic abnormalities (Chen et al., 1998). Other mutations that have been associated with this disorder are less clear-cut in terms of their physio-

logic consequences. Theoretically, reduction of sodium current would be expected to slightly shorten action potential duration and slow conduction velocity. It is also not yet clear exactly how reduction in the total number of functional sodium channels and expression of a heterogeneous population of sodium channels lead to arrhythmia. Clues may come from pharmacologic studies, which show that inhibition of sodium channel current can cause heterogeneity in action potential configuration in the right ventricular epicardium, leading to marked dispersion of repolarization and refractoriness (Krishnan and Antzelevitch, 1991). This creates a substrate for the development of reentrant arrhythmias. Some familial ventricular fibrillation mutations of *SCN5A* may cause an abnormality of conduction, and in some cases, baseline electrocardiograms in affected individuals show conduction abnormalities. In summary, both gain- and loss-of-function mutations of *SCN5A* can cause arrhythmia susceptibility. Gain of function causes prolongation of action potentials, whereas loss-of-function mutations likely shorten action potentials and slow conduction velocity.

KVLQT1 and minK Coassemble to Form Cardiac I_{Ks} Potassium Channels

KVLQT1 is located on chromosome 11p5.5 (Keating et al., 1991) in a region associated with Beckwith-Wiedemann syndrome (Lee et al., 1997). Northern analyses indicate that *KVLQT1* is expressed in the heart, placenta, lung, kidney (Wang et al., 1996), inner ear and pancreas, with greatest expression in the pancreas (Yang et al., 1997). *KVLQT1* and other genes in the region are imprinted, with paternal silencing in most tissues. However, *KVLQT1* is not imprinted in the heart (Lee et al., 1997). Two homologs of *KVLQT1* (*KCNQ2, KCNQ3*) have been identified in the brain and associated with benign familial neonatal seizures, an inherited form of epilepsy (Charlier et al., 1998; Singh et al., 1998; Yang et al., 1998).

The cDNA-predicted amino acid sequence of *KVLQT1* suggests that this gene encodes voltage-gated potassium channel α subunits. It has six putative membrane-spanning domains, S1–S6, including a voltage sensor (S4) and a potassium channel pore signature sequence between S5 and S6 (Figure 2). The intracellular N-terminal segment of KVLQT1 is short. Mutational analyses have revealed 85 mutations of *KVLQT1* coding sequences, representing ~40% of known arrhythmia-associated mutations discovered to date. Most of these mutations are missense mutations located in membrane spanning regions as well as the pore region.

Heterologous expression of *KVLQT1* in mammalian cells and *Xenopus* oocytes revealed that this gene encodes α subunits that form voltage-gated potassium channels (Barhanin et al., 1996; Sanguinetti et al., 1996b). However, the biophysical properties of the induced current were unlike any potassium current identified in cardiac myocytes. This observation led to the hypothesis that KVLQT1 subunits might assemble with subunits encoded by another gene to form a cardiac potassium channel.

minK, which is located on chromosome 21, received this name because it was thought to encode the minimal

potassium channel subunit. Only 129 amino acids long, this predicted amino acid sequence has room for one putative membrane-spanning domain. It contains no potassium channel pore signature sequence and no putative voltage-sensing domain. Although it is not a common cause of arrhythmia susceptibility, mutations in this gene have been associated with long QT syndrome and homozygous mutations cause Jervell and Lange-Nielson syndrome (Schulze-Bahr et al., 1997; Splawski et al., 1997a, 1997b). Ten arrhythmia-associated mutations of *minK* have been identified. This represents ∼5% of long QT syndrome mutations identified to date.

minK was initially cloned by functional expression in *Xenopus* oocytes (Takumi et al., 1988). The biophysical properties of the current elicited by expression of minK were similar to cardiac I_{Ks}, one of the main currents responsible for termination of the cardiac action potential. Thus, investigators concluded that *minK* encoded subunits that formed cardiac I_{Ks} channels. However, there were several problems with this hypothesis. First, as noted above, the structure of minK was unusual. The typical voltage-gated potassium channel has six membrane-spanning domains and four subunits are required for assembly and formation of functional channels. Because minK was small and only had one putative membrane-spanning domain, investigators hypothesized that many subunits might assemble to form functional channels. Some experiments, however, suggest that only two units were required for expression (Wang and Goldstein, 1995; Tai et al., 1997). Second, physiologic studies indicated that expression of minK in mammalian cells failed to induce a current. Finally, expression of increasing amounts of minK in *Xenopus* oocytes did not lead to increasing current, indicating saturability.

It is now clear that minK β subunits assemble with KVLQT α subunits to form cardiac I_{Ks} channels (Barhanin et al., 1996; Sanguinetti et al., 1996b). Heterologous expression of minK alone in mammalian cells produced no current. By contrast, heterologous expression of KVLQT1 and minK together led to a large potassium current with the biophysical properties of cardiac I_{Ks}. Although the stoichiometry of coassembly is not yet known, it is likely that four KVLQT1 α subunits assemble with four minK β subunits to form these channels.

How is it that *minK* alone can be functionally expressed in *Xenopus* oocytes? The explanation is that a homolog of *KVLQT1*, *XKVLQT1*, is constitutively expressed in *Xenopus* oocytes, but at a relatively low level (Sanguinetti et al., 1996b). This homolog can interact with minK, forming an I_{Ks}-like channel.

At least two molecular mechanisms account for reduced KVLQT1 function in the long QT syndrome (Wollnik et al., 1997; Wang et al., 1999). In the first, disease-associated intragenic deletions of one *KVLQT1* allele result in syntheses of abnormal subunits that do not assemble with normal subunits. As a result, only normal subunits form the functional tetrameric channels. This loss-of-function mechanism results in a 50% reduction in the number of functional channels. In the second mechanism, missense mutations result in synthesis of KVLQT1 subunits with subtle structural abnormalities. Many of these subunits can assemble with normal subunits, forming heterotetramers with varying stoichiometry. Channels formed from the coassembly of normal

and mutant subunits have reduced or no function. The net effect is a greater than 50% reduction in channel function, a dominant-negative effect. The severity of the dominant-negative effect varies considerably depending on the site and type of mutation. In some cases, the dominant-negative effect is relatively small whereas in others the effect is complete, leading to marked reduction of I_{Ks} even in heterozygotes. Missense mutations in the pore sequences seem to be particularly potent. The severity of the dominant-negative effect likely has an impact on the severity of arrhythmia susceptibility in individuals. However, there are many factors that affect arrhythmia susceptibility, and extensive phenotypic variability can be seen between family members carrying the same primary genetic mutations.

KVLQT1 and *minK* are both expressed in the inner ear. Here, the channel functions to produce a potassium-rich fluid known as endolymph that bathes the organ of Corti, the cochlear organ responsible for hearing. Individuals with Jervell and Lange-Nielsen syndrome have homozygous mutations of *KVLQT1* or *minK*, and therefore have no functional I_{Ks} channels. As noted above, these individuals have severe arrhythmia susceptibility and congenital neural deafness. The mechanism of deafness in these individuals is that the lack of I_{Ks} leads to inadequate endolymph production and deterioration of the organ of Corti (Vetter et al., 1996). Deafness can also result from mutations in *KCNQ4*, a gene that encodes a homolog of *KVLQT1* that is highly expressed within sensory outer hair cells of the inner ear (Kubisch et al., 1999).

HERG Encodes the α Subunit of Cardiac I_{Kr} Potassium Channels

HERG, located on chromosome 7q35-q36, is expressed primarily in the heart (Curran et al., 1995). *HERG* was originally identified from a human hippocampal cDNA library (Warmke and Ganetzky, 1994) and is also expressed in neural crest–derived neurons and microglia. Ninety-four distinct mutations of *HERG* have been identified (Splawski et al., 2000). These represent 45% of the total number of long QT syndrome mutations found to date.

Based on its predicted amino acid sequence, *HERG* was thought to encode a typical voltage-gated potassium channel α subunit with 6 membrane-spanning domains (S1–S6), a voltage sensor (S4), and a K⁺-selective pore between S5 and S6. HERG has a large intracellular C-terminal region containing a cyclic nucleotide binding domain. HERG also has a large N-terminal domain, the first 135 amino acids of which are highly conserved with comparable domains in related channels. The structure of the N-terminal domain has been solved and has structural similarity to PAS (Per-Arnt-Sim) domains (Morais et al., 1998). Proteins with PAS domains are frequently involved in signal transduction. Analysis of long QT syndrome-associated missense mutations located in the PAS domain of HERG revealed that this region is important in mediating the slow rate of channel deactivation (Chen et al., 1999).

Expression of *HERG* in heterologous systems led to the discovery that this gene encodes α subunits that form cardiac I_{Kr} potassium channels, the second of the two channels primarily responsible for termination of

the plateau phase of the action potential (Sanguinetti et al., 1995; Trudeau et al., 1995). One of the unusual biophysical properties of I_{Kr} channels that was reproduced by HERG in *Xenopus* oocytes is the bell-shaped current–voltage relationship, a rectification caused by C-type inactivation (Smith et al., 1996). This property accounts for the relative importance of I_{Kr} during phase 3 of the cardiac action potential. During repolarization of the action potential, HERG channels rapidly recover from inactivation into the open state. This results in an increase in the magnitude of I_{Kr} during the first half of phase 3 repolarization despite a decrease in the electrochemical driving force for outward flux of K^+ (Spector, 1996).

Although many of the biophysical properties of HERG current in heterologous systems were nearly identical to cardiac I_{Kr}, two properties were out of line (Sanguinetti et al., 1995; Abbott et al., 1999). First, although deactivation of cardiac I_{Kr} was relatively slow, deactivation of HERG channels was much slower. Second, the kinetics and voltage dependence of I_{Kr} block by methanesulfonanilide drugs were different than HERG channels. This problem led to the hypothesis that HERG, like KVLQT1, might assemble with an unknown β subunit to form cardiac I_{Kr} channels.

MiRP1, or *minK*-related protein 1, is located on chromosome 21, just 70 kb from *minK* (Abbott et al., 1999). The two genes have significant homology at the DNA and amino acid level and likely resulted from a recent duplication. Missense mutations of *MiRP1* have been associated with long QT syndrome, indicating that it is an arrhythmia-susceptibility gene. When MiRP1 was expressed with HERG in heterologous systems, the biophysical and pharmacologic properties of the resultant current were nearly identical to I_{Kr} in cardiac myocytes (Abbott et al., 1999). Thus, HERG α subunits assemble with MiRP1 β subunits to form cardiac I_{Kr} channels.

Many *HERG* mutations cluster around the membrane-spanning domains and the pore region. Some of these mutations, such as early nonsense mutations, have a pure loss-of-function effect. Oftentimes the encoded mutant proteins misfold and are rapidly degraded (Zhou et al., 1998), leading to a dominant-negative effect or haploinsufficiency. However, many long QT syndrome–associated mutations in *HERG* are missense mutations. Because functional I_{Kr} channels are comprised of hetero-multimers with several HERG subunits, it is possible that many of these mutations have a dominant-negative affect on channel function. Heterologous expression studies indicate that this is a common effect and that different mutations lead to a spectrum of dominant-negative suppression of channel function (Roden et al., 1996; Sanguinetti et al., 1996a; Li et al., 1997).

RyR2 Encodes the Calcium Release Channel
RyR2 encodes the ryanodine receptor, a calcium-induced calcium release channel located in cardiac sarcoplasmic reticulum. RyR2 channels are activated by Ca^{2+} that transiently enters the cell through plasma membrane–bound L-type calcium channels during depolarization of the cardiac myocyte. This Ca^{2+} triggers the release of Ca^{2+} stored in the sarcoplasmic reticulum through RyR2 channels that in turn initiates activation
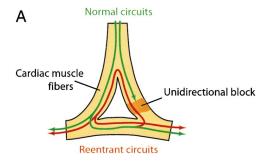
of the contractile apparatus. The four mutations identified in *RyR2* to date are missense mutations. The functional consequences of these mutations are not yet known. A likely possibility, however, is episodic, stress-induced Ca^{2+} overload in cardiac myocytes, leading to a substrate for arrhythmia.

In summary, all known arrhythmia susceptibility genes encode cardiac ion channels. *SCN5A* encodes sodium channels that are responsible for initiating cardiac action potentials. *HERG* encodes α subunits that assemble with MiRP1 β subunits to form cardiac I_{Kr} potassium channels, whereas KVLQT1 assembles with minK to form cardiac I_{Ks} potassium channels. I_{Kr} and I_{Ks} are responsible for termination of the plateau phase and contribute to final repolarization of the cardiac action potential. *RyR2* encodes the ryanodine receptor/calcium release channel crucial for excitation–contraction coupling. Mutations of *SCN5A* associated with long QT syndrome destabilize the channel inactivation gate, resulting in repetitive reopening of mutant channels and abnormal depolarizing sodium current during the plateau phase of the action potential. Thus, gain-of-function mutations of the cardiac sodium channel cause long QT syndrome. By contrast, loss-of-function mutations of the cardiac sodium channel cause idiopathic ventricular fibrillation with or without baseline conduction abnormalities. Mutations of *KVLQT1*, *HERG*, *minK*, and *MiRP1* cause a loss of function, often with a dominant-negative effect that leads to a reduction in repolarizing current. *RyR2* mutations probably lead to abnormal intracellular calcium metabolism. Taken together, these studies demonstrate that mutations of cardiac ion channels cause arrhythmia susceptibility through multiple molecular mechanisms.

Reentry, a Fundamental Mechanism of Arrhythmia
Together with previous physiologic studies, recent genetic advances provide a picture of cardiac arrhythmias at the molecular, cellular, and organ levels. Gain-of-function mutations of cardiac sodium channels and loss-of-function mutations of potassium channels delay myocyte repolarization. Loss-of-function mutations of sodium channels cause conduction abnormalities. Calcium release channel dysfunction probably causes calcium overload. These channels are expressed at varying levels in different regions of the heart, so the effect of channel dysfunction has regional variability. Regional abnormalities of cardiac repolarization or conduction provide a substrate for arrhythmia. For example, during a prolonged action potential, myocytes are relatively refractory to electrical excitation by neighboring myocytes. Dispersion of refractoriness can lead to unidirectional block of a wave of electrical excitation (Figure 4A). Thus, pockets of cells that are temporarily unable to conduct the normal flow of electrical activity in the heart create a substrate for arrhythmia.

Although unidirectional block can increase the risk of arrhythmia, it is not sufficient; a triggering mechanism is still required. The trigger for arrhythmia in the long QT syndrome is believed to be spontaneous secondary depolarizations that arise during or just following the plateau phase of action potentials. Secondary depolarizations appear as premature, small action potentials
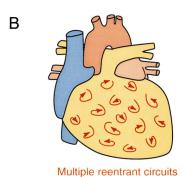
Figure 4. Unidirectional Block and Reentry, a Fundamental Mechanism of Arrhythmia

(A) Abnormal cardiac repolarization, conduction, or intracellular calcium homeostasis can lead to episodic unidirectional block, a substrate for arrhythmia. Green arrows represent the normal conduction in a bifurcated pathway. Conduction is blocked in an area of refractory tissue (unidirectional block). If conduction velocity is slowed, then reentry through area of refractory tissue can occur (red arrows). (B) Multiple reentrant circuits (multiple curved arrows) is a mechanism of ventricular fibrillation, the cause of sudden death.
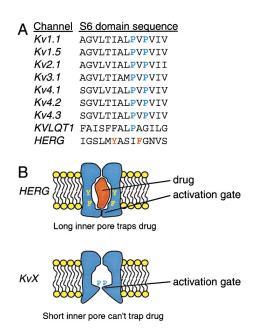

Figure 5. Unintended Block of HERG Channels by Drugs Is a Common Mechanism of Acquired Long QT Syndrome

(A) Partial sequence of the S6 transmembrane domain for several voltage-gated potassium channels. Two unusual structural features of HERG not shared by other voltage-gated K$^+$ channels are responsible for common, nonspecific drug interactions. These include a lack of proline residues in the S6 transmembrane domain, and the presence of two aromatic residues (Y, F) in this domain that face the inner cavity of the HERG channel. These aromatic residues form the drug binding site. (B) The lack of proline residues increases the size of the HERG inner cavity, thereby creating a large space for channel–drug interactions in this channel, but not other voltage-gated potassium (KvX) channels.

and are mediated by depolarizing inward calcium currents through L-type calcium channels. This cellular mechanism predicts that the autonomic nervous system can have a significant impact on arrhythmia susceptibility. Heightened sympathetic tone can substantially increase spontaneous inward current through L-type calcium channels, increasing the likelihood that the spontaneous repolarization will trigger an arrhythmia. Once triggered, the arrhythmia is maintained by a regenerative circuit of electrical activity around relatively inexcitable tissue, a phenomenon known as reentry (Figure 4A). The development of multiple reentrant circuits within the heart (Figure 4B) causes ventricular fibrillation, the arrhythmia of sudden death.

Cardiac Ion Channel Dysfunction Underlies Inherited and Acquired Arrhythmias

Abnormal cardiac repolarization, aberrant conduction, and arrhythmia susceptibility are most commonly acquired and not primarily genetic. Common acquired causes of arrhythmia include cardiac ischemia resulting from the sudden disruption of blood flow to a region of the heart, structural heart diseases like cardiomyopathy, developmental abnormalities of the heart such as arrhythmogenic right ventricular dysplasia, metabolic abnormalities like abnormal serum potassium, calcium or magnesium levels, and medications.

A common cause of acquired long QT syndrome is a side effect of numerous common medications of diverse therapeutic and structural classes (Roden, 1998). Examples of drugs associated with long QT syndrome include terfenadine, cisapride, erythromycin, amiodarone, quinidine, phenothiazines, tricyclic antidepressants, and certain diuretics (the latter mediated through drug-induced hypokalemia). Most of these medications block HERG channels, leading to reduced repolarizing potassium current and delayed myocellular repolarization. These findings show, therefore, that cardiac ion channel dysfunction underlies both inherited and acquired arrhythmias.

The problem of medication-induced long QT syndrome is a significant issue to the pharmaceutical industry and the Food and Drug Administration. Why are HERG channels so susceptible to nonspecific block by such a wide variety of medications, and why isn't acquired long QT syndrome commonly caused by block of potassium channels other than HERG that contribute to cardiac repolarization? The answer to these questions is just beginning to unfold through structural studies of HERG channels expressed in heterologous systems (Lees-Miller et al., 2000; Mitcheson et al., 2000a) (Figure 5). There are at least two important structural features that account for the unusual susceptibility of HERG channels to block by diverse drugs. First, the inner cavity of the HERG channel appears to be much larger than

any other voltage-gated K$^+$ channel. Almost all voltage-gated K$^+$ channel α subunits, except HERG, have two proline residues in the S6 domains that line the inner cavity of the channel. These prolines cause a kink in the S6 and apparently reduce the volume of the inner cavity (del Camino et al., 2000). The large inner cavity of HERG channels can accommodate and trap large drugs that other K$^+$ channels can not trap (Mitcheson et al., 2000b). Second, the S6 domains of HERG channels, but not other voltage-gated K$^+$ channels, have two aromatic residues that face into the inner cavity that may bind large aromatic drugs by a π-stacking interaction (Mitcheson et al., 2000a). The two aromatic residues located in each subunit (Tyr652, Phe656) provide a total of eight residues that can form a variable receptor site that can accommodate drugs from diverse therapeutic and structural classes. In addition, the binding affinity of drugs is enhanced by inactivation of the HERG channel (Ficker, 1998). The net effect of the structural peculiarities of HERG channels is heightened sensitivity of I_{kr} to structurally diverse drugs. Continued structural analysis of HERG channels coupled with structure–activity relationship analysis of medications will help improve our ability to predict drugs that are likely to cause a significant risk of cardiac arrhythmia.

Genetic predisposition may play an important role in drug-induced long QT syndrome. For example, less than 5% of patients receiving a drug like quinidine have arrhythmia as a side effect, irrespective of dose and other risk factors such as hypokalemia. Recent studies indicate that drug-induced arrhythmia can be associated with sporadic mutations (Abbott et al., 1999) and common polymorphisms (Sesti et al., 2000) in *MiRP1*. It is likely that mutations or polymorphisms in all of the genes associated with the inherited forms of long QT syndrome will eventually be shown to increase the risk of the acquired form of this disease.

Multiple Events Are Required to Induce Cardiac Arrhythmias
Studies of inherited and acquired arrhythmias have led us to hypothesize a multi-hit mechanism for this disease. It is clear that at least three things have to go wrong simultaneously for a life-threatening arrhythmia to occur. For example, most individuals carrying one mutant allele of an arrhythmia susceptibility gene have few, if any, arrhythmias. By contrast, individuals carrying two mutant alleles (e.g., Jervell and Lange-Nielson syndrome) have many arrhythmias and usually die during childhood unless effective treatment is implemented. That these individuals nevertheless live into early childhood, even when untreated, indicates that an additional event, such as the introduction of a medication, hypokalemia, or a sinus pause, is required for an arrhythmia. It is clear, however, that a genetic mechanism is not a prerequisite for an arrhythmia. It is known, for example, that arrhythmia can be induced in virtually anyone with the right combination of drug, hypokalemia, and a long sinus pause.

Arrhythmia genetics and physiology have converged with a growing body of evidence implicating ion channels in episodic diseases of excitable cells, including disorders of skeletal muscle, vascular smooth muscle,

and central neurons. Together, these studies have defined a new class of disease, ion channelopathies (Ashcroft, 2000).

The Future: Prediction, Prevention, and Treatment of Cardiac Arrhythmias
Despite recent advances, the fields of arrhythmia genetics, physiology, and therapy are still immature. Major problems that appear most prominent include the identification of all arrhythmia susceptibility genes, the identification of common genetic variants that contribute to arrhythmia susceptibility in the general population and the implementation of reliable, cost-effective genetic testing. Genomics and the human genome project have already had a major impact on this field, and that influence will continue to grow in the near future. Early genetic studies involved tedious methods of positional cloning, because relatively little of the human genome was mapped, genetically or physically, and few human genes were defined. The limitations of the technology available a decade ago added to the difficulties of these experiments. The early genetic linkage analyses, for example, required the use of restriction fragment length polymorphisms (RFLPs) and Southern blots. The situation is completely different today, and in the very near future genomics and the human genome project will empower these fields to an even greater extent. Genetic maps will be at the limits of resolution, all human genes will be defined and available in online databases, and genetic technologies, particularly DNA sequence analysis, will be robust, reliable, and inexpensive. DNA sequence analysis, in particular, will greatly facilitate the implementation of effective genetic testing for large populations using existing information regarding arrhythmia susceptibility genes.

Although prospects for future research are promising, at least one significant hurdle remains—ascertainment and phenotypic characterization of individuals with arrhythmia susceptibility and appropriate controls. The identification of novel arrhythmia genes and common arrhythmia susceptibility variants will involve genetic epidemiology—the genotypic characterization of large numbers of carefully phenotyped individuals. For the most part, the process of ascertaining and phenotypically characterizing individuals has been slow to change, involving a great deal of one-on-one effort in a process that is not easily scalable. Even here, however, new technology holds the promise for significant improvement. The internet revolution has connected populations of individuals separated by large distances to instantaneously and effortlessly meet and communicate about many subjects, including disease. Many for- and not-for-profit organizations have created web sites aimed at unifying, organizing, empowering, and informing individuals with virtually every imaginable health concern. By creating and working with these websites, investigators may accelerate the rate-limiting step of the human molecular genetic process.

Functional genomics, thus far largely limited to expression and protein–protein interaction studies, will also have an impact on this field. It is already clear that by examining the expression of arrhythmia genes in tissues other than the heart, one can hypothesize the

existence of pathology in other tissues that were not apparent from previous clinical studies. The clinical world tends to focus on the most severe, life-threatening, and obvious phenotypic features of the disorder. It is hard to ignore congenital deafness and sudden arrhythmic death. However, many of these genes are expressed in other tissues and evidence of pathology in these tissues is beginning to emerge. *KVLQT1*, for example, is expressed in the pancreas and it may be discovered that mutations in this gene cause a subtle risk factor for pancreatitis.

As most ion channels are heteromultimers, and may be modulated by interaction with signaling molecules, databases of protein–protein interactions will also be valuable. It is likely, however, that each interaction will require validation. It is already known, for example, that minK can interact with KVLQT1 and HERG when overexpressed in heterologous systems (McDonald et al., 1997). It is not clear, however, that the interaction of minK with HERG has physiologic relevance (Abbott et al., 1999). Nevertheless, broad functional databases will have great value, at least as a starting point.

Recent molecular and cellular studies have important implications for the prevention and treatment of arrhythmias. Identification and characterization of arrhythmia susceptibility genes provide the foundation for prevention thorough genetic testing. The observation that HERG channel function is paradoxically sensitive to extracellular potassium concentrations highlights the importance of maintaining normal electrolyte levels and provides a new strategy for treatment (Compton et al., 1996). The most important therapeutic consequence of this work, however, is the message it delivers to physicians and to the pharmaceutical industry: the observations that gain- and loss-of-function mutations of the cardiac sodium channel both cause arrhythmia susceptibility indicate that drugs that modulate cardiac ion channel function may reduce the risk of one type of arrhythmia but increase the risk of another. Thus, chronic use of cardiac ion channel blockers can be dangerous. The future of arrhythmia therapy may be devices that measure cardiac conduction and repolarization, deliver appropriate antiarrhythmic drugs when needed, and provide a safety net in the form of automatic internal defibrillation (Moss, 1997).

References

Abbott, G.W., Sesti, F., Splawski, I., Buck, M.E., Lehmann, M.H., Timothy, K.W., Keating, M.T., and Goldstein, S.A.N. (1999). MiRP1 forms I_Kr potassium channels with HERG and is associated with cardiac arrhythmia. Cell *97*, 175–187.

Abildskov, J. (1991). The sympathetic imbalance hypothesis of QT interval prolongation. J. Cardiovasc. Electrophysiol. *2*, 355–359.

Ashcroft, F.M. (2000). Ion Channels and Disease (San Diego, CA: Academic Press).

Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996). KVLQT1 and IsK (minK) proteins associate to form the IKs cardiac potassium current. Nature *384*, 78–80.

Bennett, P.B., Yazawa, K., Makita, N., and George, A.L. (1995). Mo-

lecular mechanism for an inherited cardiac arrhythmia. Nature *376*, 683–685.

Brugada, J., Brugada, R., and Brugada, P. (1997). Right bundle branch block, ST segment elevation in leads V1–V3: a marker for sudden death in patients without demonstrable structural heart disease. Circulation *96*, 1151.

Charlier, C., Singh, N.A., Ryan, S.G., Lewis, T.B., Reus, B.E., Leach, R.J., and Leppert, M. (1998). A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat. Genet. *18*, 53–55.

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

Chen, Q., Kirsch, G.E., Zhang, D., Brugada, R., Brugada, J., Brugada, P., Potenza, D., Moya, A., Borggrefe, M., Breithardt, G., et al. (1998). Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature *392*, 293–296.

Compton, S.J., Lux, R.L., Ramsey, M.R., Strelich, K.R., Sanguinetti, M.C., Green, L.S., Keating, M.T., and Mason, J.W. (1996). Gene derived therapy in inherited long QT syndrome: correction of abnormal repolarization by potassium. Circulation *94*, 1018–1022.

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

del Camino, D., Holmgren, M., Liu, Y., and Yellen, G. (2000). Blocker protection in the pore of a voltage-gated K$^+$ channel and its structural implications. Nature *403*, 321–325.

Duggal, P., Vesely, M.R., Wattanasirichaigoon, D., Villafane, J., Kaushik, V., and Beggs, A.H. (1998). Mutation of the gene for IsK associated with both Jervell and Lange-Nielsen and Romano-Ward forms of Long-QT syndrome. Circulation *97*, 142–146.

Dumaine, R., Wang, Q., Keating, M.T., Hartmann, H.A., Schwartz, P.J., Brown, A.M., and Kirsch, G.E. (1996). Multiple mechanisms of sodium channel-linked long QT syndrome. Circ. Res. *78*, 914–924.

Ficker, E. (1998). Molecular determinants of dofetilide block of HERG K+ channels. Circ. Res. *82*, 386–395.

Gellens, M., George, A., Chen, L., Chahine, M., Horn, R., Barchi, R., and Kallen, R. (1992). Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc. Natl. Acad. Sci. USA *89*, 554–558.

Hoorntje, T., Alders, M., van Tintelen, P., van der Lip, K., Sreeram, N., van der Wal, A., Mannens, M., and Wilde, A. (1999). Homozygous premature truncation of the HERG protein: the human HERG knockout. Circulation *100*, 1264–1267.

Jervell, A., and Lange-Nielsen, F. (1957). Congenital deaf-mutism, functional heart disease with prolongation of the QT interval, and sudden death. Am. Heart J. *54*, 59–68.

Jiang, C., Atkinson, D., Towbin, J., Splawski, I., Lehmann, M., Li, H., Timothy, K., Taggart, R., Schwartz, P., Vincent, G., et al. (1994). Two long QT syndrome loci map to chromosome 3 and 7 with evidence for further heterogeneity. Nat. Genet. *8*, 141–147.

Kannel, W., Cupples, A., and D'Agostino, R. (1987). Sudden death risk in overt coronary heart diseases: the Framingham study. Am. Heart J. *113*, 799–804.

Keating, M., Atkinson, D., Dunn, C., Timothy, K., Vincent, G.M., and Leppert, M. (1991). Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. Science *252*, 704–706.

Krishnan, S.C., and Antzelevitch, C. (1991). Sodium channel block produces opposite electrophysiological effects in canine ventricular epicardium and endocardium. Circ. Res. *69*, 277–291.

Kubisch, C., Schroeder, B.C., Friedrich, T., Lutjohann, B., El-Amraoui, A., Marlin, S., Petit, C., and Jentsch, T.J. (1999). KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell *96*, 437–446.

Lazzara, R. (1997). Twisting of the points. J. Am. Coll. Cardiol. *29*, 843–845.

Lee, M.P., Hu, R.-J., Johnson, L.A., and Feinberg, A.P. (1997). Human *KVLQT1* gene shows tissue-specific imprinting and encompasses

Beckwith-Wiedemann syndrome chromosomal rearrangements. Nat. Genet. *15*, 181–185.

Lees-Miller, J.P., Duan, Y., Teng, G.Q., and Duff, H.J. (2000). Molecular determinant of high-affinity dofetilide binding to HERG1 expressed in Xenopus oocytes: involvement of S6 sites. Mol. Pharmacol. *57*, 367–374.

Li, X., Xu, J., and Li, M. (1997). The human delta1261 mutation of the HERG potassium channel results in a truncated protein that contains a subunit interaction domain and decreases the channel expression. J. Biol. Chem. *272*, 705–708.

Marks, M., Whisler, S., Clericuzio, C., and Keating, M. (1995). A new form of long QT syndrome associated with syndactyly. J. Am. Coll. Cardiol. *25*, 59–64.

Martini, B. (1989). Ventricular fibrillation without apparent heart disease: description of six cases. Am. Heart J. *118*, 1203–1209.

McDonald, T., Yu, Z., Ming, Z., Palma, E., Meyers, M.B., Wang, K.W., Goldstein, S.A., and Fishman, G.I. (1997). A minK-HERG complex regulates the cardiac potassium current I-Kr. Nature *388*, 289–292.

Mitcheson, J.S., Chen, J., Lin, M., Culberson, C., and Sanguinetti, M.C. (2000a). A structural basis for drug-induced long QT syndrome. Proc. Natl. Acad. Sci. USA *97*, 12329–12333.

Mitcheson, J.S., Chen, J., and Sanguinetti, M.C. (2000b). Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. J. Gen. Physiol. *115*, 229–239.

Morais, J., Lee, A., Cohen, S., Chait, B., Li, M., and Mackinnon, R. (1998). Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell *5*, 649–655.

Moss, A.J. (1995). ECG T-wave patterns in genetically distinct forms of the hereditary long QT syndrome. Circulation *92*, 2929–2934.

Moss, A.J. (1997). Update on MADIT: the Multicenter Automatic Defibrillator Implantation Trial. The long QT interval syndrome. Am. J. Cardiol. *79*, 16–19.

Neyroud, N., Tesson, F., Denjoy, I., Leibovici, M., Donger, C., Barhanin, J., Faure, S., Gary, F., Coumel, P., Petit, C., et al. (1997). A novel mutation in the potassium channel gene *KVLQT1* causes the Jervell and Lange-Nielsen cardioauditory syndrome. Nat. Genet. *15*, 186–189.

Priori, S.G., Napolitano, C., Tiso, N., Memmi, M., Vignati, G., Bloise, R., Sorrentino, V., and Danieli, G.A. (2000). Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholamine polymorphic ventricular tachycardia. Circulation *102*, r49–r53.

Roden, D.M. (1998). Mechanisms and management of proarrhythmia. Am. J. Cardiol. *82*, 49I–57I.

Roden, D.M., Lazzara, R., Rosen, M., Schwartz, P.J., Towbin, J., and Vincent, G.M. (1996). Multiple mechanisms in the long-QT syndrome. Current knowledge, gaps, and future directions. The SADS Foundation Task Force on LQTS. Circulation *94*, 1996–2012.

Romano, C., Gemme, G., and Pongiglione, R. (1963). Artimie cardiach rare dell'eta pediatrica. II. Accessi sincopali per fibrillazione ventricolare parossitica. Clin. Pediatr. *45*, 656–683.

Sanguinetti, M.C., and Jurkiewicz, N.K. (1990). Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class-III antiarrhythmic agents. J. Gen. Physiol. *96*, 195–215.

Sanguinetti, M.C., Jiang, C., Curran, M.E., and Keating, M.T. (1995). A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell *81*, 299–307.

Sanguinetti, M.C., Curran, M.E., Spector, P.S., and Keating, M.T. (1996a). Spectrum of HERG K^+ channel dysfunction in an inherited cardiac arrhythmia. Proc. Natl. Acad. Sci. USA *93*, 2208–2212.

Sanguinetti, M.C., Curran, M.E., Zou, A., Shen, J., Spector, P.S., Atkinson, D.L., and Keating, M.T. (1996b). Coassembly of KVLQT1 and minK (IsK) proteins to form cardiac IKs potassium channel. Nature *384*, 80–83.

Schott, J., Charpentier, F., Peltier, S., Foley, P., Drouin, E., Bouhour, J., Donnelly, P., Vergnaud, G., Bachner, L., Moisan, J., et al. (1995). Mapping of a gene for long QT syndrome to chromosome 4q25-27. Am. J. Hum. Genet. *57*, 1114–1122.

Schulze-Bahr, E., Wang, Q., Wedekind, H., Haverkamp, W., Chen, Q., Sun, Y., Rubie, C., Hordt, M., Towbin, J., Borggrefe, M., et al. (1997). *KCNE1* mutations cause Jervell and Lange-Nielsen syndrome. Nat. Genet. *17*, 267–268.

Schwartz, P., Moss, A., Vincent, G., and Crampton, R. (1993). Diagnostic criteria for the long QT syndrome. Circulation *88*, 782–784.

Sesti, F., Abbott, G.W., Wei, J., Murray, K.T., Saksena, S., Schwartz, P.J., Priori, S.G., Roden, D.M., George, A.L., Jr., and Goldstein, S.A. (2000). A common polymorphism associated with antibiotic-induced cardiac arrhythmia. Proc. Natl. Acad. Sci. USA *97*, 10613–10618.

Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V., et al. (1998). A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat. Genet. *18*, 25–29.

Smith, P.L., Baukrowitz, T., and Yellen, G. (1996). The inward rectification mechanism of the HERG cardiac potassium channel. Nature *379*, 833–836.

Spector, P. (1996). Fast inactivation causes rectification of the IKr channel. J. Gen. Physiol. *107*, 611–619.

Splawski, I., Timothy, K.W., Vincent, G.M., Atkinson, D.L., and Keating, M.T. (1997a). Molecular basis of the long-QT syndrome associated with deafness. N. Engl. J. Med. *336*, 1562–1567.

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

Splawski, I., Shen, J., Timothy, K.W., Lehmann, M.H., Priori, S., Robinson, J.L., Moss, A.J., Schwartz, P.J., Towbin, J.A., Vincent, G.M., and Keating, M.T. (2000). Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5A, KCNE1, and KCNE2. Circulation *102*, 1178–1185.

Tai, K.K., Wang, K.-W., and Goldstein, S.A.N. (1997). MinK potassium channels are heteromultimeric complexes. J. Biol. Chem. *272*, 1654–1658.

Takumi, T., Ohkubo, H., and Nakanishi, S. (1988). Cloning of a membrane protein that induces a slow voltage-gated potassium current. Science *242*, 1042–1045.

Trudeau, M.C., Warmke, J.W., Ganetzky, B., and Robertson, G.A. (1995). HERG, a human inward rectifier in the voltage-gated potassium channel family. Science *269*, 92–95.

Vetter, D.E., Mann, J.R., Wangemann, P., Liu, J., McLaughlin, K.J., Lesage, F., Marcus, D.C., Lazdunski, M., Heinemann, S.F., and Barhanin, J. (1996). Inner ear defects induced by null mutation of the isk gene. Neuron *17*, 1251–1264.

Vincent, G.M., Timothy, K., Leppert, M., and Keating, M. (1992). The spectrum of symptoms and QT intervals in carriers of the gene for the long QT syndrome. N. Engl. J. Med. *327*, 846–852.

Wang, K., and Goldstein, S. (1995). Subunit composition of MinK potassium channels. Neuron *14*, 1303–1309.

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

Wang, W., Shen, J., Li, Z., Timothy, K., Vincent, G., Priori, S., Schwartz, P., and Keating, M. (1995b). Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. Hum. Mol. Genet. *4*, 1603–1607.

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., et al. (1996). Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat. Genet. *12*, 17–23.

Wang, Z., Tristani-Firouzi, M., Xu, Q., Lin, M., Keating, M.T., and Sanguinetti, M.C. (1999). Functional effects of mutations in KvLQT1 that cause long QT syndrome. J. Cardiovasc. Electrophysiol. *10*, 817–826.

Ward, O. (1964). A new familial cardiac syndrome in children. J. Ir. Med. Assoc. *54*, 103–106.

Warmke, J., and Ganetzky, B. (1994). A family of potassium channel

genes related to *eag* in *Drosophila* and mammals. Proc. Natl. Acad. Sci. USA *91*, 3438–3442.

Willich, S., Levy, D., Rocco, M., Tofler, G., Stone, P., and Muller, J. (1987). Circadian variation in the incidence of sudden cardiac death in the Framingham heart study population. Am. J. Cardiol. *60*, 801–806.

Wollnik, B., Schroeder, B.C., Kubisch, C., Esperer, H.D., Wieacker, P., and Jentsch, T.J. (1997). Pathophysiological mechanisms of dominant and recessive KVLQT1 K^+ channel mutations found in inherited cardiac arrhythmias. Hum. Mol. Genet. *6*, 1943–1949.

Yang, W.P., Levesgue, P.C., Little, W.A., Conder, M.L., Shalaby, F.Y., and Blanar, M.A. (1997). KvLQT1, a voltage-gated potassium channel responsible for human cardiac arrhythmias. Proc. Natl. Acad. Sci. USA *94*, 4017–4021.

Yang, W.P., Levesque, P.C., Little, W.A., Conder, M.L., Ramakrishnan, P., Neubauer, M.G., and Blanar, M.A. (1998). Functional expression of two KvLQT1-related potassium channels responsible for an inherited idiopathic epilepsy. J. Biol. Chem. *273*, 19419–19423.

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