Cardiovascular diseases are caused by a combination of environmental and genetic factors. Deciphering these genetic factors should provide fundamental new insights into their pathogenesis, diagnosis, prevention, and treatment. Previous large-scale association studies have been able to identify patterns of DNA sequence variations that confer susceptibility to cardiovascular diseases or alter pharmacological responsiveness. The most common type of genetic variant, a single nucleotide polymorphism (SNP), is a difference between chromosomes in the base present at a particular site in the DNA sequence. Nonsynonymous SNPs (ie, those resulting in amino acid substitution) have the potential to alter the functional properties of the protein encoded by the polymorphic gene, thereby giving rise to a distinct phenotype. The simultaneous presence of multiple SNPs within the same gene (eg, in the gene encoding the human β-adrenergic receptor) or the occurrence of a novel mutation within a polymorphic gene may result in a more complex phenotypic alteration, with each variant individually contributing to its manifestation.

In this issue of Circulation, Crotti and coworkers provide compelling clinical and genetic evidence that co-inheritance of a novel, low-penetration mutation in the KCNH2 gene (ie, the gene encoding the pore-forming α-subunit of the channel mediating the rapidly activating component of the cardiac delayed-rectifier potassium current, I\textsubscript{Kr}) and a common, nonsynonymous single nucleotide polymorphism in the same gene is associated with increased QT duration and sudden death from cardiac arrhythmia, whereas heterozygous carriers of either gene variant alone were asymptomatic or exhibited only transient and mild QT prolongation. Furthermore, using a heterologous expression system and conventional voltage-clamp technology, the authors elegantly demonstrated that paired expression of the mutant and polymorphic KCNH2 channel genes, but not coexpression of either allele with the wild-type gene, gives rise to markedly reduced I\textsubscript{Kr} amplitude. Given the critical role of I\textsubscript{Kr} magnitude in setting the time course of cardiac repolarization, these in vitro findings support the concept that common nonsynonymous SNPs can unmask clinical manifestation of a low-penetration long-QT syndrome mutation, most likely via alteration in the level of functional I\textsubscript{Kr} channel expression in the outer cell membrane. The results additionally indicate that both the mutant and polymorphic allele are recessive, in excellent agreement with the absence of major QT abnormalities in heterozygous carriers of either KCNH2 allele.

Although previous in vitro studies have demonstrated that a variety of common nSNPs in the KCNH2 gene can give rise to functional alterations in heterologously expressed channels, direct evidence that these alterations are responsible for clinical manifestation of the LQTS type 2 was lacking. Similarly, Tan and coworkers showed that common nSNPs in the human SCN5A gene (ie, the gene encoding the pore-forming α-subunit of the ion channel hNav1.5, which carries inward Na current, I\textsubscript{Na}) have altered electrophysiological characteristics when heterologously expressed in different SCN5A splice backgrounds. Again, no clinical correlate of their experimental findings was provided. The present study is the first to convincingly establish a causal role for a common nSNP in a long-QT gene in determining the severity of a long-QT syndrome phenotype, and therefore should be considered a model for future studies aimed at probing the significance of nSNP for long-QT–associated arrhythmia susceptibility, or for predisposition to other monogenic disorders in general.

Crotti and coworkers limited their genetic analyses to the exons of 5 candidate genes associated with the long-QT syndrome, the potassium channel genes KCNQ1, KCNH2, KCNE1, and KCNE2, and the Na channel gene SCN5A. Gene variants conferring differences in gene regulation or function, such as intronic or promoter variants of these candidate genes, may also influence cardiac repolarization similar to exonic nSNPs. Recently published results of a large-scale linkage disequilibrium-based SNP association study discovered a not-previously described nSNP in intronic sequences of the KCNQ1 gene, which were associated with QT prolongation in the general population living in southern Germany. This result indicates that common intronic nSNPs in long-QT genes may modify repolarization to a similar extent as common exonic gene variants and could determine the severity of a low-penetration long-QT mutation in exonic DNA sequences of the same gene. Future genotype-phenotype association studies should therefore consider including intronic DNA sequences of long-QT syndrome candidate genes in the genetic analyses.

From a clinical point of view, the major purpose of linkage analyses is to identify gene variants that confer susceptibility to a particular disease and thereby to facilitate risk stratifica-
tion of the individual patient. Detailed knowledge of the underlying mechanism is not required to implement this goal. In contrast, if discovery of genetic risk factors is to enhance our ability to choose novel targets for therapeutic interventions, then insights into the underlying pathogenesis are needed. Our present understanding of the molecular mechanisms responsible for genotype-specific ion channel dysfunction to a large degree derives from electrophysiological measurements that were conducted on heterologously expressed mutant or polymorphic ion channels. Heterologous expression assays, however, have some inherent limitations. First, heterologously expressed channels may undergo differential posttranscriptional and/or post-translational modifications, depending on the cell type used (e.g., mammalian versus nonmammalian), thereby giving rise to nonspecific (i.e., nondiagnostic) alterations in channel function. Second, it is difficult to extrapolate functional consequences of genotypic variations at the cellular/whole-heart level from electrophysiological measurements in single nonmyocytes. To circumvent the latter problem, experimentally observed alterations in channel properties have been implemented in models of human cardiac action potentials. This approach may lack sensitivity and/or specificity, however, depending on the cell model used. Third, the assay is time-consuming, and given the expected increase in both the number and complexity of discovered gene variants causing cardiac ion channel dysfunction (see below), this approach may not be practical in the future. One alternative strategy to more directly probe the functional consequences of mutant or polymorphic ion channel expression (or any genotypic combination) in a physiologically more relevant context would be the use of human embryonic stem (ES) cell–derived cardiomyocytes in culture. Kamp’s group has recently demonstrated that human ES-derived cardiomyocytes maintained in culture display complex electrophysiological properties typical of cardiac muscle, including rate adaptation of action potential duration, high levels of IKr expression, increased contractility in response to β-adrenergic receptor stimulation, and provoked early and late afterdepolarizations. ES cells are readily amenable to genetic manipulations, which should facilitate the generation of cardiomyocyte clones expressing any combination of mutant and/or polymorphic ion channel genes. Furthermore, in conjunction with high-throughput electrophysiological technology (e.g., automated multiplex voltage-clamp/current-clamp systems), it may become possible to assess the functional impact of a large number of genetic variations in a relatively short time, not only under baseline conditions but also during adrenergic stimulation and exposure to compounds that are known to induce long-QT arrhythmias.

Use of stringent experimental design should also help promote our understanding of functional implications of ion channel gene variants. For example, ion channel mutations may exert a dominant negative effect on wild-type current expression, or produce no or intermediate effects, respectively, when occurring in heterozygous condition with the contrasting allele (recessive and codominant inheritance). To differentiate between these types of functional interactions, it is mandatory to coexpress wild-type channel genes with the mutant/polymorphic gene, as was done by Crotti and coworkers. Whatever approach is used, future studies aimed at probing the role of DNA sequence variants in determining arrhythmia susceptibility should employ a high degree of experimental stringency that allows conclusive statements on the functional implications of particular DNA sequence variations.

Whereas previous projects as well as the study by Crotti et al have been limited to sequencing functional parts of candidate genes that had been selected on the basis of previous functional or genetic hypotheses, large-scale efforts are well under way to search the entire human genome for common DNA sequence variation and their associations to diseases in a number of populations of various ethnic origins. It is only a matter of time until new, more complex patterns of DNA sequence variations (i.e., SNPs) are discovered that are linked to QT prolongation, predisposition to cardiac arrhythmias, and risk of sudden death. These sequence variants, whether or not they have functional effects, could then serve as genetic markers to compile a patient’s risk profile and thereby contribute to primary prevention of fatal arrhythmias. Probing the causative role of these complex genetic variations in determining disease susceptibility and gaining insight into the mechanisms underlying genotype-phenotype association will be extremely challenging because each nSNP individually may contribute only modestly to the complex phenotype. Pedigree analyses, as in the study by Crotti et al, have been highly successful for single-gene disorders but are not applicable to complex quantitative traits. Therefore, one major goal of genome-wide linkage studies should be to develop research tools that will help investigators determine the contribution of complex DNA variants to disease susceptibility, prevention of illness, and drug response. The preeminent challenge ahead will be to design reductionist approaches that will enable researchers to dismantle the complexity of genotype-phenotype associations, but without losing the experimental stringency that was so exemplarily executed in the study by Crotti et al. Our present arsenal of strategies to prevent sudden death from cardiac arrhythmia is limited primarily to chronic β-blockade and implantation of implantable cardioverter-defibrillator. Genome-wide association studies hold promise as a powerful tool for discovery of novel targets for more specific therapeutic interventions. Close partnership between basic scientists and clinicians will be required if the wealth of genetic information is to benefit patients at risk of dying suddenly from cardiac arrhythmia.

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References

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