Inherited Arrhythmogenic Diseases
The Complexity Beyond Monogenic Disorders

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Abstract—Twelve years after the identification of the molecular bases of the long-QT syndrome, it is now possible to express some considerations on the impact that genetic findings have had in the understanding of inherited arrhythmogenic diseases. Along with the excitement for the emerging data on genotype/phenotype correlation and for the development of the first recommendations for gene-specific management of patients, it is also important to acknowledge the unexpected complexity that has emerged. The focus of this article is to analyze the elusive aspects of the relationship between genetic defects and clinical manifestations and to propose some research directions that may provide the needed answers to move forward in the understanding of the genetics of heart rhythm abnormalities. (Circ Res. 2004;94:140-145.)

Key Words: arrhythmias ■ genetics ■ sudden cardiac death ■ ion channels ■ heart

Diseases caused by a single genetic defect are referred to as monogenic disorders: they follow Mendelian inheritance and are classified as autosomal dominant, autosomal recessive, or X-linked. Approximately 5000 monogenic diseases have been identified, and more than 1000 genes responsible for these disorders are known. Monogenic diseases are rare with prevalence ranging from 1 patient in 1000 inhabitants in the most common forms and 1 in 200,000 in the rarest. The identification and the study of the genetic substrate of these diseases are relevant because they contribute to the development of diagnostic tests for affected individuals and also because they help understanding the function of different proteins in humans. Accordingly genetic testing is nowadays applied not only for diagnostic purposes, but also as a research tool for the study of the pathophysiology of inherited diseases. In cardiology there are two major clusters of monogenic disorders: (1) the cardiomyopathies due to alterations in sarcomeric and in cytoskeletal proteins and (2) the arrhythmogenic diseases that are caused by mutations in ion channels and ion channel-controlling proteins such as the long-QT syndromes (LQTS), the Brugada syndromes (BrS), catecholaminergic polymorphic ventricular tachycardias (CPVT), and Andersen syndrome.

What We Have Learned From the Genetics of Monogenic Arrhythmogenic Syndromes
The identification of the genes underlying the inherited arrhythmogenic syndromes has greatly contributed to the
understanding of the substrate for arrhythmias development, but more importantly, it has provided major practical information that are helpful when managing affected individuals. The identification of a mutation allows us to establish the diagnosis independently from the electrocardiographic features and the arrhythmic manifestations. When screening family members of a genotyped proband, an unexpectedly large number of carriers of the genetic defect is identified among relatives that were considered nonaffected based on clinical evaluation. Among those patients genotyped at our institution, 32% of carriers of LQTS-related mutations had a QTc within normal limits (S.G. Priori, unpublished data, 2004). The information of being the carrier of a clinically silent defect is relevant and it has at least two practical implications. First, silent carriers have a 50% probability of transmitting long-QT syndrome to their offspring; secondly, they are more susceptible than an age-matched population to develop cardiac arrhythmias. The risk of experiencing a cardiac event by age 40 among the carriers of LQT1, LQT2, or LQT3 is proportional to the duration of the QTc interval; therefore, carriers of mutations with a QTc $\geq$500 ms have 70% probability of experiencing their first cardiac event by age 40. However, even the group of patients with normal QTc (ie, silent carriers of a genetic defect that are missed at clinical diagnosis) have a risk of 20% of becoming symptomatic. Based on current evidence it seems that the most immediate contribution of genetic information to clinical practice is that of representing a novel and useful parameter for risk stratification that can be used alone or in combination with other clinical variables. This is true for the long-QT syndrome that is the only inherited arrhythmogenic disorders in which data on several hundreds of genotyped patients with long clinical followup have become available. Similar data however are emerging for other diseases such as CPVT, in which preliminary data in a relatively small number of patients have shown that the combination of gender and genotype may influence the likelihood of experiencing cardiac events. Although genotyping is already worth being pursued in all patients with inherited genetic disorders, it is expected that its contribution to medical practice will increase when some of the gaps in understanding of the links between DNA defects and clinical phenotype are filled.

**Complexity Beyond Monogenic Arrhythmogenic Syndromes**

Since Keating’s initial discovery of mutations in KCNQ1, KCNH2, and SCN5A genes in large families affected by long-QT syndrome, hundreds of mutations have been identified in various ion channel subunits and in proteins important for the proper functioning of cardiac ion channels. It was initially assumed that all carriers of pathogenic mutations would manifest the corresponding phenotype: it became rapidly evident however that the clinical consequences of genetic defects are by far more variable than expected.

Two features of monogenic diseases, namely penetrance and expressivity, had been identified since the inception of molecular genetics. The penetrance of a monogenic disease is defined as the percentage of individuals with a mutant allele who develop the phenotype of the related disease and it can vary from 10% to 100%. The expressivity of a disease is defined as the different phenotypical manifestations that can be observed among carriers of the same genetic defect. Accordingly, combining variable penetrance and expressivity, it becomes clear that carriers of a DNA mutation may manifest either no clinical phenotype or phenotypes that are not typical of the “textbook” description of the disease. As a consequence, the understanding of the clinical implications of genotyping in patients affected by “simple” monogenic diseases becomes less straightforward than desired. In analogy with what happens in the genetics of inherited cardiomyopathies, in arrhythmogenic disorders, the “same” phenotype is associated with multiple genetic defects: this phenomenon is called “genetic heterogeneity.” At present, there are six genes that can cause the phenotype of the long-QT syndrome: they explain 50% to 60% of clinically diagnosed cases; similarly two genes are known to cause catecholaminergic polymorphic VTs and they also account for half of the patients with the phenotype. Only one gene is known to cause the Brugada syndrome, but several other genes are likely to be implicated in the disease because so far only 20% of clinical diagnoses result positive at genetic testing. The first question that stems from these considerations is whether it is still appropriate to refer to all the genetic variants with the same name (such as long-QT syndrome). Is it still meaningful to group all the individuals with the same ECG marker (prolonged QT interval, ST-segment elevation in the right precordial leads, adrenergically mediated polymorphic VT) under the same diagnosis, when we know that each genetic variant has a distinguishing clinical profile? The question may be more than semantic, as data are emerging that suggest that the genetic substrate is a major determinant of prognosis in patient harboring different genetic defects. In the future, we may prefer to consider each genetic variant as a separate disease based on the specific defect as this would avoid the difficulty of trying to fit the complex and overlapping phenotypes into clinical categories (Figure). To clarify this concept, we should simply think of the several definitions used to explain overlapping clinical profiles: Brugada syndrome with conduction defects, Brugada syndrome with atrial arrhythmias, conduction defects with sinus abnormalities, Brugada syndrome with prolonged QT interval, etc. All these conditions could be grouped in the category of sodium channel disease and its full spectrum of abnormalities. To complicate the issue even further, it is now established that mutations in one gene may lead not only to variable phenotypes within the same disease, but also to profoundly different diseases. The most emblematic example of this diversity is represented by mutations in the lamin A/C gene, which are known to cause at least eight phenotypes (allelic diseases) as different as dilated cardiomyopathy, Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy, Charcot-Mary-Tooth disease, mandibuloacral dysplasia, Hutchinson-Gilford progeria, lipoatrophy with diabetes, hepatic steatosis hypertrophic cardiomyopathy, leukomalanodermic papules, and limb girdle muscular dystrophy type 1B. In the field of inherited arrhythmogenic disorders, three diseases have been associated with mutations of the cardiac sodium channel gene (long-QT syndrome, Bru-
gada syndrome, and progressive conduction disturbances) and two diseases (long-QT syndrome and familial atrial fibrillation) have been associated with mutations in the KCNQ1 gene encoding for the α-subunit of the potassium channel conducting the slow component of the delayed rectifier (I_{Ks}). It is therefore obvious that the identification of a mutation in a given gene cannot establish the diagnosis of a single disease and that also the identification of a mutation in an individual with a known disease is not enough to predict the phenotype of that individual.

We will now venture into other aspects of inherited arrhythmogenic diseases that are still poorly understood and warrant further studies.

What Current Knowledge Cannot Explain in the Inherited Arrhythmogenic Syndromes

Overlapping Phenotypes

Functional characterization of mutations in the SCN5A gene encoding the human cardiac sodium channel gene has been instrumental for the investigation of at least three human diseases that are caused by defects in this gene: initially the link between the mutation and the phenotype seemed rather straightforward. SCN5A mutations identified in patients with the long-QT syndrome cause a “gain of function” in the sodium channel leading to an increase in the inward current and therefore to a prolongation of the QT interval, whereas mutations identified in patients with Brugada syndrome or with progressive conduction disturbances produce the opposite defect and determine a “loss of function.” This straightforward interpretation has been complicated by the demonstration that there are mutations in the SCN5A gene (as for example 1795 Ins D and Del K 1500) that lead to hybrid phenotypes that present with both Brugada syndrome and long-QT syndrome (1795 Ins D) phenotypes or with overlapping Brugada syndrome, long-QT syndrome, and progressive conduction disturbances phenotypes (Del 1500 K). Functional in vitro characterization of 1795 Ins D showed that the mutation disrupts fast inactivation, causing sustained Na⁺ current throughout the action potential plateau and prolonging cardiac repolarization at slow heart rates: at the same time, it augments slow inactivation, delaying recovery of Na⁺ channel availability between stimuli and reducing the Na⁺ current at rapid heart rates.

Further insights for the understanding of the overlapping phenotypes are provided by the functional characterization of the deletion of the lysine at position 1500. When studied in 293 EBNA cells, the mutation enhanced closed-state inactivation rate by more than 10-fold, resulting in a marked reduction of Na⁺ channel availability at the normal resting potential of cardiac cells. The mutation also increased the open-state inactivation rate overall, reducing the Na⁺ current to an extent considered sufficient to account for the Brugada syndrome and the conduction disturbance at the sinoatrial and atrioventricular junction. Furthermore, the late component of Na⁺ current resulting from a return from the inactivated state is increased almost 3-fold in the ΔK1500 mutant, thus accounting for the action potential prolongation and for the abnormally prolonged QT interval. What the functional studies however cannot explain is why selected family members within this kindred preferentially presented with one of the three phenotypes: factors such as age and gender were unrelated to the electrocardiographic pattern. It is therefore interesting to speculate that so far “unknown” factors interfere with a primary genetic defect and may ultimately determine the severity of the clinical manifestations observed in each family member. The concept that the primary mutation may be pivotal to the phenotype, but by no means the only determinant of the clinical manifestations, emerges also from other poorly understood characteristics of inherited arrhythmogenic diseases that will be discussed.

Paroxysmal Manifestations

Although identification of the genetic defects has disclosed the nature of the electrical instability of patients with inherited arrhythmogenic diseases, one of the most intriguing questions remains unanswered. It is still unknown why in patients born with a genetic defect, the clinical phenotype presents paroxysmal exacerbation such as sudden QT interval...
prolongation in long-QT syndrome or intermittent ST segment elevation in Brugada syndrome. Even the arrhythmic episodes have paroxysmal nature and are frequently clustered within few days interspersed by a long asymptomatic interval that may last decades. Even more puzzling is the evidence that the mean age of occurrence of first symptoms seems to be typical of the different diseases. In LQTS, the mean age of occurrence of first symptoms is in the early teens, in CPVT, it is in childhood, and in Brugada syndrome, it extends into the fourth decade of life. Interestingly, allelic diseases such as LQT3 and Brugada syndrome share defects on the same gene but show a different age-dependency of the first clinical manifestation. The cause for the age-dependent penetrance of inherited arrhythmogenic diseases is not understood and is likely to be multifactorial. The observation that in LQT1 male patients have earlier onset of symptoms\(^1,22\) suggests that gender may play a role in modulating clinical manifestations: however, whether this is related to differences in lifestyle (physical activity) or in susceptibility to catecholamines has not been clarified.

The erratic occurrence of arrhythmias has often become a matter of debate as a long symptom-free intervals may be attributed to the efficacy of a specific treatment, but it may also simply reflect the unpredictable occurrence of cardiac events that may be observed even in the absence of therapeutic efficacy. Several factors have been implicated to account for “electrical storms” and for sudden worsening of the electrocardiographic characteristics. Gender,\(^1\) hormonal changes,\(^23\) electrolyte abnormalities, concomitant drugs, and food intake\(^23\) have been considered responsible for QT interval prolongation and for paroxysmal torsades de pointes in previously asymptomatic LQTS individual. Fever,\(^25\) vagal reflexes,\(^26\) age, and gender\(^27,28\) have been implicated to precipitate arrhythmias and to worsen the electrocardiographic pattern in patients with the Brugada syndrome. None of these cofactors however has been conclusively related to the severity of the clinical phenotype. Therefore the term “modifiers” is presently used to refer to all those factors that may be responsible for modulating the clinical manifestations. Identification of the nature of modifiers of the clinical phenotype maybe considered the next goal in the understanding of inherited arrhythmogenic syndromes.

Genetic Modifiers

The incomplete prediction of the clinical phenotype derived from in vitro study is probably more logical than it is unexpected. We need to depart from the cellular electrophysiology setup and look at the models that we are using from some distance, remembering the complexity of a cardiac cell. We will therefore realize that too much emphasis is posed on the significance of data obtained by the characterization of a primary mutation in the artificial setting of currently available expression systems. These models utilize cells that are very different from cardiac myocytes, and therefore, they do not incorporate key elements such as intracellular coupling, second messengers, membrane receptors, and other key proteins that in the physiological environment concur to the clinical phenotype. In real life, mutations exert their action on an environment that is profoundly affected by interactions with dozens of other proteins that activate positive and negative feedback in response to perturba-

tions derived from the environment, the autonomic nervous system, or the metabolism. Based on these considerations, it is easier to understand why we cannot yet link a mutation to the clinical manifestations of the disease in our patients. The few studies that have attempted to gain a deeper insight in the consequence of mutations by incorporating in their models at least another factor that may influence the phenotype, have shed new light on the interplay between the inherited mutation and its modulation.\(^29–31\) Based on the hypothesis that environmental factors may explain the paroxysmal manifestations of inherited diseases and that genetic modifiers more likely account for the interindividual variability among patients harboring the same primary mutation, few authors have tested the effects of introducing a polymorphism as a background for studying primary mutations in vitro. Baroudi et al\(^30\) were the first to introduce the concept that the interaction of polymorphisms and mutations may exert profound effect on the functional consequences. These authors demonstrated that the T1620M mutation that if expressed alone produces gating abnormalities in the cardiac sodium channel current, when coexpressed with a common polymorphism of the SCN5A gene (R1232W) in tsA201 cells the mutants affect protein trafficking, resulting in retention within the endoplasmic reticulum of the mutant. Along the same line, Ye et al\(^32\) recently showed that the presence of the H558R polymorphisms in the SCN5A gene was able to rescue normal trafficking and normal current for the M1766L mutants protein that was otherwise associated with an LQTS phenotype.

If we now consider the hundreds of genes (ion channel subunits, promoters, receptors, second messengers, transcription factors, etc) that may eventually determine the impact of a primary mutations on the clinical phenotype, we immediately realize that a more integrated in vivo approach is needed to dissect the modifiers relevant to inherited arrhythmogenic diseases.

Another interesting implication that stems from the data showing that common polymorphisms modulate the activity of a primary mutation is the concept that, even in the absence of a disease causing mutation, polymorphisms may influence susceptibility to arrhythmias in the general population. The more robust data along this line come from the work of Splawski et al\(^33\) who suggested that the polymorphism at position Y1102 that present in 13.2% of the African American population is strongly associated with the development of arrhythmias in the absence of a clinical phenotype of LQTS of Brugada syndrome or of Lev Lenegre. These data would encourage the systematic search for polymorphisms in genes encoding cardiac ion channels in patients with common cardiac diseases such as ischemia and hypertrophy to test whether single nucleotide changes may be risk factors for arrhythmias in these settings.

As a final step in this excursus of the complexity of inherited arrhythmogenic diseases, we need to spend a few words on the potential consequences that mutations in cardiac ion channel genes may exert on different types of cardiac cells. Based on the notion that the heart that is composed of several cellular types, it becomes clear that very little is known on the role that mutations may have in atrial myocytes, in specialized conduction system cells, in sinus node cells,\(^34\) and in Purkinje fibers. Very preliminary data may suggest that torsades de pointes may develop in the atria as
well as in the ventricles\textsuperscript{35} and that mutations in the SCN5A gene may also affect automaticity in the sinus node.\textsuperscript{36} Similarly, when functional abnormalities are introduced in computer models that mimic the heterogeneity of the ventricular myocardium (epicardial, endocardial, and M cells), otherwise elusive aspects of the clinical phenotype can be explained.\textsuperscript{37}

**Structural Abnormalities of the Heart**

Another debated aspect of inherited arrhythmogenic diseases relates to the link between ion channel mutations and structural abnormalities of the heart. The first link between ion channel defects and cardiomyopathies originated from the evidence that few families with mutations in the ryanodine receptor type 2 (RyR2), beside presenting adrenergically mediated polymorphic arrhythmias similar to those of CPVT patients,\textsuperscript{38,39} also show minimal structural abnormalities of the right ventricle compatible with the diagnosis of arrhythmogenic right ventricular cardiomyopathy (ARVC) of type 2.\textsuperscript{40} Data from Tiso et al\textsuperscript{11} suggested that RyR2 mutations identified in ARVC2 patients cause a decreased affinity of the ryanodine receptor for the FKBP12.6 regulatory protein and therefore lead to intracellular calcium overload, apoptosis, and cell death. In the same study, mutations associated with CPVT and normal heart caused an increased affinity of the ryanodine receptor for FKBP12.6, thus accounting for the lack of structural derangement.\textsuperscript{41} Unfortunately, the latter data have not been confirmed by other investigators,\textsuperscript{42,43} and therefore, the link between RyR2 defects and cardiomyopathies remain elusive. What is interesting however is the concept that mutations in ion channels may lead to the disruption of the intracellular environment and produce regional fibrosis, apoptosis, or cell death. The understanding of these mechanisms is a top priority as it will provide novel insight on the link between the electrical and mechanical structures in the heart.

**Extracardiac Manifestations**

These observations prompt even more adventurous thoughts as it is well known that many ion channels that are mutated in the inherited arrhythmogenic syndromes are also expressed in extra cardiac tissues where they are likely to cause abnormalities that may or may not interfere with the cardiac phenotype. It is known for example that homozygous mutations in the KCNQ1 gene and in the KCNE1 gene, encoding respectively for the $\alpha$-subunit and for the $\beta$-subunit of the rapid component of the delayed rectifier, may affect the production of the endolymph and cause deafness that is part of the phenotype of the recessive variant of the long-QT syndrome.\textsuperscript{44–46} It would be not surprising to discover that mutations in KCNQ1 that are also expressed in several other organs such as the adrenal medulla, the pancreas, the bronchi, etc, may alter metabolism or electrolyte in a subtle but still physiologically relevant way and may therefore contribute to the clinical phenotype. Experimental evidences of this concept have already been provided by the demonstration that KCNQ1 disruption in knockout mice may also cause gastric hyperplasia.\textsuperscript{47}

**Conclusions**

It is an exciting time in the study of inherited arrhythmogenic disorders: in the past decade, we have learned a lot about the primary defect underlying these diseases. Yet the excitement is not over, as we realize that most challenging issues still have to be addressed. Development will come from novel studies that will be able to assess the function of mutant proteins in preparations that are more closely related to the physiological environment in which these proteins are distributed, such as expression of mutant ion channels in cardiac myocytes\textsuperscript{48} and development of transgenic models in animal species that have an action potential that is more closely related to that present in humans.\textsuperscript{49} These more physiological models will be useful not only to characterize individual mutations, but also to elucidate the effect of mutations on the complex physiology of cardiac cells and in that of cells outside the heart. It is expected that the results of these future studies will provide novel therapeutic strategies for patients that will eventually encompass gene therapy.\textsuperscript{50,51}

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