Genetics of Cardiac Arrhythmias and Sudden Cardiac Death

SILVIA G. PRIORI AND CARLO NAPOLITANO

Molecular Cardiology, IRCCS Fondazione Salvatore Maugeri, and University of Pavia, 27100 Pavia, Italy

ABSTRACT: This presentation deals with the molecular substrates of the inherited diseases leading to genetically determined cardiac arrhythmias and sudden death. In the first part of this article the current knowledge concerning the molecular basis of cardiac arrhythmias will be summarized. Second, we will discuss the most recent evidence showing that the picture of the molecular bases of cardiac arrhythmias is becoming progressively more complex. Thanks to the contribution of molecular genetics, the genetic bases, pathogenesis, and genotype–phenotype correlation of diseases—such as the long QT syndrome, the Brugada syndrome, progressive cardiac conduction defect (Lenegre disease), catecholaminergic polymorphic ventricular tachycardia, and Andersen syndrome—have been progressively unveiled and shown to have an extremely high degree of genetic heterogeneity. The evidence supporting this concept is outlined, with particular emphasis on the growing complexity of the molecular pathways that may lead to arrhythmias and sudden death, in terms of the relationships between genetic defect(s) and genotype(s), as well as gene-to-gene interactions. The current knowledge is reviewed, focusing on the evidence that a single clinical phenotype may be caused by different genetic substrates and, conversely, a single gene may cause very different phenotypes acting through different pathways.

KEYWORDS: sudden death; ventricular arrhythmias; ventricular tachycardia; genetics; molecular substrates

INTRODUCTION

The identification of the molecular determinants of inherited arrhythmogenic diseases has been pivotal to the understanding of several aspects of cardiac arrhythmias and sudden death. Following the seminal papers on long QT syndrome carried out by Mark Keating and his group in the early 1990s, it became clear that there is a wide spectrum of clinical phenotypes caused by abnormal genes encoding for transmembrane cardiac ion channels. More recently, it has been observed that not only transmembrane ion channels cause cardiac arrhythmias, but also intracellular channel and non-ion conducting proteins may be pathophysiologically linked to inherited arrhythmias and sudden death (TABLE 1).

Address for correspondence: Prof. Silvia G. Priori, M.D., Ph.D., Molecular Cardiology, IRCCS Maugeri Foundation, Via Ferrata 27100, Pavia, Italy. Fax: +39-0382-592059.
spriori@fsm.it

**Ann. N.Y. Acad. Sci. 1015: 96–110 (2004). © 2004 New York Academy of Sciences.
doi: 10.1196/annals.1302.008**

TABLE 1. Genes and proteins causing inherited arrhythmogenic diseases

Protein family	Phenotype	Locus	Gene	Protein	Protein function	Functional abnormality
Ion channels	LQT1	11p15.5	*KCNQ1*	KVLQT1	I_{Ks} alpha subunit	loss of function
	LQT5	21q22.1–22.2	*KCNE1*	MinK	I_{Ks} beta subunit	loss of function
	LQT-JLN1	11p15.5	*KCNQ1*	KVLQT1	I_{Ks} alpha subunit	loss of function
	AFIB1	11p15.5	*KCNQ1*	KVLQT1	I_{Ks} alpha subunit	gain of function
	LQT-JLN2	21q22.1–22.2	*KCNE1*	MinK	I_{Ks} beta subunit	loss of function
	LQT2	7q35–q36	*HERG*	HERG	I_{Kr} alpha subunit	loss of function
	LQT6	21q22.1–22.2	*KCNE2*	MiRP1	I_{Kr} beta subunit	loss of function
	LQT3	3p21–23	*SCN5A*	SCN5A	I_{Na} alpha subunit	gain of function
	BrS1	3p21–23	*SCN5A*	SCN5A	I_{Na} alpha subunit	loss of function
	Lenegre	3p21–23	*SCN5A*	SCN5A	I_{Na} alpha subunit	probable loss of function
	mixed phenotypes	3p21–23	*SCN5A*	SCN5A	I_{Na} alpha subunit	loss or gain are possible
Anchoring proteins	And1	17q23	*KCNJ2*	Kir2.1	I_{K1} channel	loss of function
Ca^{2+}	LQT4	4q25–27	*ANK2*	ankyrin B	ion channel targeting	loss of function
	CPVT1	1q42–43	*RyR2*	RyR2	calcium release	loss of function
	CPVT2	1p11–13.3	*CASQ2*	CASQ2	calcium storage	unknown

As a parallel development to the identification of novel genes, genotype–phenotype correlation studies are highlighting distinguishing features of each genetic variant, suggesting that each of them should be regarded as a separate disease. As a consequence of this integration of molecular and clinical studies, it has become apparent that a single genetic defect may cause a clinical phenotype not only through a direct effect on the protein function (e.g., loss of transmembrane current), but also by altering the interaction with other regulatory proteins (e.g., beta subunits and targeting proteins). Thus, cardiac ion channels may no longer be considered as "stand alone" structures, but have to be regarded as macromolecular complexes that need the integration of several components in order to work properly.

Here we review the current knowledge on the molecular substrates of the inherited diseases leading to arrhythmias and sudden death. We will concentrate our assessment on the evidence that a single clinical phenotype may be caused by different genetic substrates and, conversely, that a single gene may cause very different phenotypes acting through different pathways.

INHERITED ARRHYTHMIAS AND SUDDEN DEATH: THE PHENOTYPES AND THE SUBSTRATES

Long QT Syndrome

The long QT syndrome (LQTS) is an inherited arrhythmogenic disease characterized by susceptibility to life-threatening arrhythmias. Two phenotypic variants have been initially identified, the autosomal-dominant Romano Ward syndrome and the autosomal-recessive Jervell and Lange-Nielsen syndrome, in which the cardiac phenotype is associated with neurosensory deafness.[1,2] Prolonged ventricular repolarization (i.e., a prolonged QT interval) is the electrocardiographic marker of LQTS, of abnormal morphology of the T wave, and of a characteristic polymorphic ventricular tachycardia, called *torsades des pointes*, which is most often induced by activation of the sympathetic nervous system.[3]

Syncope and fainting are the typical manifestations of LQTS, and their occurrence is often precipitated by physical or emotional stress (e.g., fear, anger, loud noises, or sudden awakening).[4] Onset of symptoms is typically in the first two decades of life, including the neonatal period; but the first symptoms may appear later in life, especially among females.[4] The severity of the clinical manifestations of LQTS is highly variable, ranging from full-blown disease with markedly prolonged QT interval and recurrent syncope, to subclinical forms with borderline QT interval prolongation and no arrhythmias or syncopal events.[5] Thus, risk stratification becomes a crucial step for clinical management.

Genetic Bases

Following the initial studies that took advantage of linkage analysis to map the major genetic loci involved in the pathogenesis of LQTS,[6,7] positional cloning and the "candidate gene approach" allowed the identification of mutations in three genes: KCNQ1 (LQT1, 11p15.5, OMIM no. 192500), KCNH2 (LQT2, 7q35–36, OMIM no. 152427), and SCN5A (LQT3, 3p21–23, OMIM no. 603830).[8–10] These genes also make up the vast majority of genotyped LQTS patients. Subsequently,

mutations in two additional genes, both located on chromosome 21 (21q22.1–22.2) and called KCNE1 (LQT5; OMIM no. 176261) and KCNE2 (LQT6; OMIM no. 603796), were identified (FIG. 1). All the genes associated with LQT1–3 and LQT5–6 encode for cardiac ion channel subunits.[11] On the basis of this evidence LQTS was initially considered a "channelopathy." However, the recent finding of a mutation in the ANK2 gene encoding for ankyrin B[12] (see below) at the LQT4 locus (4q25–27, OMIM no. 600919)[13] demonstrated that the phenotype of LQTS can be caused by abnormal proteins other than cardiac ion channels (FIG. 1).

Pathogenesis

KCNQ1 (LQT1-JLN1) and KCNE1 (LQT5-JLN2). The cardiac delayed rectifier current (I_K) is a major determinant of phase 3 of the cardiac action potential. It comprises two independent components: one rapid (I_{Kr}) and one slow, catecholamine-sensitive component (I_{Ks}). The KCNQ1 gene and the KCNE1 gene encode, respectively, for the alpha and beta subunits of the potassium channel conducting the I_{Ks}. LQT1 is the most prevalent genetic form of LQTS, being responsible for approximately half of genotyped patients; while LQT5 (I_{Ks} beta subunit), caused by mutations in the KCNE1 gene, is a rather uncommon variant, accounting for approximately 2–3% of cases. Homozygous or compound heterozygous mutations of KCNQ1 (JLN1) and KCNE1 (JLN2) have also been associated with the recessive Jervell and Lange-Nielsen form of LQTS.[14,15]

Expression studies of mutated proteins suggested multiple mechanisms of functional failure. Defective proteins may coassemble with wild-type protein and exert a dominant-negative effect. Other mutations lead to defective proteins that do not assemble with wild-type peptides, resulting in a loss of function that reduces the I_{Ks} current by 50% (haploinsufficency). Finally, defective peptides may not even reach the membrane of the cardiac cell because the mutations interfere with intracellular protein trafficking.[11,16]

KCNH2 (LQT2) and KCNE2 (LQT6). The KCNH2 and KCNE2 genes encode, respectively, for the alpha and beta subunits of the potassium channel conducting the I_{Kr} current. LQT2 is the second most common variant of LQTS, accounting for 35–40% of mutations in LQTS genotyped patients. Functional expression studies have demonstrated that mutations in the KCNH2 gene cause a reduction of I_{Kr} current. Defective proteins may either cause a dominant-negative effect on the wild-type subunits or they may not interfere with the function of the normal subunits, thus causing haploinsufficiency.[17] Trafficking abnormalities have also been reported as a consequence of KCNH2 mutations.[18,19] Mutations in the KCNE2 gene are found in the LQT6 variant of LQTS, the rarest variant of the disease, and only few KCNE2 mutations have been associated with LQTS <http://pc4.fsm.it:81/cardmoc>.

SCN5A (LQT3). The SCN5A gene encodes for the cardiac sodium channel. The first SCN5A mutations were clustered in the regions that regulate the inactivation of the channel (delKPQ, R1623Q, and N1325S).[8] *In vitro* expression studies showed that these mutations cause an increased late inward sodium current (I_{Na}).[20] It was concluded that Na^+ channel mutations originate the LQTS phenotype by inducing a "gain of function" leading to an increase in the Na^+ inward current that prolongs action potential duration. The prevalence of LQT3 among LQTS patients is estimated to be 10–15%.

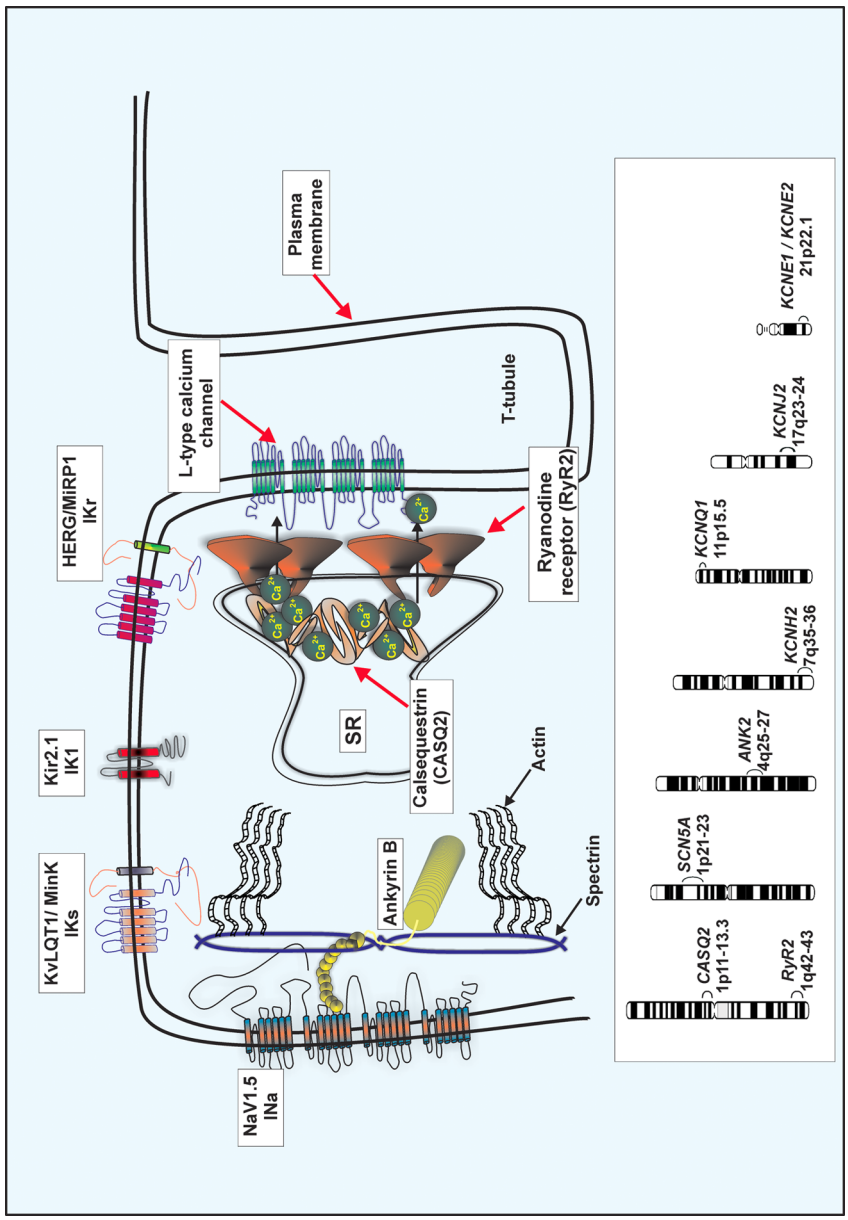

FIGURE 1. Genes and proteins causing inherited arrhythmogenic disorders. Schematic representation of the proteins involved in the pathogenesis of inherited arrhythmogenic diseases. The corresponding genes with their chromosomal localization are depicted in the **inset** at the bottom.

ANKB (LQT4). Only one family linked to this locus (4q25-q27) has been reported so far. Interestingly, the phenotype of the LQT4 patient differs from the typical LQTS. Most of the affected individuals, besides QT interval prolongation, also present with severe sinus bradycardia and paroxysmal atrial fibrillation (detected in >50% of the patients). Experimental data based on ankyrin B knockout mice suggested that this protein, located in the LQT4 critical region, was a plausible candidate gene for LQT4. Subsequently, a missense mutation in the ANK2 gene was identified in the family linked to the critical region on chromosome 4, confirming that the gene for LQT4 is the ankyrin gene.[12]

At variance with the other genes, ANK2 does not encode an ion channel but does encode a structural protein called ankyrin B that is most likely implicated in ion channels anchoring to the cellular membrane.[12]

Genotype–Phenotype Correlation Studies in LQTS

Genotype–phenotype correlation in the LQTS has been one of the most active lines of research in the last few years. For a detailed description of such studies, which is beyond the scope of this chapter, we refer the readers to other publications.[21,22] Here we will use the available evidence only to point out the remarkable degree of phenotypic variability that has been reported as part of LQT clinical presentation.

Gene-specific differences have been reported in terms of morphology of the ST-T wave complex,[23] triggers for cardiac events,[4,24,25] and risk of cardiac events.[26] The increased availability of data collected among genotyped patients has allowed the development of risk stratification models based on the genetic substrate. Priori *et al*. recently reported information on 647 LQTS patients from 193 genotyped families.[26] This study showed a lower cumulative event-free survival among LQT2 versus LQT1 patients, and a similar trend was present among LQT3 versus LQT1. These findings allowed us to propose the first genotype-based risk stratification scheme.[26] In the same study it was also observed that the percentage of genetically affected patients with a normal QT interval ("silent mutation carriers") differed strikingly among genotypes, being much more frequent ($P < 0.001$) among LQT1 (36%) than LQT2 (19%), and especially than LQT3 (10%). Risk stratification may be further refined when the location of a mutation is taken into consideration. In 2002 Moss *et al.* studied 201 LQT2 patients and showed that individuals with mutations in the pore region were at considerably greater risk for cardiac events than patients with nonpore mutations, even though the difference in the incidence of aborted cardiac arrest and sudden death was not statistically significant.[27]

Genetic and Phenotypic Heterogeneity

Initially, the evidence that at least five genes are responsible for LQTS implied that the classification of the disease into two phenotypes (Romano-Ward and Jervell and Lange-Nielsen) was insufficient to completely describe the disease. Subsequently, more findings have led to the conclusion that LQTS is no longer a cardiac channelopathy since it may also be caused by mutation intracellular proteins. Therefore, genetic heterogeneity and multiplicity of mechanisms are distinguishing features of LQTS. Furthermore, besides the remarkable number of mutations reported so far, <http://pc4.fsm.it:81/cardmoc>, it is also evident that the clinical manifestations

may span from completely asymptomatic individuals to fully penetrant and symptomatic forms, even among patients harboring the same mutation. This phenomenon is defined as variable penetrance, and it represents a landmark feature of LQTS, which may have a profound effect on clinical presentation and management. The first evidence of variable penetrance was provided by Vincent *et al.,*[28] who demonstrated a large range of QT duration among genotyped patients and proposed the existence of "silent gene carriers." We further characterized incomplete penetrance[5] by reporting that families with as low as 17% of carriers show clinical signs of the disease and, as extreme example of low penetrance, by demonstrating that subclinical mutations may generate the substrate for drug-induced *torsades de pointes*.[29]

The determinants of incomplete penetrance in LQTS are far from being elucidated. Several modifier factors, genetic and/or environmental, may come into play, including genetic polymorphisms in the same gene carrying the primary genetic defect (see below). Independently from its causes, variable penetrance brings about the consequence of hampering the possibility of reliably predicting the clinical outcome in each specific subject.

Brugada Syndrome

Brugada and Brugada[30] described a novel autosomal-dominant disease occurring in the structurally normal heart and characterized by ST segment elevation in the right precordial leads (V1 to V3) and right bundle branch block, and susceptibility to ventricular tachyarrhythmias.[30] This disease is now referred to as *Brugada syndrome* (BrS). The age at onset of clinical manifestations (syncope or cardiac arrest) is the third to fourth decade of life, although malignant forms with earlier onset and even neonatal manifestations have been reported.[31] Cardiac events typically occur during sleep or at rest.[32]

Pathogenesis

The understanding of the molecular basis of BrS is still limited; so far, only one gene has been identified, and it accounts for less than 25% of cases[33]—SCN5A, the same gene responsible for LQT3 (FIG. 1). However, at variance with LQT3, *in vitro* functional characterization of SCN5A mutations showed that the overall effect is that of a loss of channel function. With this in mind, a plethora of different biophysical mechanisms, all leading to fewer functional channels, have been described, from haploinsufficiency to changes in channel inactivation and activation kinetics.[34]

Prognostic Indicators and Management

Robust genotype–phenotype correlation data are not yet available for Brugada syndrome; therefore, the clinical management of the disease must rely upon the clinical findings. Initial data[30,35] pointed to estimates of a very high lethality of the disease, which led to a widespread use of the implantable cardioverter defibrillator (ICD) to prevent life-threatening events, given the lack of effective pharmacological treatment for this disease. More recently, with the collection of larger groups of patients, the estimated lethality of the disease is much lower than initially thought.[36] Despite the lack of genetic data supporting risk stratification, we showed that a careful, noninvasive clinical evaluation of the affected patients may gather sufficient

information for a satisfactory risk profiling.[33] These data have been subsequently confirmed by other groups.[37]

PROGRESSIVE CARDIAC CONDUCTION DEFECT AND OVERLAPPING PHENOTYPES

Phenotypic Characteristics and Pathogenesis

The clinical features of Lenegre disease (OMIM no. 113900) are still ill defined. Lenegre disease, also called progressive cardiac conduction defect (PCCD), is one of the most common cardiac conduction disturbances. It is characterized by progressive alteration of cardiac conduction through the atrioventricular node, His-Purkinje system, with right or left bundle branch block. These abnormalities may lead to complete atrioventricular block, causing syncope and sudden death. An autosomal dominant pattern of transmission has been reported.

Schott *et al*.[38] described two families with conduction defects and identified, in both, a mutation in the SCN5A gene, thus suggesting for the first time that progressive conduction disease is a novel subtype of sodium channel disease. Thus, the cardiac sodium channel gene, SCN5A, is a major player in the pathogenesis of cardiac conduction defect, the third phenotype associated with this gene (FIG. 1).

Three missense mutations and one splicing mutation in the SCN5A gene have been linked to progressive cardiac conduction defect (Lenegre disease) <http://pc4.fsm.it:81/cardmoc>. As in the case of LQT3 and Brugada syndrome, the electrophysiological consequences of Lenegre-associated mutations induce a wide spectrum of cellular phenotypes: faster decay of the current, positive shift of activation and inactivation, enhancement of slow inactivation, a pronounced closed-state inactivation, and slower recovery from inactivation. Interestingly some of the abovementioned functional abnormalities have also been identified in mutations found in patients with a Brugada syndrome phenotype. The complexity of the genotype–phenotype correlation of SCN5A mutations is further emphasized by the evidence that one Lenegre mutation (G514C) shows faster recovery from the inactivated state and a reduced closed-state inactivation—that is, features previously identified in patients with LQT3 phenotype.[39]

Recently, Kyndt *et al*. reported a family with SCN5A mutation and an overlapping phenotype of Brugada syndrome and conduction defects.[40] Overlapping phenotypes between LQT3 and Brugada syndrome have been reported.[40–42] Bezzina *et al*. described the simultaneous presence of QT prolongation and ST segment elevation in a family where an in-frame insertion in the SCN5A gene (InsD1795) was present.[41] Furthermore, in a family with an in-frame SCN5A deletion that we reported, LQT3, BrS, PCCD, and sinus pauses requiring pacemaker implant were concomitantly present.[43] *In vitro* expression showed I_{Na} abnormalities compatible with both LQT3 and Brugada syndrome.[34]

Catecholaminergic Polymorphic Ventricular Tachycardia

The first formal description of catecholaminergic polymorphic ventricular tachycardia (CPVT; OMIM no. 604772) was provided by Coumel and coworkers[44,45] in 1978 and in 1995. More recently, CPVT has been recognized as a genetically deter-

mined arrhythmogenic disease, and its pathophysiological mechanisms are being progressively unveiled.

Physical activity or acute emotions are the specific triggers for arrhythmias among CPVT patients, and, at variance with LQTS (which is characterized by the unpredictability of arrhythmias onset), the CPVT-related arrhythmias are easily reproduced during an exercise stress test. A 180° alternating QRS axis on a beat-to-beat basis, the so-called bidirectional VT, is often the distinguishing presentation of CPVT-related arrhythmias.[45] However, more recent observations have pointed to the fact that CPVT patients may also show irregular polymorphic VT without a "stable" QRS vector alternans. Supraventricular arrhythmias are also frequently observed during exercise among CPVT patients.

Syncope, triggered by exercise or acute emotion, is often the first manifestation of CPVT, even if sudden cardiac death may occur in previously asymptomatic subjects. In approximately 30% of cases, the family history reveals one or multiple premature sudden deaths, which usually occur during childhood, even though later onset (after age 20) events have been reported. These sudden deaths occurring in individuals without cardiac structural abnormalities may lead to the postmortem diagnosis of idiopathic ventricular fibrillation.

Genetic Bases

The autosomal dominant CPVT was mapped by Swan *et al*. to chromosome 1q42–43,[46] and the first genetic defects in CPVT patients were reported for the first time by Priori *et al.,*[47] who demonstrated mutations of the human ryanodine receptor gene (RyR2) in four families (FIG 1). Other groups confirmed this finding soon afterwards.[48] Lahat *et al.*[49,50] provided the first evidence for a variant of CPVT, inherited as an autosomal-recessive trait. They mapped the disease in seven consanguineous Bedouin families in a 16-centimorgan interval on the short arm of chromosome 1 (1p23-21). Subsequently the same group identified CASQ2 as the gene for this variant of CPVT. More recently, other investigators[51] have reported CASQ2 mutations in autosomal-recessive CPVT (FIG. 1).

RyR2 mutations are found in approximately 50% of the patients presenting with a clinical diagnosis of CPVT, while the prevalence of CASQ2 mutations is not known. An appealing, but still unproven, hypothesis is that the CASQ2 mutation could also cause the autosomal-dominant variant of CPVT. Overall, as with other inherited forms of cardiac arrhythmias, CPVT shows genetic heterogeneity.

Pathophysiology

Both RyR2 and CASQ2 are involved in intracellular calcium homeostasis and the excitation–contraction coupling. RyR2 is a large tetrameric pore-forming protein that controls the calcium release channel from the sarcoplamic reticulum in the myocardial cells.[52] After the initial demonstration of RyR2 mutations in CPVT1 patients, an increasing number of mutations has been reported[52] <http://pc4.fsm.it:81/cardmoc> in patients with severe ventricular arrhythmias and sudden death, thus confirming the central role of RyR2 in intracellular Ca^{2+} handling in arrhythmogenesis.

CASQ2 encodes calsequestrin, a highly expressed protein in the heart, which is functionally and spatially closely linked to RyR2. Calsequestrin serves as a major

Ca^{2+} binding protein located in the terminal cisternae of the SR of cardiac muscle cells, and it binds Ca^{2+} with high capacity and with moderate affinity.

While the functional consequences of CSQ2 mutations are not yet clear, recent data have started to shed light on the functional effects of RyR2 mutations.[53] Phosphorylated CPVT-associated mutant RyR2 channels exhibit increased activity at a low cytosolic concentration of calcium. This may promote calcium leak from the sarcoplasmic reticulum. Thus, the adrenergically mediated triggered activity[54] is the likely mechanism of arrhythmias in CPVT patients.

Andersen Syndrome

Andersen *et al.*[55] reported in 1971 the case of an eight-year-old boy with short stature, hypertelorism, broad nasal root, and defect of the soft and hard palates. The definition of Andersen syndrome (And1) was used for the first time in 1994 by Tawil *et al*.[56] to describe a clinical disorder consisting of three major features: potassium-sensitive periodic paralysis, ventricular arrhythmias, and dysmorphic features. The presence of a variable degree of QT interval prolongation was pointed out in the first systematic description of the disease,[56] and subsequently, Sansone *et al*.[57] strengthened its crucial diagnostic significance. Besides QT interval prolongation, And1 patients may also present repolarization abnormalities consisting of a late repolarization component resembling a "giant" U wave. Bidirectional ventricular tachycardia has also been reported as a distinguishing pattern of arrhythmias in And1. Despite the reported sudden death,[58] arrhythmias do not appear to be a major cause of death in And1, and the disease often presents a benign outcome.[57,59]

Genetic Bases and Pathophysiology

The genetic background of LQT-And has been recently elucidated by genome-wide linkage analysis by Plaster *et al*.,[60] who successfully linked this disorder to the locus 17q23 in a large family. A candidate gene screening was carried out in the critical region, and missense mutations were identified in the KCNJ2 gene (FIG. 1). KCNJ2 encodes an inward rectifier potassium channel, Kir2.1, highly expressed in the heart, where it appears to control the resting membrane potential of cardiac myocytes. *In vitro* expression of the mutant Kir2.1 channels show[61] a reduced I_{K1} with a dominant negative effect on the wild-type subunits. Interestingly, since dysmorphic facial appearance constitutes a distinctive trait of LQT-And, the data provided by Plaster *et al*.[60] also strongly suggest that Kir2.1 plays a major role in developmental signaling.

THE GROWING COMPLEXITY OF GENETIC BASES OF ARRHYTHMIAS AND SUDDEN DEATH

One Gene, Different Diseases

The case of the cardiac sodium channel gene constitutes a paradigmatic example of the complexity of pathways leading from the genetic defect to the clinical phenotype. Following the standard investigational approach that considers a clinical phenotype to find the causes, SCN5A mutations have been identified in LQTS patients

using the results of genetic linkage studies. The evidence of the gain of channel function induced by LQT3 mutants made it rational to hypothesize that the loss-of-function mutations in this channel could exist; and consequently, SCN5A mutations associated with BrS were demonstrated. Afterwards and quite unexpectedly, SCN5A mutations were associated with a third phenotype, the progressive cardiac conduction defect, and patients/families presenting with overlapping (between LQT3, BrS, and PCCD) phenotypes have been reported. As already mentioned, the available functional data did not provide information sufficient to fully clarify the mechanisms leading to the clinical phenotype.

Another ion channel–encoding gene that may generate differential phenotypes is KCNQ1, a major cause of LQTS, but KCNQ1 has also recently been implicated in the pathogenesis of familial atrial fibrillation. In this latter case, the genetic defect causes the synthesis of protein subunits having a gain of function, as compared with the loss-of-function mutants of KCNQ1-related LQTS.[62]

Gene-to-Gene Interactions

Genetic defects on the same gene may thus cause different diseases. However, it is also becoming clear that the primary genetic defect is only one among several determinants of the phenotype. The first evidence of the role of ancillary proteins in regulating ion channel activity and in modulating the effect of mutations was provided by An *et al.* in 1998.[63] These authors showed that the D1790G mutation induces a negative shift of steady state inactivation when assessed on heteromeric SCN5A channels (coexpression of alpha- and beta-1 subunits). Interestingly, the same effect was not found in monomeric channels (alpha subunit only).[63] Mounting evidence shows that genetic polymorphisms may have a substantial impact on the clinical manifestations and that the proper function of the ion channels requires the coordination of other ancillary molecules. The first concept is well exemplified by the recent report demonstrating that the SCN5A R555H blunts the electrophysiological effects of a nearby mutation (T512I), causing conduction block and, *in vitro*, a hyperpolarizing shift of activation and inactivation.[64] An example of proteins having a remarkable, albeit indirect, effect on ion channel and cardiac excitability is provided by the study Marx and coworkers.[65] The control of KCNQ1 function through the activation of the adrenergic nervous system is relevant to the modulation of action potential duration during acute stress or exercise. It has been shown[66] that in order for this system to work properly, the intracellular adrenergic signaling requires a specific protein, yotiao, which targets the effects of beta-adrenergic receptor activation to the channel. Yotiao binds to a specific leucine zipper motif in the carboxy-terminus of KCNQ1, and LQTS mutations may disrupt this binding, thus tapering off the physiological activation of KCNQ1 by catecholamines.[66]

Additional evidence concerning the important role of protein interaction in the pathogenesis of cardiac arrhythmias has recently been provided. Ankyrins are a family of intracellular proteins that target several transmembrane proteins to their proper localization in the membrane (e.g., sodium channel, rayanodine receptor, sodium/calcium exchanger, and sodium/potassium ATPase). Experimental evidence[12] shows that ANKB causes the LQT4 variant of LQTS not through a direct effect on cardiac repolarization but probably through inducing abnormalities of the coordinated

expression of different targeted molecules, such as the sodium/calcium exchanger and sodium/potassium ATPase.

SUMMARY

The available evidence underlines the multiplicity of mechanisms that may cause inherited forms of cardiac arrhythmias. The electrical activity of the heart is finely regulated so that not only may ion channels, when altered, form a substrate for arrhythmias, but also other regulatory proteins that interact with those ion channels may be important players. In this scenario the presence of polymorphisms may take part in the modulation of the phenotype.

REFERENCES

1. ROMANO, C., G. GEMME & R. PONGIGLIONE. 1963. Aritmie cardiache rare in eta' pediatrica. Clin. Ped. **45:** 656–657.
2. JERVELL, A. & F. LANGE-NIELSEN. 1957. Congenital deaf mutism, functional heart disease with prolongation of the QT interval and sudden death. Am. Heart J. **54:** 59–61.
3. SCHWARTZ, P.J., S.G. PRIORI & C. NAPOLITANO. 2000. The long QT syndrome. *In* Cardiac Electrophysiology: From Cell to Bedside. D.P. Zipes & J. Jalife, Eds.: 597–615. W.B. Saunders. Philadelphia.
4. SCHWARTZ, P.J., S.G. PRIORI, C. SPAZZOLINI, *et al*. 2001. Genotype-phenotype correlation in the long-QT syndrome : gene-specific triggers for life-threatening arrhythmias. Circulation **103:** 89–95.
5. PRIORI, S.G., C. NAPOLITANO, P.J. SCHWARTZ. 1999. Low penetrance in the long-QT syndrome: clinical impact. Circulation **99:** 529–533.
6. KEATING, M.T., D. ATKINSON, C. DUNN, *et al*. 1991. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. Science **252:** 704–706.
7. JIANG, C., D. ATKINSON, J.A. TOWBIN, *et al*. 1994. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. Nat. Genet. **8:** 141–147.
8. WANG, Q., J. SHEN, I. SPLAWSKI, *et al*. 1995. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. Cell **80:** 805–811.
9. SANGUINETTI, M.C., C. JIANG, M.E. CURRAN, *et al*. 1995. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell **81:** 299–307.
10. WANG, Q., M.E. CURRAN, I. SPLAWSKI, *et al*. 1996. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. Nat. Genet. **12:** 17–23.
11. KEATING, M.T. & M.C. SANGUINETTI. 2001. Molecular and cellular mechanisms of cardiac arrhythmias. Cell **104:** 569–580.
12. MOHLER, P.J., J.J. SCHOTT, A.O. GRAMOLINI, *et al*. 2003. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. Nature **421:** 634-639.
13. SCHOTT, J.J., F. CHARPENTIER, S. PELTIER, *et al*. 1995. Mapping of a gene for long QT syndrome to chromosome 4q25-27. Am. J. Hum. Genet. **57:** 1114–1122.
14. NEYROUD, N., F. TESSON, I. DENJOY, *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.
15. SCHULZE-BAHR, E., Q. WANG, H. WEDEKIND, *et al*. 1997. KCNE1 mutations cause Jervell and Lange-Nielsen syndrome. Nat. Genet. **17:** 267–268.
16. PRIORI, S.G., I. RIVOLTA & C. NAPOLITANO. 2003. Genetics of long QT, Brugada and other channelopathies. *In* Cardiac Electrophysiology, 4th edit. D.P. Zipes & J. Jalife, Eds. Elsevier. Philadelphia. In press.
17. SANGUINETTI, M.C., M.E. CURRAN, P.S. SPECTOR, *et al*. 1996. Spectrum of HERG K^+-channel dysfunction in an inherited cardiac arrhythmia. Proc. Natl. Acad. Sci. USA **93:** 2208–2212.

18. ZHOU, Z., Q. GONG, M.L. EPSTEIN, *et al*. 1998. HERG channel dysfunction in human long QT syndrome. Intracellular transport and functional defects. J. Biol. Chem. **273:** 21061–21066.
19. FICKER, E., D. THOMAS, P.C. VISWANATHAN, *et al*. 2000. Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. Am. J. Physiol. Heart Circ. Physiol. **279:** H1748–1756.
20. DUMAINE, R., Q. WANG, M.T. KEATING, *et al*. 1996. Multiple mechanisms of Na^+ channel–linked long-QT syndrome. Circ. Res. **78:** 916–924.
21. PRIORI, S.G. & C. NAPOLITANO. 2003. The long QT syndrome. *In* Cardiac Repolarization. I. Gussak & C. Antzelevitch, Eds.: 169–187. Humana Press. Totowa, NJ.
22. SCHWARTZ, P.J. & S.G. PRIORI. 2003. Long QT syndrome—phenotype genotype considerations. *In* Cardiac Electrophysiology, 4th edit. D.P. Zipes & J. Jalife, Eds. Elsevier. Philadelphia. In press
23. MOSS, A.J. 2002. T-wave patterns associated with the hereditary long QT syndrome. Card. Electrophysiol. Rev. **6:** 311–315.
24. ACKERMAN, M.J., D.J. TESTER, C.J. PORTER. 1999. Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. Mayo Clin. Proc. **74:** 1088–1094.
25. MOSS, A.J., J.L. ROBINSON, L. GESSMAN, *et al*. 1999. Comparison of clinical and genetic variables of cardiac events associated with loud noise versus swimming among subjects with the long QT syndrome. Am. J. Cardiol. **84:** 876–879.
26. PRIORI, S.G., P.J. SCHWARTZ, C. NAPOLITANO, *et al*. 2003. Risk stratification in the long-QT syndrome. N. Engl. J. Med. **348:** 1866–1874.
27. MOSS, A.J., W. ZAREBA, E.S. KAUFMAN, *et al*. 2002. Increased risk of arrhythmic events in long-QT syndrome with mutations in the pore region of the human ether-a-go-go–related gene potassium channel. Circulation **105:** 794–799.
28. VINCENT, G.M., K.W. TIMOTHY, M. LEPPERT, *et al*. 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.
29. NAPOLITANO, C., P.J. SCHWARTZ, A.M. BROWN, *et al*. 2000. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. J. Cardiovasc. Electrophysiol. **11:** 691–696.
30. BRUGADA, P. & J. BRUGADA. 1992. Right bundle branch block, persistent ST segment elevation, and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. J. Am. Coll. Cardiol. **20:** 1391–1396.
31. PRIORI, S.G., C. NAPOLITANO, U. GIORDANO, *et al*. 2000. Brugada syndrome and sudden cardiac death in children. Lancet **355:** 808–809.
32. BRUGADA, J., P. BRUGADA & R. BRUGADA. 1999. The syndrome of right bundle branch block ST segment elevation in V1 to V3 and sudden death—the Brugada syndrome. Europace **1:** 156–166.
33. PRIORI, S.G., C. NAPOLITANO, M. GASPARINI, *et al*. 2002. Natural history of Brugada syndrome. Insights for risk stratification and management. Circulation **105:** 1342–1347.
34. NAPOLITANO, C., I. RIVOLTA & S.G. PRIORI. 2003. Cardiac sodium channel diseases. Clin. Chem. Lab. Med. **41:** 439–444.
35. BRUGADA, J. & P. BRUGADA. 1997. Further characterization of the syndrome of right bundle branch block, ST segment elevation, and sudden cardiac death. J. Cardiovasc. Electrophysiol. **8:** 325–331.
36. PRIORI, S.G., C. NAPOLITANO, M. GASPARINI, *et al*. 2000. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. Circulation **102:** 2509–2515.
37. BRUGADA, J., R. BRUGADA, C. ANTZELEVITCH, *et al*. 2002. Long-term follow-up of individuals with the electrocardiographic pattern of right bundle-branch block and st-segment elevation in precordial leads V1 to V3. Circulation **105:** 73–78.
38. SCHOTT, J.J., C. ALSHINAWI, F. KYNDT, *et al*. 1999. Cardiac conduction defects associate with mutations in *SCN5A*. Nat. Genet. **23:** 20–21.
39. TAN, H.L., M.T. BINK-BOELKENS, C.R. BEZZINA, *et al*. 2001. A sodium-channel mutation causes isolated cardiac conduction disease. Nature **409:** 1043–1047.
40. KYNDT, F., V. PROBST, F. POTET, *et al*. 2001. Novel SCN5A mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. Circulation **104:** 3081–3086.

41. BEZZINA, C., M.W. VELDKAMP, M.P. VAN DEN BERG, *et al*. 1999. A single Na(+) channel mutation causing both long-QT and Brugada syndromes. Circ. Res. **85:** 1206–1213.
42. ELDAR, M., E. PRAS & H. LAHAT. 2003. A missense mutation in the casq2 gene is associated with autosomal-recessive catecholamine-induced polymorphic ventricular tachycardia. Trends Cardiovasc. Med. **13:** 148–151.
43. GRANT, A.O., M.P. CARBONI, V. NEPLIOUEVA, *et al*. 2002. Long QT syndrome, Brugada syndrome, and conduction system disease are linked to a single sodium channel mutation. J. Clin. Invest. **110:** 1201–1209.
44. COUMEL, P., J. FIDELLE, V. LUCET, *et al*. 1978. Catecholaminergic-induced severe ventricular arrhythmias with Adams-Stokes syndrome in children: report of four cases. Br. Heart J. **40:** 28–37.
45. LEENHARDT, A., V. LUCET, I. DENJOY, *et al*. 1995. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. Circulation **91:** 1512–1519.
46. SWAN, H., K. PIIPPO, M. VIITASALO, *et al*. 1999. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. J. Am. Coll. Cardiol. **34:** 2035–2042.
47. PRIORI, S.G., C. NAPOLITANO, B. COLOMBO, *et al*. 2001. Mutations of the cardiac Ryanodine receptor (RyR2) gene are polymorphic ventricular tachycardia in Bedouin families from Israel. Am. J. Hum. Genet. **69:** 1378–1384.
48. LAITINEN, P.J., K.M. BROWN, K. PIIPPO, *et al*. 2001. Mutations of the cardiac ryanodine receptor (RyR2) gene in familial polymorphic ventricular tachycardia. Circulation **103:** 485–490.
49. LAHAT, H., E. PRAS, T. OLENDER, *et al*. 2001. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced. Am. J. Hum. Genet. **69:** 1378–1384.
50. LAHAT, H., M. ELDAR, E. LEVY-NISSENBAUM, *et al*. 2001. Autosomal recessive catecholamine- or exercise-induced polymorphic ventricular tachycardia. Circulation **103:** 2822–2827.
51. POSTMA, A.V., I. DENJOY, T.M. HOORNTJE, *et al*. 2002. Absence of calsequestrin 2 causes severe forms of catecholaminergic polymorphic ventricular tachycardia. Circ. Res. **91:** E21–26.
52. MARKS, A.R., S. PRIORI, M. MEMMI, *et al*. 2002. Involvement of the cardiac ryanodine receptor/calcium release channel in catecholaminergic polymorphic ventricular tachycardia. J. Cell. Physiol. **190:** 1–6.
53. WEHRENS, X.H., S.E. LEHNART, F. HUANG, *et al*. 2003. FKBP12.6 deficiency and defective calcium release channel (ryanodine receptor) function linked to exercise-induced sudden cardiac death. Cell **113:** 829–840.
54. PRIORI, S.G. & P.B. CORR. 1990. Mechanisms underlying early and delayed afterdepolarizations induced by catecholamines. Am. J. Physiol. **258:** H1796–1805.
55. ANDERSEN, E.D., P.A. KRASILNIKOFF & H. OVERVAD. 1971. Intermittent muscular weakness, extrasystoles, and multiple developmental anomalies. A new syndrome? Acta Paediatr. Scand. **60:** 559–564.
56. TAWIL, R., L.J. PTACEK, S.G. PAVLAKIS, *et al*. 1994. Andersen's syndrome: potassium-sensitive periodic paralysis, ventricular ectopy, and dysmorphic features. Ann. Neurol. **35:** 326–330.
57. SANSONE, V., R.C. GRIGGS, G. MEOLA, *et al*. 1997. Andersen's syndrome: a distinct periodic paralysis. Ann. Neurol. **42:** 305–312.
58. LEVITT, L.P., L.I. ROSE & D.M. DAWSON. 1972. Hypokalemic periodic paralysis with arrhythmia. N. Engl. J. Med. **286:** 253–254.
59. GUTMANN, L. 2000. Periodic paralyses. Neurol. Clin. **18:** 195–202.
60. PLASTER, N.M., R. TAWIL, M. TRISTANI-FIROUZI, *et al*. 2001. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. Cell **105:** 511–519.
61. TRISTANI-FIROUZI, M., J.L. JENSEN, M.R. DONALDSON, *et al*. 2002. Functional and clinical characterization of KCNJ2 mutations associated with LQT7 (Andersen syndrome). J. Clin. Invest. **110:** 381–388.
62. CHEN, Y.H., S.J. XU, S. BENDAHHOU, *et al*. 2003. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science **299:** 251–254.

63. AN, R.H., X.L. WANG, B. KEREM, *et al*. 1998. Novel LQT-3 mutation affects Na+ channel activity through interactions between alpha- and beta1-subunits. Circ. Res. **83:** 141–146.
64. VISWANATHAN, P.C., D.W. BENSON & J.R. BALSER. 2003. A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. J. Clin. Invest. **111:** 341–346.
65. MARX, S.O., J. KUROKAWA, S. REIKEN, *et al*. 2002. Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. Science **295:** 496–499.
66. SANGUINETTI, M.C., N.K. JURKIEWICZ, A. SCOTT & P.K. SIEGL. 1991. Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. Circ. Res. **68:** 77–84.