Understanding the pathogenetic chain of events between genotype and phenotype is critical to the appropriate diagnosis and treatment of heritable diseases. Although our knowledge of the molecular substrate of many diseases continues to increase, identifying the mechanistic links between a susceptibility variant and disease expression remains a major challenge. A minority of mendelian diseases display complete clinical penetrance in individuals harboring known disease-causing mutations, with rare examples such as achondroplasia and Huntington disease. Even in the most well-characterized hereditary arrhythmia syndrome, the long-QT syndrome (LQTS), in which genotype-phenotype relationships and gene-specific management strategies1 exist for the most common molecular subtypes (LQTS 1 to 3), the reasons for incomplete penetrance and variable expression in this disease remain largely unknown. In fact, LQTS has relatively low penetrance; however, in those LQTS genotype-positive individuals who do exhibit symptoms, the initial presentation can be catastrophic, and all too often, families come to clinical attention as a result of aborted cardiac arrest or sudden cardiac death. Therefore, identification of disease-associated mutations in individuals at risk for heritable arrhythmogenic disorders is imperative for subsequent risk reduction in their family members and them.

Over the past 10 to 15 years, it has been shown that mutations in SCN5A, the gene encoding the α subunit of the human cardiac sodium channel (Na, 1.5), have pleiotropic effects, including risk for type 3 LQTS, Brugada syndrome, progressive cardiac conduction disease, atrial fibrillation, sick sinus syndrome, sudden infant death syndrome, and dilated cardiomyopathy. This phenotypic plasticity is not unique to SCN5A, but is also observed with other cardiovascular disease-associated genes. For example, mutations in the cardiac ryanodine receptor gene, RYR2, are associated with catecholaminergic polymorphic ventricular tachycardia and arrhythmogenic right ventricular dysplasia/cardiomyopathy.

In the current issue of Circulation, Watanabe et al2 performed an elaborate series of in vivo studies that demonstrate the functional significance of a previously described missense mutation in SCN5A, resulting in the substitution of an aspartic acid by an asparagine (D1275N). This mutation also exhibits phenotypic plasticity. An initial report linked it to a phenotype of atrial standstill in a large Dutch family and observed this phenotype exclusively in individuals with coinheritance of polymorphisms in the regulatory region of the atrium-specific gap junction protein connexin40.3 Soon after, a second group published D1275N in a kindred segregating an autosomal dominant phenotype of dilated cardiomyopathy with conduction disease, with arrhythmias including atrial flutter/fibrillation and sick sinus syndrome and right and left ventricular dilation and dysfunction.4 No correlation with the connexin40 polymorphisms was identified; these polymorphisms were also absent from the proband reported in the present study, discussed later. Collectively, these data suggest that the connexin40 variants are not necessary for clinical expression of the D1275N mutation. However, it is plausible that variants affecting this atrium-specific protein may influence the development of atrial disease by modulating other genetic factors.

Watanabe et al identified a 19-year-old white man with the D1275N mutation who presented with recurrent syncope on exertion. An ECG showed atrial flutter, and the patient was treated with catheter ablation, after which he had atrial standstill, sinus node dysfunction, and high-degree atrioventricular block. The patient received an implantable cardioverter-defibrillator, and 10-year follow-up has shown the patient to be asymptomatic with normal echocardiogram. The D1275N mutation displayed incomplete penetrance; the proband’s mother and son also tested positive but were asymptomatic.

Notably, although this and other studies have reported several varied human phenotypes associated with the D1275N mutation, prior studies using heterologous expression systems had not identified aberrant functional effects of this variant on Na, 1.5 properties.3-5 A major contribution of the new work is the use of recombinase-mediated cassette exchange to generate mice with wild-type and/or mutant human Na, 1.5 to resolve the relationship between the D1275N variant and clinical phenotypes. In fact, the authors found that the D1275N variant causes abnormal
phenotypes in mice, including bradycardia, sinus node dysfunction, progressive cardiac conduction defects, and tachyarrhythmias, in a gene-dose–dependent fashion. The mice also consistently showed end-diastolic and end-systolic left ventricular dilation and dysfunction, again similar to reported human phenotypes. Although minimal to no change was observed between wild-type and D1275N channels in heterologous cells, sodium current amplitudes and channel gating showed marked changes between wild-type cardiomyocytes and those with 1 or 2 D1275N mutations.

This study demonstrates the potential limitations of using heterologous expression systems alone when studying phenotypic effects of human genetic variants associated with ion channel disease. In vivo functional studies performed in genetically engineered mice displayed a much more significant phenotype than what was observed with in vitro assays. Unfortunately, heterologous cell–based systems may not necessarily replicate the physiological milieu of the native myocyte. Indeed, the present study found elevated levels of SCN5A transcript in mice with the DN allele yet reduced levels of total and membrane-associated Na\(_{\text{i,1.5}}\). So, although transcription may be proceeding normally, the D1275N mutation may affect the posttranscriptional and/or posttranslational modifications or interactions necessary for Na\(_{\text{i,1.5}}\) localization and function.

What, then, are the precise mechanisms of pathogenesis of variants in SCN5A and other arrhythmia-related genes? And what triggers are involved in determining why some individuals with SCN5A mutations will have severe cardiac phenotypes but others will have minor symptoms or none at all? The penetrance of SCN5A–associated Brugada syndrome is only \(\approx 30\)%. We also do not fully understand the reasons for substantial intrafamilial and interfamilial clinical variability between individuals with identical pathogenic mutations. Recent data suggested for the first time that the SCN5A mutation type can anticipate the severity of loss-of-function mutations linked to Brugada syndrome and that those with a more deleterious loss of sodium current produce a more severe phenotype of syncope and conduction disease.\(^6\) Notably, the phenotypic severity in Scn5a\(^{-/-}\) mice correlates with the ability of the normal allele to produce functional sodium channel proteins.\(^7\) Moreover, there is also a role for compound heterozygosity (ie, 2 different SCN5A mutations in the same individual) in the phenotypic expression of Brugada syndrome.\(^8\) In addition, genetic variants in the same or different genes have been shown to modulate the phenotypic expression of known deleterious mutations associated with SCN5A–associated Brugada syndrome and other heritable arrhythmia syndromes.\(^9\)\(^10\) Crotti et al\(^11\) reported that common variants in NOS1AP, previously found to be associated with the QT-interval duration in the general population, also influence risk for sudden cardiac death in patients with LQTS. To add another layer of complexity, it is now widely recognized that human arrhythmia syndromes are not due solely to mutations in cardiac ion channel genes alone but that mutations in channel-interacting proteins responsible for proper expression, subcellular localization, and local regulation of ion channels are also central to the pathogenesis of cardiac arrhythmogenic disease.\(^12\) Future identification of common variants and epigenetic modifiers affecting ion channel proteins and their cooperative partners will likely provide additional insight into the potential substrates for phenotypic expression of mutations associated with heritable arrhythmia syndromes and general cardiovascular disease.

In the interim, how do we apply genetic variant information clinically, especially when the variant is novel? Determining the functional significance of these variants is critical for the proper application of clinical genetic testing results to patients with, or at risk for, diseases associated with sudden cardiac death. Clinical genetic testing is now widely available for most heritable arrhythmia syndromes and cardiomyopathies. Many of these testing platforms incorporate the latest in genetic technologies such as next-generation sequencing, which allows faster, more comprehensive, and more cost-efficient analysis of up to 30 or more genes that have been found to be common or rare causes of these heritable arrhythmogenic diseases. When the disease-associated mutation has been identified in the proband, cascade screening of at-risk relatives results in immediate prophylactic treatment of inherited arrhythmia syndromes.\(^13\) However, to perform such predictive testing and subsequent risk reduction, clinicians must be sure that they are testing for the causative, disease-associated mutation and not a benign unassociated variant, especially in these conditions in which sudden cardiac death can be the first presentation of disease.

Although remarkable progress has been made in the field of cardiovascular genetics over the past 10 years, the interpretation of clinical genetic test results can be complex and challenging in many cases, especially because a significant percentage of variants identified through clinical testing will be novel\(^14\) and may be deemed by the testing laboratory to be so-called variants of uncertain clinical significance. There is also the complicating factor of benign background genetic noise (ie, genetic variation in arrhythmia-associated genes found in healthy individuals), the frequency of which is currently being studied for many arrhythmia- and cardiomyopathy-associated genes and has been shown to be relatively high for certain conditions such as arrhythmogenic right ventricular dysplasia/cardiomyopathy.\(^15\) Unfortunately, it is currently not feasible to perform detailed in vivo analysis as performed by the authors to test all potentially pathogenic gene variants. However, in the absence of in vivo models, multiple approaches may be used to aid in the interpretation of genetic variants. These include cosegregation of genotype with phenotype in large kindreds; analysis of amino acid conservation across species; the screening of large, ethnically matched control populations to determine the frequency of the variant; expression and functional analyses of the gene variant in primary cardiomyocytes and heterologous cells; and, in applicable cases, investigation of the type and location of the variant within the protein. Those variants that cosegregate with the phenotype, have a high degree of cross-species conservation, are located in a highly significant protein domain, and are absent from matched control subjects have the highest likelihood to be pathogenic.

In closing, genetic information should be considered probabilistic rather than absolute. Our ability to unravel DNA variation data and, in many cases, provide meaningful inter-
pretations and recommendations for anticipated phenotypes is currently being outpaced by the explosion of available genetic testing technologies. As Watanabe et al have demonstrated, deciphering the complex pathway from genetic variant to clinical cardiovascular phenotype will require the addition of more sophisticated tools, including and in addition to the in vivo assays used here, the application of systems genetics, and mathematical modeling to study cardiovascular networks. These innovative tools will allow identification of the mechanisms linking genetic variants to multiple clinical phenotypes by integrating information regarding rare and common variants, their interactions with each other, and epigenetic and environmental modulators. In this way, we may one day find ourselves in a better position to predict, prevent, and personalize treatment for arrhythmogenic diseases.

**Sources of Funding**

We acknowledge support from the National Institutes of Health (HL084583, HL083422 to Dr Mohler), Pew Scholars Trust (Dr Mohler), and Fondation Leducq Award to the Alliance for CaMKII Signaling in Heart Disease (Dr Mohler).

**Disclosures**

A.C. Sturm is a scientific speaker consultant for Bio-Reference Laboratories in relation to GeneDx.

**References**


**Key Words:** Editorial ■ genetic testing ■ genetic variation ■ genetics ■ ion channels ■ polymorphism, genetic
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*Circulation*. 2011;124:993-995
doi: 10.1161/CIRCULATIONAHA.111.051466
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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