A Modern Approach to Classify Missense Mutations in Cardiac Channelopathy Genes

Hugues Abriel, MD, PhD; Elena V. Zaklyazminskaya, MD, PhD

Ion channels are essential membrane proteins that allow for the passage of ions across the plasma membrane in virtually all cell types. The 3 intrinsic features of ion channels are their gating, ionic selectivity, and modulation properties. Many of these ion channels are opened on depolarization of the cell membrane and are hence said to be voltage-gated. Excitability of neurons and myocytes is a fundamental cellular property that is governed by the activity of many different types of such voltage-gated channels.

For >20 years, it has been established that numerous human disorders are caused by dysfunctional channels, either because the genes coding for their subunits are mutated or because of nongenetic acquired causes, thus defining, respectively, genetic or acquired channelopathies. In 1995, in a series of seminal articles, it has been shown that congenital long-QT syndrome (LQTS) patients were carriers of mutations in several of the key genes coding for voltage-gated channels that are involved in the generation of the action potential of cardiomyocytes. In this field of genetic cardiac channelopathies, LQTS and Brugada syndrome are the 2 main phenotypes characterized by specific ECG alterations and the occurrence of ventricular arrhythmias that may lead to sudden cardiac death. As of today, 13 susceptibility genes have been linked to congenital LQTS and 12 genes to Brugada syndrome. The prevalence of these genetic cardiac channelopathies in the general population has been difficult to estimate, but it is now accepted that, for these 2 main phenotypes, they cannot be classified as rare anymore because the frequencies are ≈1:2000. With the use of the most recent DNA sequencing technologies, and similar to what has been found in many other genes, it is clear that the cardiac channelopathy genes are very polymorphic. Furthermore, several studies, as well as different databases, such as the National Heart, Lung, and Blood Institute (NHBLI) exome sequencing project, demonstrate that many genetic variants are found in the general control populations of various ethnic origins. Thus, 1 of the big challenges of cardiologists and medical geneticists who are taking care of cardiac channelopathy patients and families is to make sense of this genetic heterogeneity and, above all, to find the causative (pathogenic) genetic variants. This task is further complicated by the fact that, in most cases, the penetrance and expressivity of these variants have been shown to be variable.

Currently, several approaches are used to address the question of pathogenicity of novel variants. As a matter of fact, a set of standards to assist in the determination of the significance of variants identified in routine testing in clinical diagnostic laboratories has been published. They include the testing of ethnically matched controls, cosegregation in family with disease (if possible), occurrence of de novo variant with the sporadic incidence of disease, species conservation, in silico (bioinformatic) prediction of pathogenic effect and splicing site, and functional studies of variants. Note that all these analyses are not specific to any particular disease. In addition, these tools, taken altogether or in various combinations, can be useful for any gene of interest, such as genes encoding ion channels or sarcomeric proteins. Bioinformatic analyses of variants of unknown clinical significance is a complex and time-consuming process that can, however, provide important insights on pathogenicity and even propose directions for experimental validation. Despite being considered to be among the most informative tools, functional studies, using reexpression of ion channels in cellular systems, have their own clear limitations. For example, it has been shown that 1 Brugada syndrome SCN5A missense variant displayed no phenotype when expressed in HEK293 cells, whereas there was a clear loss-of-function when expressed in cultured cardiomyocytes. In another study, it was only by generating a knock-in mouse line that the SCN5A p.D1275N variant could demonstrate its pathogenicity. Finally, cellular functional experiments sometimes may even be misleading, as in the case of the KCNQ1 p.A341V variant, for which the clinical phenotype could be classified as severe, whereas the cellular expression analyses initially suggested only subtle biophysical alterations.

As a consequence, there is presently a clear need to develop alternative strategies to deal with the finding of new genetic variants in channelopathy patients, particularly for the frequent missense mutations. This is exactly what has been attempted in the study by Giudicessi et al, from the group of Dr M.J. Ackerman at Mayo Clinic, published in this edition of Circulation: Cardiovascular Genetics. Briefly, the authors of this study used and tested 4 bioinformatic tools, that is, conservation analysis, Grantham matrix, and the software tools Sorting Intolerant From Tolerant From Tolerant and Polymorphism Phenotyping to infer about the possible pathogenicity of missense variants in the 2 genes, KCNQ1 and KCNH2 (coding for the voltage-gated K+ channels K,7.1 and K,11.1/hERG), that
are most commonly found to be mutated in LQTS patients.\(^3\) By analyzing missense variants found in a well-characterized LQTS population (cases) and in a control population (controls), the authors could demonstrate that the 4 tools are able to distinguish between case-derived and control-derived variants in both genes, with the exception of the Grantham matrix value for KCNH2. Remarkably, when ≥3 of the 4 tools agreed on the pathogenic status of C-terminal missense variants, located outside the cyclic nucleotide-binding domain of KCNH2, the computed estimated predicted value improved from 56% to 91%. Taking into account the observation that the location of the variant in the specific topology of both ion channels has been previously shown to be predictive for pathogenicity,\(^15\) the authors have integrated their finding of the usefulness of the 4 tools in a decision tree (Figure 3 of Giudicessi et al)\(^14\) that will help clinicians classify the variants in patients or the general population.

This study is, without a doubt, helping this field to move ahead, but one can also mention limitations, as was also thoughtfully discussed by the authors. For instance, the possible influence of the variants on splicing has not been investigated, which may be relevant, because intronic synonymous and nonsynonymous variants in genes encoding ion channels\(^16\) or other proteins\(^17\) can also affect messenger RNA stability and lead to splicing defects. Furthermore, both the bioinformatic tools, Sorting Intolerant From Tolerant and Polymorphism Phenotyping, are integrating in their algorithm multiple sequence alignments. Thus, 3 of the 4 tools used (Sorting Intolerant From Tolerant, Polymorphism Phenotyping, and multiple alignment) basically represent advanced modifications of the same principle, which may decrease the power of using parallel approaches.

In contrast, one of the remarkable findings of this study is that the same combination of tools was found to produce different estimated predictive values for the 2 K\(^+\) channel genes and even for different domains of the channels. This may suggest that it could be even more effective to develop gene-specific or domain-specific algorithms. For example, one could use selected lists of species for alignment, taking into account the phylogenetic information of channel genes and specific regions of the channel. Another interesting point to be mentioned is that because the estimated predictive power for KCNQ1 is higher than that for KCNH2,\(^14\) it may be suggested that this gene tolerate less variation. However, this is in contrast with the clinical observation, showing that LQTS patients carrying mutations in KCNQ1 require implanted cardioverter-defibrillator treatment less frequently compared with patients with KCNH2 mutations.\(^18\) It may be that this apparent paradox reflects redundant cellular mechanisms underlying the essential function of the K\(_\text{v}7.1\)-mediated current in cardiac cells.

For the cardiac channelopathy field, this study is just the beginning of a journey. The authors have shown, however, a clear and useful approach for variant analysis of genes that are involved in cardiac arrhythmias. In this article, Giudicessi et al\(^14\) have studied the 2 most prevalent LQTS genes encoding voltage-gated K\(^+\) channels. It would be of great interest to continue this work and to apply the proposed approach to the highly polymorphic SCN5A gene (encoding the voltage-gated Na\(^+\) channel, Na\(_\text{v}1.5\)), where hundreds of common variants and rare mutations with ≥8 distinct phenotypes have been found.\(^19\) Also, it could be of interest to compare predictive value analyzing genes that code for ion channel accessory subunits and regulatory proteins that interact with ion channels.

Finally, this study represents a very promising attempt to increase the accuracy of interpretation of molecular findings in clinical practice. The need for such tools is clearly increasing, because there is an unparalleled development of molecular diagnostics in regulated healthcare systems as well as commercial direct-to-consumer genetic tests. The availability of an unprecedented amount of genetic information will most likely be very problematic in the absence of reliable ways to translate these data into clinically relevant information.\(^20\) At this stage, this study,\(^14\) published in Circulation Cardiovascular Genetics, represents a nice proof of concept for which the potential clinical usefulness is obvious and that will have to be further developed and refined for the better care of thousands of patients and families with genetically determined cardiac arrhythmias.

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**References**

to ankyrin-G and expression of Nav1.5 on the surface of cardiomyocytes. 


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