This Review is the first in a thematic series on *Inherited Arrhythmogenic Syndromes: The Molecular Revolution*, which includes the following articles:

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**The Fifteen Years of Discoveries That Shaped Molecular Electrophysiology**

**Time for Appraisal**

**Silvia G. Priori**

**Abstract:** This article serves as an introductory overview to a thematic review series that will present the latest advancements in the field of inherited arrhythmias. This area of cardiac electrophysiology started approximately 15 years ago thanks to the contribution of Mark Keating and coworkers, who discovered the molecular basis of long QT syndrome. The field rapidly expanded when clinicians, molecular biologists, geneticists, and cellular electrophysiologists, who undertook an impressive collaborative effort to clarify the genetic basis of “cardiac channelopathies.” As a result of this hard work, the paradigms for diagnosis and management of patients with inherited arrhythmogenic diseases were substantially modified, demonstrating once more the value of “translational research.” As more and more genes have been implicated in the genesis of inherited arrhythmias, we keep broadening our understanding of the complexity of ion channels and their multifaceted regulatory processes. Despite the fact that several discoveries have already been made, the field is facing new challenges that are attracting young investigators who share with the pioneers the ambitious goal of finding new therapies and even a cure for these conditions. *(Circ Res. 2010;107:451-456.)*

**Key Words:** arrhythmia ■ genetic testing ■ genetics ■ ion channels ■ ventricular fibrillation ■ ventricular tachycardia

The impressive advancements in gene discoveries that has occurred in the past 20 years has been greatly facilitated by the introduction of the Sanger method for sequencing DNA.1 The so called “chain termination” method for sequencing DNA was introduced by Dr Sanger in the 1970s and allowed him to sequence the first DNA-based genome (phi X 174 bacteriophage),2 inspiring, a decade later, the initiation of the humane genome project.1
The commercial exploitation of the Sanger method allowed the introduction of automatic sequencing in the research laboratories around the world and accelerated the rate of discovery of human genes. The search for disease-causing mutations started in the second half of the 1980s, when diseases such as Duchenne muscular dystrophy and cystic fibrosis were associated with DNA abnormalities that segregated with the clinical phenotype. In the following 25 years, the causative genes for most major inherited diseases were identified allowing the introduction of diagnostic genotyping in the clinics.

Like several other fields of medicine, clinical electrophysiology has been strongly impacted by the innovation brought by molecular genetics: the search for the genetic bases of inherited arrhythmogenic diseases was initiated by the inspiring work of Mark Keating that pioneered the identification of genomic mutations able to disrupt structure and function of cardiac ion channels and cause life threatening arrhythmias. The Keating group identified, for the first time, mutations in the KCNQ1, KCNH2, and SCN5A genes responsible for long QT syndrome (LQTS). These genes encode for the α subunit of the ion channels that conduct the potassium delayed rectifier currents (I\textsubscript{Kr} and I\textsubscript{Ks}) and the cardiac sodium current (I\textsubscript{Na}). Before the “genetic era,” LQTS, a familial disease described in the late fifties and associated with juvenile sudden cardiac death, was explained as an abnormality of cardiac innervation. The discoveries of Keating and coworkers designed the new “road map” to fundamental changes in the understanding of the disease.

Thanks to the work of a large number of clinicians dedicated to the cure of patients with inherited arrhythmias and to the perseverance of molecular biologists, geneticists, and the cellular electrophysiologists who were determined to clarify the genetic basis of these conditions, the concept of “cardiac channelopathies” emerged and thrived (Figure 1), redefining the paradigms for management of patients with inherited arrhythmogenic diseases.

As more and more DNA samples were collected and sequenced, it became apparent that most of the mutations identified in the genes encoding ion channel subunits disrupt the function of channels and modify their biophysical properties. These mutations are called “loss-of-function” defects to distinguish them from a minority of the genetic defects that result in a “hyperactive channel” and are therefore called “gain-of-function” mutations.

Over a short time, the list of diseases caused by mutations in ion channels expanded: investigators abandoned linkage analysis and started using the “candidate gene approach” to identify novel “ion channels” and “ion channel–related proteins” associated with clinical phenotypes. It became clear that almost every protein that forms or regulates the function of an ion channel is associated with one or more clinical phenotypes. LQTS and short QT syndrome (SQTS) represent a typical example of how different defects in the same genes may cause opposite phenotypes.

Gussak et al identified SQTS in 2000, and shortly afterward, it was hypothesized that gain-of-function mutations in the genes that cause LQTS through loss-of-function mutations, were the most logical candidates for SQTS. As a matter of fact, gain-of-function mutations in KCNH2 (all-
ready known as the gene for LQT2) account for SQTS type 1 (SQTS1) and mutations in the KCNQ1 and KCNJ2 genes, known to cause LQT1 and LQT7, are also responsible for 2 forms of SQTS: SQTS2 and SQTS3. In less than 5 years after the first description of SQTS, its molecular background was already largely dissected. Such a rapid advancement of science has been made possible by the strong interaction between clinicians and basic scientists that is becoming a new feature in contemporary medicine.

A striking proof of the pivotal role of the interplay between physicians and researchers is represented by the clinical value of in vitro functional characterizations of DNA variants of uncertain clinical meanings found in genes encoding ion channels subunits.

When the diagnostic laboratory identifies a new mutation in a small family or in a sporadic case, it is hard to define the probability that the mutation cause the clinical phenotype of the carrier. Basic scientists may help the cardiologist by using different experimental approaches that span from patch clamp studies of mutations expressed in heterologous systems to the preparation of minigene systems that can clarify the effect of intronic variants. In selected cases, the in vitro characterization of mutations may be useful also to explain a novel and puzzling phenotypic manifestation or an abnormal response to therapy seen in a patient.

Basic scientists may also provide clinically relevant genetically engineered mice models that may act as surrogate of clinical investigations and contribute to discovering the arrhythmogenic mechanisms of inherited syndromes and even allow the identification and testing of novel therapeutic strategies.

Most of the initial research on arrhythmogenic syndromes focused on the identification of mutations in proteins encoding subunits of voltage-dependent ion channels. However, in the last few years, this view greatly evolved based on the evidence that ion channels are not simple structures formed by the assembly of 1 or 2 proteins; rather, they function as highly sophisticated macromolecular complexes. As the attention of investigators moved toward the search for mutations in genes encoding for new groups of cardiac proteins, the field was ripe for more advancements.

In the year 2000, the identification of mutations in the cardiac ryanodine receptor (RyR2) introduced the concept that genetic abnormalities of proteins of the sarcoplasmic reticulum are responsible for arrhythmogenic syndromes caused by abnormal intracellular calcium handling. Shortly after the identification of RyR2 as a disease gene, mutations in the gene encoding a closely related protein, cardiac calsequestrin, were identified. RyR2 and CASQ2 are now recognized as the 2 leading genes responsible for a highly malignant disease called catecholaminergic polymorphic ven-
tricular tachycardia. From a mechanistic point of view, it has been important to understand that the arrhythmogenic substrate of CPVT are delayed afterdepolarizations (DADs) that develop as a consequence of mutation-mediated diastolic calcium release. Despite the fact that DADs had been described in vitro and in vivo, the demonstration that they were responsible for arrhythmogenesis in humans is largely a consequence of genetic research. Because of the malignant outcome of affected children CPVT, has stimulated the development of pharmacology of intracellular calcium handling, bringing the filed to new advancements such as the recent discovery that sodium channel blockers may be important modulators of the ryanodine receptor.

The identification of mutations in the Ankyrin gene in patients affected by an uncommon form of LQTS (LQT4) has been another contribution that, besides allowing for the understanding of additional human diseases, opened up a new understanding of the biology of cardiac excitability. In a few years, ankyrins moved from being poorly characterized proteins to being seen as major regulators of localization of cardiac proteins.

Whereas ANK2 mutations have been associated with LQT4, later renamed as “ankyrin-B syndrome” because of its peculiar phenotypic features, mutations in the gene that encodes ankyrin-G ultimately alter pathways for voltage-gated Nav channel localization, leading to the phenotype of Brugada syndrome. The importance of these studies extends beyond the identification of another causative gene for inherited arrhythmias and clearly impacts cell biology and understanding of the determinants of electric properties of cardiac cells.

The discovery of the gene for LQT11 is also worth mentioning despite the fact that the disease is likely to be extremely rare. To describe the basis of the discovery of the gene for LQT11, it is necessary to go back to LQT1 and LQT5. LQT1 was the first genetic variant of LQTS to be described. In this form of LQTS, the defective gene is KCNQ1 that encodes for the α subunits of the channel that conducts IkS current. The α subunit of this channel binds at least 1 β subunit encoded by the gene KCNE1 that is mutated in LQTS5. The molecular mechanisms that link the 2 subunits of the IkS channel to the adrenergic pathway were analyzed in an enlightening article by the group of Kass. In this study, published in Science in 2002, Kass and coworkers reported that modulation of the IkS current through the β-adrenergic pathway requires targeting of cAMP-dependent protein kinase (PKA) and protein phosphatase (PP)1 to KCNQ1: this effect is mediated by the targeting protein AKAP9 (A-kinase anchoring protein 9), also known as Yotiao.

Elaborating on this discovery, the authors became interested in testing the hypothesis that Yotiao, itself, could be a candidate gene for LQTS. The authors screened 50 LQTS patients for mutations on the gene encoding Yotiao and were able to identify a point mutations at position 1570 (S1570L) localized to the KCNQ1-binding domain that was absent in 1320 reference alleles. The mutation was shown to blunt the interaction between KCNQ1 and Yotiao, thus reducing PKA mediated phosphorylation of the IkS channel, leading to action potential prolongation in silico.

More recently, Postma et al further extended the group of proteins that may predispose to arrhythmias development when the authors suggested that mutations in complex genes such as the T-box transcription factor 5 that cause Holt–Oram syndrome (a disease characterized by upper limb and cardiac malformations) may also predispose to paroxysmal atrial fibrillation.

All of the above-mentioned experiments represent an important cornerstone as they extend the proteins implicated in the pathogenesis cardiac “channelopathies” beyond the subunits that form the core of an ion channel, highlighting the importance in arrhythmogenesis of the assembly and proper targeting and localization of regulatory protein that turn an ion channel into a macromolecular complex.

Despite the fact that most of the inherited arrhythmogenic syndromes do not cause structural abnormalities of the heart, arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease of the cardiac muscle but usually manifests with a syncopal phenotype superimposable to that of “channelopathies.” The disease has a very rare recessive variant called Naxos disease that is highly malignant and presents cutaneous manifestations such as palmoplantar keratoderma and woolly hair. Most patients, however, are affected by the autosomal dominant form of the disease that is characterized by incomplete penetrance and variable expressivity of the cardiac phenotype. It was the discovery of the genetic basis of the recessive form that opened the field to the next level, when McKoy et al reported in a family with Naxos disease a 2-bp deletion in the plakoglobin gene (JUP) that causes a premature truncation of the protein. Despite the fact that the search for the genes of ARVC had been actively pursued for several years, the discovery of plakoglobin as the first ARVC gene came as a surprise because the link between the phenotype and a protein responsible for cell–cell connection was highly unexpected. As it always happens when a scientific breakthrough occurs, several confirmatory findings followed that of McKoy and coworkers and implicated additional desmosomal proteins to the ARVC phenotype.

The field rapidly moved ahead, proposing a novel pathogenetic hypothesis for right ventricular cardiomyopathy and placing the effect of the mechanical contractile force acting on the diseased muscle at the center of the degenerative process that causes myocardial derangement, inflammation, fibrosis, and cell death. Furthermore, analogous to what happened after Mark Keating’s discovery of ion channel mutations in LQTS, the identification of mutations in genes encoding for desmosomal proteins stimulated basic science investigations of the structure and function of cell–cell coupling in the heart that produced important advancements in the understanding for cell adhesion and its importance in preservation of cardiac contractility.

The field of cardiac arrhythmology is now ready to move forward to the next challenge represented by molecular therapy. This is not an easy task, and it will likely engage scientists for several years. The complexity of the electric architecture of the heart is a major challenge to the development of strategies to compensate loss-of-function defects. Cardiac excitability, in fact, is not simply regulated by the amount of currents but, instead, requires the fine-
tuning of regional gene expression profiling. A remarkable example of regional control of electric properties has been provided by Maguy et al., who showed that in failing hearts, a specific rearrangement of electric properties occurs inducing a decrease of connexin 40 and connexin 43 and of sodium channel (Nav 1.5) and potassium channels (Kv 4.3 and Kv 3.4). How disease state may alter expression profiling of the heart is unknown; recent data from Gyorke and colleagues suggested that microRNA may be implicated in the process. Overall, it is clear that, as molecular therapy is advancing in all fields of medicine, cardiac electrophysiology is actively filling knowledge gaps that will enable entrance into the “curative” era for arrhythmogenic disorders.

This short narrative has provided a succinct overview of the contribution of molecular genetics applied to arrhythmogenic syndromes in advancing not only clinical management of the diseases but also fundamental understanding of electrical and mechanical functions of the heart. To acknowledge the advancements occurred in the last 15 years of research on arrhythmogenic syndromes, the Editors of Circulation Research have invited some of the scientists who have contributed to the field to write a series of review articles that will be coauthored by a clinical geneticist/cardiologist and a basic scientist to provide a comprehensive overview of current knowledge.

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**References**


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