long QT syndrome (LQTS) is a clinically and genetically heterogeneous disorder of myocardial repolarization that often manifests clinically as heart rate–corrected QT interval (QTc) prolongation on 12-lead ECG and increased risk of syncope and sudden cardiac death. Among phenotypically robust nonsyndromic LQTS cases (ie, persistent QTc prolongation $\geq 480$ ms or Schwartz diagnostic score $\geq 3.5$), $\approx 75\%$ are anticipated to harbor a heterozygous pathogenic variant in 1 of the 3 major LQTS-susceptibility genes ($\text{KCNQ1}/\text{LQT1}$, $\approx 35\%$; $\text{KCNH2}/\text{LQT2}$, $\approx 30\%$; and $\text{SCN5A}/\text{LQT3}$, $\approx 10\%$).

As a result of established genotype-phenotype correlations, the identification of a putative pathogenic variant enables use of genotype-guided approaches to risk stratification and clinical management. As reflected by the widely adopted 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology classification and reporting standards (Figure), ascertainment of variant pathogenicity/causality is largely dependent on 3 factors: (1) inheritance (degree of cosegregation or de novo status), (2) rarity (absence in public exomes or over-representation in cases versus controls), and (3) function (in vitro or in vivo functional studies that display a damaging effect on the gene or gene-product). Without $\geq 2$ of these factors, the pathway to pathogenicity for a given rare, never-before-seen, missense variant becomes increasingly daunting. Unfortunately, the information needed to assess inheritance is often incomplete or unavailable and functional characterization notoriously expensive and cumbersome. As a result, efforts over the past decade have focused on the development and refinement of sequence (ie, conservation scores, physicochemical properties, etc)—and structure (ie, tertiary/secondary structure, solvent accessible surface area, etc)—based in silico prediction tools designed to serve as a surrogate to in vitro/in vivo functional studies. Although individual in silico prediction tools have a tendency to overcall rare missense variants as deleterious, the agreement of multiple in silico tools are used commonly in rare variant adjudication to fulfill either pathogenic supporting (PP3) or benign supporting (BP4) criteria in the current American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines (Figure).

In this issue of Circulation: Cardiovascular Genetics, Li et al13 look to improve on existing nonspecific in silico prediction tools such as sorting intolerant from tolerant (SIFT), polymorphism phenotyping version 2 (PolyPhen2),15 and protein variant effect analyzer (PROVEAN)16 commonly used in rare variant adjudication by introducing Q1VarPred, a novel sequence parameter-based, artificial neural network-driven, $\text{KCNQ1}$-encoded K$_v$7.1 potassium channel-specific in silico prediction tool. Although machine learning algorithms underlie most in silico prediction tools in use today,14,15 and voltage-gated potassium channel-specific tools (ie, KvSNP) have been described previously,17 the efforts of Li et al13 represent the first attempt to use a channel-specific machine learning approach to predict the potential functional impact of rare $\text{KCNQ1}$ genetic variants.

To this end, Li et al13, aided by a carefully curated compendium of 107 previously functionally characterized $\text{KCNQ1}$ missense variants, demonstrate that $\text{KCNQ1}$ variants with severely dysfunctional in vitro electrophysiological phenotypes preferentially localize to certain highly conserved K$_v$7.1 subdomains. Because of a large number of shared $\text{KCNQ1}$
variants, it is not surprising that the Kv7.1 subdomains enriched with dysfunctional KCNQ1 variants overlap substantially with the Kv7.1 topology-based estimated predicted values put forward in previous large case-control studies. That said, the division of Kv7.1 into 24 distinct subdomains appears to provide additional granularity, particularly within the difficult-to-interpret Kv7.1 N- and C-terminal regions. However, because of a paucity of functionally characterized KCNQ1 variants and potential bias introduced by the tendency of individual functional studies to focus on a specific region(s) in the Kv7.1 channel, great caution should be exercised if the subdomains proposed by Li et al, especially those relatively conserved transmembrane and C-terminal subdomains (S1, S2, S3–S4, S5-pore, B, and B–C) not shown to be enriched with dysfunctional KCNQ1 variants, are used in probability of pathogenicity assessments/rare variant adjudication.

Nevertheless, armed with evidence that the position-specific rate of evolution is likely a strong predictor of in vitro electrophysiological phenotype, Li et al use a novel position-specific rate of evolution measurement that takes into account both topology and phylogenetic tree branch length as well as a conventional position-specific scoring matrix that assesses the anticipated physicochemical radicalness of a given amino acid substitution as predictive/input features to train the 3-layer feedforward artificial neural network that underlies Q1VarPred. After repeated cross-validation to assure generalizability, the predictive performance of Q1VarPred was compared with 8 existing in silico prediction tools in parallel. Interestingly, the Kv7.1-specific Q1VarPred outperformed the 7 nonspecific and single potassium-channel specific in silico prediction tools assessed. However, it remains to be seen whether the enhanced performance of Q1VarPred, which was only modestly superior to PROVEAN, is because of (1) the Kv7.1-specific approach, (2) the divergent method used for calculating the position-specific rate of evolution, or (3) a combination of these and other factors.

At present, the utility of Q1VarPred is likely limited to working hand-in-hand with time-tested nonspecific in silico prediction tools to determine whether a given KCNQ1 variant meets American College of Medical Genetics and Genomics/Association for Molecular Pathology variant classification and reporting guidelines. Multiple lines of computational/in silico evidence criteria are highlighted with yellow boxes. BA indicates benign stand-alone; BP, benign supporting; BS, benign strong; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; and PVS, pathogenic very strong. Adapted from Richards et al.

| Variants | Benign | Supporting | Pathogenic |
|----------|--------|------------|------------|
| MAF > 5% in public exome databases (BA1) | Strong | Supporting | Absent in public exome databases (PM2) |
| MAF is too high for disorder (BS3) | Supporting | Moderate | Predicated null variant in a gene where LOF is a known mechanism of disease (PV51) |
| Multiple lines of computational evidence suggest no impact on gene/gene product (BP4) | Strong | Supporting | Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before (PM5) |
| Silent variant with no predicted splice impact (BP7) In-frame indels in repeat w/o known function (BP3) | Supporting | Moderate | Protein length changing variant (PM4) |
| Missense gene where only truncating cause disease (BP1) | Absent in public exome databases (PM2) | Supporting | Same amino acid change as an established pathogenic variant (PS1) |
| Missense in gene with low rate of benign missense variants and path, missense common (PP2) | Moderate | Supporting | Well-established functional studies show a deleterious effect (PS3) |
| Co-segregation with disease in multiple affected family members (PP1) | Supporting | Strong | De novo (without paternity & maternity confirmed) (PM4) |
| Increased segregation data | Moderate | Supporting | De novo (paternity & maternity confirmed) (PS2) |
| Observed in trans with a dominant variant (BP2) | Supporting | Pathogenic moderate | For recessive disorders, detected in trans with a pathogenic variant (PM3) |
| Observed in cis with a pathogenic variant (BP2) | Supporting | Pathogenic strong | For recessive disorders, detected in cis with a pathogenic variant (PM3) |
| Reputable source w/o shared data = benign (BP4) | Supporting | Benign strong | For recessive disorders, detected in cis with a pathogenic variant (PM3) |
| Reputable database = pathogenic (PP5) | Supporting | Benign strong | For recessive disorders, detected in cis with a pathogenic variant (PM3) |
| Found in case with an alternate cause (BP5) | Absent in public exome databases (PM2) | Supporting | Pathogenic very strong |
| Patient’s phenotype or FH highly specific for gene (PP4) | Supporting | Pathogenic strong |

**Figure.** Role of in silico prediction algorithms in the 2015 American College of Medical Genetics and Genomics/Association for Molecular Pathology variant classification and reporting guidelines. Multiple lines of computational/in silico evidence criteria are highlighted with yellow boxes. BA indicates benign stand-alone; BP, benign supporting; BS, benign strong; PM, pathogenic moderate; PP, pathogenic supporting; PS, pathogenic strong; and PVS, pathogenic very strong. Adapted from Richards et al.
functional impact, observed in vitro electrophysiological effect(s), and actual disease phenotype. If feasible, there is no doubt that such approaches could improve the utility of in silico prediction tools and help to solve the variants of uncertain significance crisis that actively plagues genetic testing for LQTS and other sudden cardiac death-predisposing genetic disorders.

**Disclosures**

None.

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