

ORIGINAL RESEARCH ARTICLE

Multimodal *KCNQ1* Variant-Effect Maps Define Ion Channel Mechanisms and Disease Risk

M. Lorena Harvey¹ PhD; Ellen Osborn² MS; Megan C. Lancaster³ MD, PhD; Jeremy E. Smith⁴ MS; Qianyi Shen, MMolBiol; Temidayo A. Abe⁵ MD; Ruoyi Cai⁶ PhD; Ayesha Muhammad⁷ MD, PhD; Rose Kannankeril, BS; Tao Yang⁸ MD, PhD; Maria E. Calandranis⁹ BS; Daniel J. Blackwell¹⁰ PhD; Richard E. Dolder¹¹ III, BS; Jessa L. Aldridge¹² PhD; Matthew R. Fleming¹³ MD, PhD; Marcia A. Blair¹⁴ MS; Eduardo Soria, BS; Jack W. O'Sullivan¹⁵ MBBS, DPhil; Giovanni Davogustto¹⁶ MD, MSc; Brett M. Kroncke¹⁷ PhD; Calum A. MacRae¹⁸ MD, PhD; Bjorn C. Knollmann¹⁹ MD, PhD; Frederick P. Roth²⁰ PhD; Victoria N. Parikh²¹ MD; Euan A. Ashley²² MRCP, DPhil; Jamie I. Vandenberg²³ MBBS, PhD; Chai-Ann Ng²⁴ PhD; Dan M. Roden²⁵ MD*; Andrew M. Glazer²⁶ PhD*

BACKGROUND: Variants in the ion channel gene *KCNQ1* are the leading cause of congenital long QT syndrome (LQTS), a disorder characterized by QT prolongation and risk of fatal arrhythmias. The expanding use of genetic testing has led to the identification of numerous variants of uncertain significance, complicating clinical interpretation and risk stratification. Functional characterization of *KCNQ1* variants is essential to define their role in long QT syndrome and advance precision medicine in cardiovascular care.

METHODS: We generated a nearly comprehensive variant library of 13 403 protein-coding variants in *KCNQ1*. Using 4 pooled assays followed by high-throughput sequencing, we characterized the variants' effects on cell surface abundance and channel function in both the variant-only and heterozygous states. Case-control analyses and data from large-scale biobanks were used to assess the clinical impact of loss-of-function variants on QT prolongation and long QT syndrome. Heterozygous assay results were used to determine dominant-negative effects, which were also evaluated for association with arrhythmia risk. Protein structural clustering of distinct classes of deleterious variants was analyzed.

RESULTS: Across the 4 assays, we generated 49 305 functional measurements of *KCNQ1* variants. Of the 13 403 *KCNQ1* studied variants, 1757 demonstrated strong pathogenic evidence, and 6660 showed moderate benign evidence. Loss-of-function variants were significantly associated with QT prolongation and long QT syndrome in case-control and biobank analyses. Deleterious variants clustered within distinct structural domains based on their functional classification. A majority of pathogenic variants exhibited dominant-negative effects; these variants were linked to a higher risk of arrhythmia diagnoses than were haploinsufficient variants.

CONCLUSIONS: This comprehensive *KCNQ1* functional atlas provides novel functional evidence for most *KCNQ1* variants of uncertain significance. It also reveals mechanistic principles linking genetic variation to ion channel biology and arrhythmia susceptibility, advancing cardiovascular precision medicine.

Key Words: arrhythmia ■ arrhythmias, cardiac ■ genetic testing ■ *KCNQ1* potassium channel ■ long QT syndrome

Editorial, see p XXX

The human genome has ≈400 ion channel genes, with >100 linked to ion channelopathy disorders.¹ Variants can impair channel function through

diverse mechanisms, including disrupted protein folding, defective membrane trafficking, or altered channel gating.² In addition, multimeric channels can exert either

Correspondence to: Andrew M. Glazer, PhD, Vanderbilt Genetics Institute, Ashley Medical Center, 2215B Garland Ave, 1275 MRB IV, Nashville, TN 37232. Email andrew.m.glazer@vumc.org

*D.M. Roden and A.M. Glazer contributed equally.

Supplemental Material is available at <https://www.ahajournals.org/doi/suppl/10.1161/CIRCULATIONAHA.126.080900>.

This manuscript was invited by the editorial team led by Dr Joseph Hill and jointly handled and accepted by the editorial team led by Dr Bradley Maron.

© 2026 American Heart Association, Inc.

Circulation is available at www.ahajournals.org/journal/circ

Clinical Perspective

What Is New?

- A near-comprehensive functional atlas of 13 403 *KCNQ1* coding variants was generated using 4 high-throughput assays measuring surface abundance and channel function in variant-only and heterozygous states.
- These assays identified trafficking-defective, gating-defective, gain-of-function, haploinsufficient, and dominant-negative variants, providing new mechanistic resolution for *KCNQ1* variant interpretation.
- Experimentally measured loss of function was strongly associated with QT prolongation and long QT syndrome risk across case-control and biobank analyses.

What Are the Clinical Implications?

- High-throughput data sets provided calibrated functional evidence for thousands of *KCNQ1* variants, including many variants currently classified as variants of uncertain significance.
- Dominant-negative *KCNQ1* variants were associated with higher arrhythmia risk than haploinsufficient variants, suggesting that molecular mechanism may inform clinical risk stratification.
- *KCNQ1* functional maps may help translate genetic testing results into more precise diagnosis, counseling, and management for patients with suspected long QT syndrome.

Nonstandard Abbreviations and Acronyms

B/LB	benign or likely benign
DN	dominant-negative
JLNS	Jervell and Lange-Nielsen syndrome
LoF	loss of function
GoF	gain of function
LQTS	long QT syndrome
MAVE	multiplexed assay of variant effect
P/LP	pathogenic or likely pathogenic
RR	risk ratio
VUS	variants of uncertain significance
WT	wild-type

haploinsufficient or dominant-negative (DN) effects in heterozygous individuals, further complicating variant interpretation.³ These mechanisms are difficult to measure at scale and are poorly predicted by existing computational approaches.

KCNQ1 is a canonical channelopathy gene that encodes $K_v7.1$, the pore-forming subunit of a voltage-gated potassium channel. $K_v7.1$ assembles as a homo-

tetramer and functions in a macromolecular complex with calmodulin and the KCNE1 β -subunit.^{4–7} This complex generates the slow delayed rectifier current (I_{Kr}), which plays a central role in cardiac electrophysiology. *KCNQ1* loss-of-function (LoF) variants are the most common cause of congenital long QT syndrome (LQTS), characterized by QT prolongation on the ECG and an increased risk of life-threatening ventricular arrhythmias.^{8,9} Because arrhythmic death from LQTS is preventable, *KCNQ1* is designated as an actionable gene by the American College of Medical Genetics and Genomics.^{10,11}

Accurate clinical classification of *KCNQ1* variants is key for precision cardiovascular medicine. However, 70% of *KCNQ1* missense variants in ClinVar are classified as variants of uncertain significance (VUS) or have conflicting interpretations.¹² Functional assessment of ion channels relies on low-throughput manual patch-clamp or medium-throughput automated patch-clamp measurements.^{13–15} As clinical sequencing has expanded, this limited functional capacity has contributed to the growing burden of VUS.

Here, we applied 4 high-throughput multiplexed assays of variant effect (MAVEs) to comprehensively measure the functional impact of *KCNQ1* coding variants. We studied 13 403 variants (94% of possible variants) using fluorescence-activated cell sorting and cell-fitness assays in both variant-only and heterozygous contexts. More than 2500 variants showed altered function, including trafficking-defective, gating-defective, and DN variants. Functional scores strongly predicted clinical classifications and LQTS risk in biobank and case-control analyses. These data provide new insights into ion channel biology, enable prospective identification of deleterious variants, and demonstrate the value of high-throughput functional genomics for precision medicine and biologic function.

METHODS

Overview of the MAVE Experimental Approach

A myc epitope tag was inserted into the extracellular region of *KCNQ1* to facilitate the measurement of protein surface abundance. Saturation mutagenesis was performed to generate a comprehensive plasmid library of 13 706 single amino acid *KCNQ1* variants (obtaining 97% of 14 196 possible nonsense, synonymous, and missense variants). Random 18-bp barcodes were cloned into the plasmid library, resulting in many barcodes corresponding to each variant. Deep sequencing of the plasmid pool established correlations between the barcodes and their respective variants. HEK293 landing pad cells were transfected with the *KCNQ1*-myc variant library, which integrated into the landing pad (1 plasmid/cell). Surface abundance was evaluated by staining live cells with a fluorophore-conjugated anti-myc antibody, followed by fluorescence-activated cell sorting into 4 bins based on fluorescence intensity and deep sequencing for quantification. Channel function was assessed by treating the cells with triple-drug media over a 28-day period, with cell pellets collected every 7 days for deep sequencing and barcode quantification.

In heterozygous studies, HEK293 landing pad cells expressing an HA (hemagglutinin)-tagged copy of wild-type (WT) *KCNQ1* were transfected with the *KCNQ1* variant library, and cells were assessed for channel function or WT *KCNQ1* (potassium voltage-gated channel subfamily KQT member 1)-HA surface abundance as described previously. After quality control, at least one MAVE score was obtained for 13 403 variants. Detailed Methods are available in the Supplemental Material.

Statistical Analyses

All statistical analyses were performed in R or Python using built-in functions unless otherwise indicated. Comparisons of MAVE abundance scores among charge-substitution classes in the N-terminus and HA-HB linker were assessed using Kruskal-Wallis tests. Correlations between MAVE scores, replicate measurements, individual validation assays, and computational predictions were assessed using Spearman correlation. Receiver operating characteristic curves were used to evaluate the ability of MAVE scores to distinguish pathogenic or likely pathogenic (P/LP) from benign or likely benign (B/LB) variants. Functional evidence was calibrated using binary OddsPath calculations and a continuous kernel density log-likelihood ratio framework. Case-control enrichments were summarized as risk ratios (RRs) with 95% CIs calculated on the log-transformed risk-ratio scale. Biobank associations with QTc or LQTS diagnoses were assessed using regression models as described in the cohort-specific methods.

Institutional Approval of Human Studies

All 3 biobank projects were conducted using de-identified participant data sets. The BioVU study protocol was approved by the Vanderbilt University Medical Center institutional review board (approval 251056). The UK Biobank research was conducted under approved application numbers 24983 and 22282. The All of Us protocol was approved by the institutional review board of the All of Us Research Program.

Data, Code, and Materials Availability

All unique and stable reagents generated in this study are available from the corresponding author with a completed materials transfer agreement. The MAVE data sets are available in the Supplemental Material and will be deposited in MaveDB and ClinVar upon publication. The analysis code is available at https://github.com/GlazerLab/KCNQ1_MAVE. Any additional information required to reanalyze the data reported in this article is available from the corresponding author upon request.

RESULTS

Overview of *KCNQ1* MAVE Assays

We assessed *KCNQ1* in 4 MAVE assays in human embryonic kidney (HEK293) cells. We generated a comprehensive barcoded plasmid library comprising 13 706 *KCNQ1* variants represented by 266 868 unique barcodes (19.5 barcodes/variant). Across the 4 assays (Tables S1 and S2), we obtained measurements for 13 403 variants, covering 94% of possible *KCNQ1* coding variants (12 223 missense, 544 synonymous, and 636 non-

sense). Scores were scaled so 1 represents WT and 0 complete LoF (File S1).

MAVE of *KCNQ1* Surface Abundance

Many membrane protein variants disrupt folding or trafficking, causing reduced cell-surface abundance.¹⁶ To quantify *KCNQ1* surface abundance, we introduced the variant library into landing pad HEK293 cells,¹⁷ ensuring single-variant expression at uniform levels. Live cells were labeled with an antibody targeting an extracellular myc tag,¹⁸ sorted by fluorescence intensity, and barcodes were deep sequenced (Figure S1). Surface abundance scores were obtained for 93% of all possible *KCNQ1* coding variants (Figure 1A) and were highly reproducible across replicates (Spearman $\rho=0.89-0.92$; Table S2). We defined early nonsense (residues 1–610) and late nonsense (611–676) variants and identified 2321 loss-of-abundance, 1333 partial loss-of-abundance, and 305 gain-of-abundance missense variants (Figure 1D).

MAVE abundance scores correlated strongly ($\rho=0.87$) with single-variant surface abundance measurements for 134 published variants^{2,19,20} and 24 additional variants assessed here by flow cytometry (Figure 1E, Figure S3H, and Table S3). A small fraction of synonymous variants (21 of 541 [3.9%]) had abundance scores <0.5 ; follow-up single-variant testing of 7 such variants showed near-normal surface abundance, indicating experimental noise (Figure S2 and Table S3). A total of 93% of ClinVar-designated B/LB variants (26 of 28) had normal surface abundance, whereas only 27% of P/LP variants (59 of 217) had normal abundance (Figure 1F).

Structure-function analysis revealed pronounced mutational sensitivity across key regions (zoomed-in heatmaps presented in Figure S3). Although *KCNQ1* lacks a canonical signal peptide, its first transmembrane segment functions as a signal anchor,²¹ and additional N-terminal motifs showed strong sensitivity, including an LEL_{38–40} trafficking motif²² and previously unrecognized endoplasmic reticulum retention signals (RKR_{12–14} and RR_{24–25}; Figure S3D). The N-terminal 40 residues and a region between helices HA and HB exhibited strong charge dependence, with negatively charged substitutions increasing surface abundance and positively charged substitutions decreasing it (Figures S3D and S4). Transmembrane helices S1 through S6 were broadly intolerant to charged and polar substitutions, and the pore-lining P helix and P loop were especially sensitive (Figure S3C and S3D). Cytosolic HA and HB helices had mutational sensitivity in calmodulin-binding IQ and 1-5-10 motifs (Figure S3E).²³ Coiled-coil helices HC and HD displayed periodic mutational sensitivity consistent with a heptad repeat architecture, and disruption of residues mediating HD intersubunit salt bridges caused loss of abundance (Figure S3E and S5).^{18,24,25} Nearly every variant in a C-terminal PY motif (LPxYxxx_{659–665}) caused gain

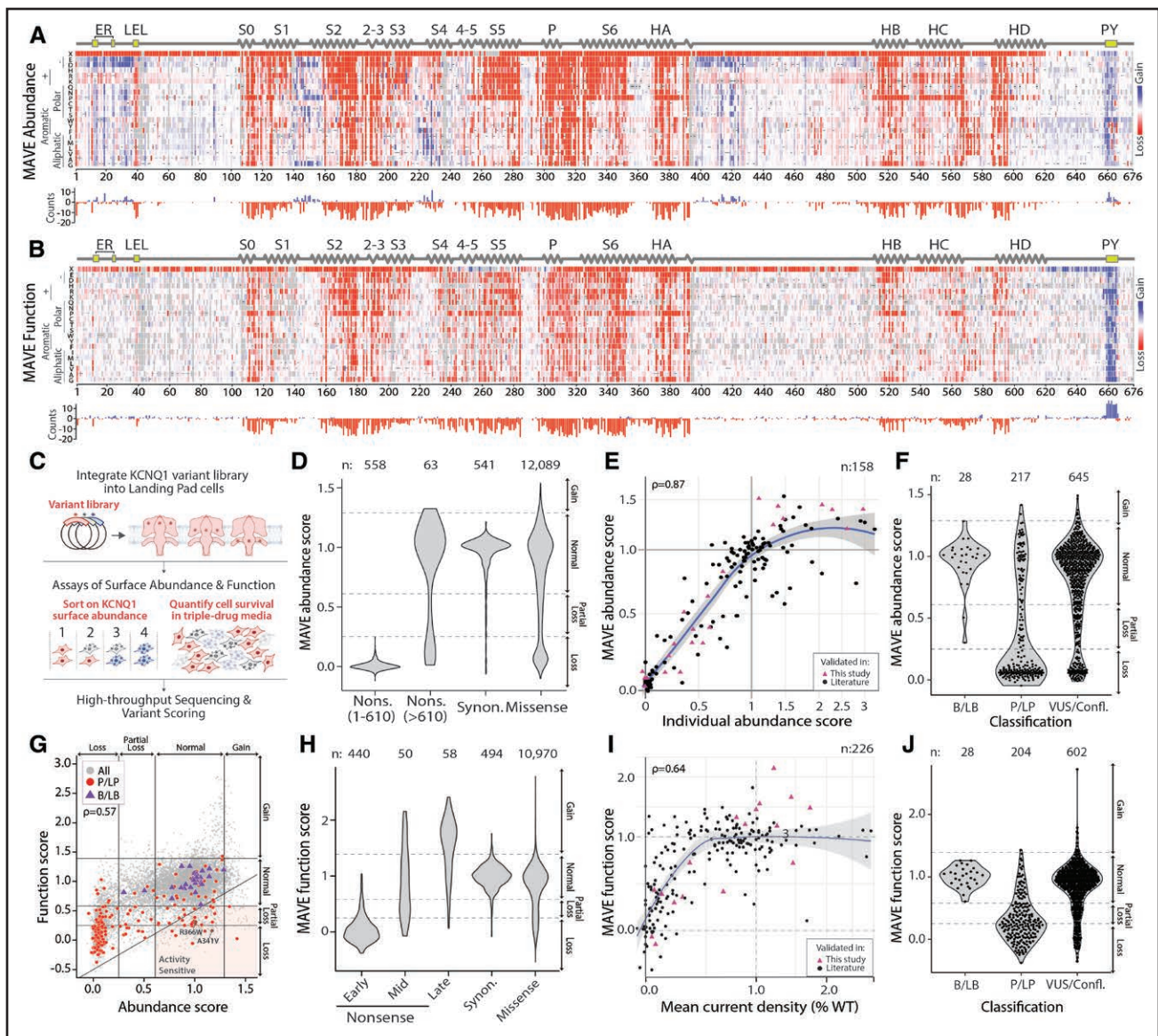


Figure 1. Multiplexed assays of variant effect of *K_v7.1* surface abundance and function.

A and **B**, Heatmaps of multiplexed assay of variant effect (MAVE) scores for surface abundance (**A**) and function (**B**) across *KCNQ1* coding variants. Secondary structure and motifs are annotated. White, wild-type-like; red, loss; blue, gain; black dashes, wild-type residues. **C**, Schematic of the MAVE workflow. **D**, Distribution of surface abundance scores by variant class. **E**, Correlation of MAVE surface abundance scores with individual measurements for 158 missense variants (24 from this study, 134 from the literature). One outlier value ($x=5.01$, $y=1.29$) was removed from the plot. **F**, Surface abundance scores stratified by clinical classification (benign/likely benign [B/LB], pathogenic/likely pathogenic [P/LP], or variant of uncertain significance [VUS]/conflicting). **G**, Function scores versus abundance scores, highlighting activity-sensitive variants (impaired function, normal surface abundance) among all variants (gray), P/LP variants (red), and B/LB variants (purple), with 2 known activity-sensitive variants (A341V, R366W) labeled. **H**, Distribution of function scores by variant class. **I**, Correlation of MAVE function scores with individual patch clamp measurements for 226 missense variants (including 19 from this study). **J**, Function scores stratified by clinical classification. ER indicates endoplasmic reticulum.

of abundance (Figure S3A and S3B). This degron motif is a binding site for E3 ubiquitin ligases, such as Nedd4-2, which ubiquitinate *K_v7.1*, causing degradation.²⁶

MAVE of *KCNQ1* Function

The surface abundance assay cannot identify variants that have normal surface abundance but impaired chan-

nel activity (such as A341V and R366W).^{20,27,28} To capture these effects, we developed a function assay that measures a growth disadvantage of *KCNQ1*-expressing cells (Figure S1F). Over 28 days, WT *KCNQ1* caused a modest fitness defect (61% fitness compared with non-expressing cells). This effect was enhanced (to 17% fitness) by treatment with 2 channel activators (ML277 and R-L3) and ouabain, which inhibits the Na^+/K^+ -ATPase

responsible for maintaining transmembrane sodium and potassium gradients. We applied this “triple-drug” function assay to the *KCNQ1* variant library (Figure 1B). Across 5 timepoints, LoF nonsense variants increased in frequency compared with synonymous variants, whereas PY motif gain-of-function (GoF) variants were rapidly depleted (Figure S6). Function scores, derived from changes in frequency between initial and final pools, were obtained for 85% of possible variants and were reproducible across 6 replicates ($p=0.46$ – 0.54 ; Table S2). LoF variants clustered in transmembrane and cytoplasmic helices, whereas GoF variants localized predominantly to the PY ubiquitination motif. Early nonsense variants (1–610) were largely LoF, whereas late nonsense variants (≥ 611) showed mixed effects, with many exhibiting GoF scores due to loss of the PY motif (Figure 1H). Unexpectedly, nonsense variants between residues 239 and 307 showed elevated function scores. Follow-up experiments indicated that this effect arises from cryptic stop-codon readthrough in specific contexts and is not physiologically relevant (Figure S6). We did not observe a global elevation of missense function scores in this region: 85% of P/LP missense variants spanning residues 239 through 307 had reduced function scores, similar to the 82% observed for P/LP missense variants gene-wide, supporting their inclusion in downstream analyses (Figure S6I).

Overall, the assay identified 1362 LoF, 1259 partial LoF, and 339 GoF missense variants (Figure 1H). MAVE function scores correlated well ($p=0.64$) with patch-clamp measurements from 47 published studies and 19 additional variants assessed here by automated patch-clamp measurements (Figure 1I and Figures S7 and S8; $n=226$ total variants). The correlation was slightly higher when restricted to automated patch-clamp data sets ($p=0.68$; $n=174$), and was strongest for variants with paired MAVE and automated patch-clamp data generated in this study ($p=0.80$; $n=19$). Surface abundance and function scores were moderately correlated ($p=0.57$); however, 339 variants showed near-normal surface expression but partial or complete LoF (Figure 1G). These activity-sensitive variants, which likely affect channel gating, included 22 known P/LP variants, including A341V and R366W. Function scores aligned more closely with clinical classifications than abundance scores: all B/LB variants (28 of 28) had normal function, whereas 82% of P/LP variants (168 of 204) showed abnormal function (Figure 1J).

Heterozygous *KCNQ1* MAVES Reveal DN Effects

Most MAVES are conducted in a variant-only context, but variants can exhibit DN effects with considerable clinical impact, especially in multimeric proteins, such as $K_v7.1$. To address this, we aimed to perform heterozygous *KCNQ1* MAVE experiments. We engineered HEK293 landing pad cells stably expressing HA-tagged WT *KCNQ1* and

coexpressed myc-tagged variants using the landing pad. We validated the system with known DN and haploinsufficient variants (Figure S10).²

We then performed surface abundance and function MAVE assays in this heterozygous context (Figure 2A–2C and Figure S11). The heterozygous abundance assay specifically measured WT–HA surface expression, whereas the heterozygous function assay measured the combined activity of the WT and variant alleles. Heterozygous abundance and function scores correlated with previous single-variant measurements ($p=0.87$ and $p=0.47$, respectively).^{2,15,29,30} Integrating all 4 MAVE scores (variant-only/heterozygous×abundance/function), we identified 290 “severe DN” missense variants (LoF in all 4 assays) and 548 “strong DN” variants (LoF in 3 of 4 assays). Nearly all B/LB variants had normal heterozygous scores, whereas 69 P/LP variants and 38 ClinVar VUS or conflicting variants exhibited DN behavior (Figure 2C, Figure S3L, and Table S5). Of the 319 activity-sensitive variants with measured heterozygous function scores, 114 (36%) also had reduced heterozygous function, suggesting that activity-sensitive variants include a mixture of DN and haploinsufficient mechanisms.

Distinct Structural Clustering of LoF, Channel Activity, and DN Variants

Mapping of MAVE results onto the $K_v7.1$ structure revealed distinct spatial clustering of functional variant classes (Figure 2D). Transmembrane and cytosolic helices were highly enriched for LoF variants. Activity-sensitive variants with near-normal surface abundance but impaired function localized to regions essential for channel activation: the voltage-sensing S4 helix, the S4–S5 linker (which binds essential activator PIP_2), and the pore domain (S5, pore helix, and S6). Within the pore domain, 118 of 129 variants (91%) within 5 Å of the pore axis showed partial or complete LoF (Figure S9).

In contrast, a striking hotspot of DN variants was present in the voltage-sensing domain encompassing the S0, S2, and S2–S3 helices (Figure 2D and Table S5). In this region, 11 residues each harbored ≥ 10 DN variants, all of which mediate interhelix contacts within the voltage-sensing domain (Figure S3K and Table S5).² The pore selectivity filter $GYGD_{314-317}$ also showed marked DN enrichment (mean 11 DN variants/residue), including 10 known P/LP variants (Figure S3K and Table S5). Dominant-negative variants in both hotspot regions strongly reduced heterozygous surface abundance and heterozygous function scores (Figure S11). Together, these patterns define discrete structural determinants of surface abundance, channel activity, and DN behavior in *KCNQ1*.

Combining Assays and Calibration with Benign and Pathogenic Variants

To integrate variant mechanisms across the 4 assays (variant-only and heterozygous abundance and

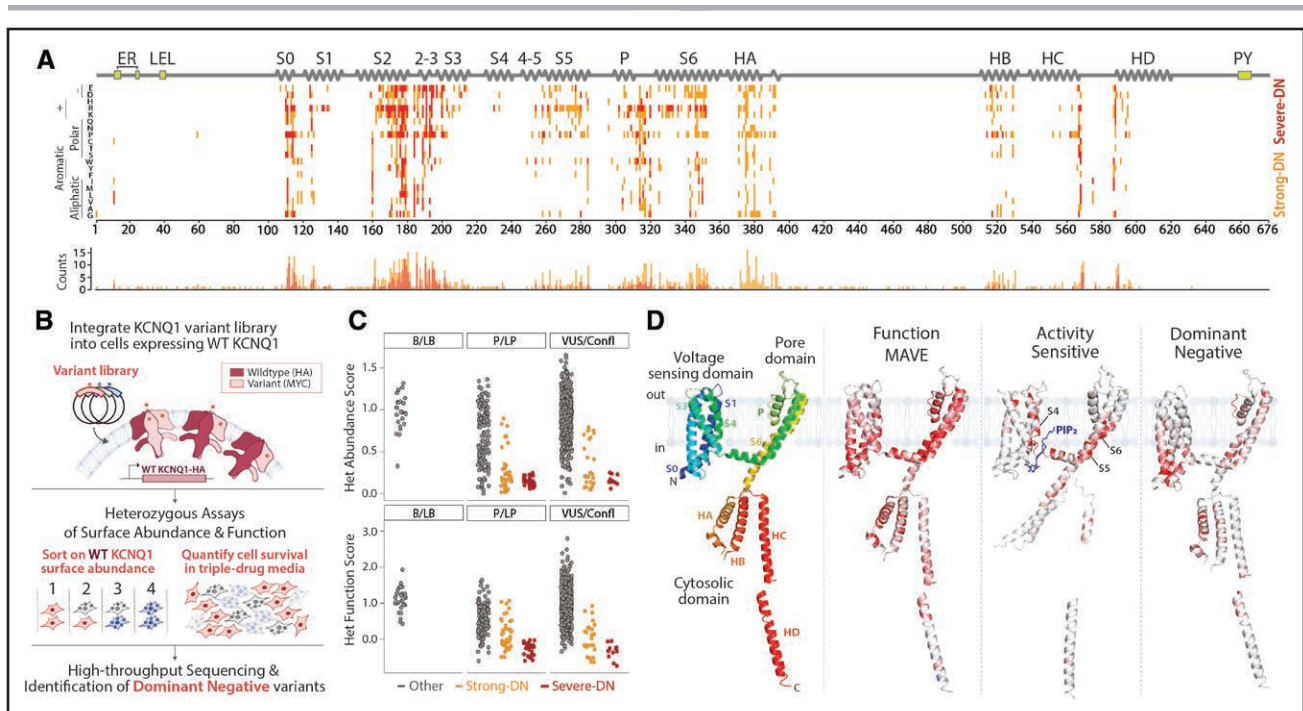


Figure 2. Heterozygous multiplexed assays of variant effect identify dominant-negative *KCNQ1* variants.

A, Heatmap of dominant-negative (DN) effects across *KCNQ1*, integrating heterozygous abundance and function multiplexed assays of variant effect (MAVEs). Variants classified as strong DN (orange) or severe DN (red) are shown across annotated secondary structure and functional motifs; the histogram below indicates the density of DN variants along the sequence. **B**, Schematic of the heterozygous MAVE workflow. **C**, Heterozygous abundance (top) and function (bottom) scores stratified by clinical classification (benign/likely benign [B/LB], pathogenic/likely pathogenic [P/LP], or variant of uncertain significance [VUS]/conflicting), highlighting that many known P/LP variants have DN effects. **D**, Structural mapping of *KCNQ1* variants with different classes of MAVE scores. Function-disrupting variants localize to transmembrane and cytosolic helices. Activity-sensitive variants cluster near S4, the S4–S5 linker, and the PIP₂-binding site. Dominant-negative variants are clustered in the voltage-sensing domain and the selectivity filter (Figure S3). Cryo-EM structures are from Protein Data Bank 6UZZ, except for the PIP₂-containing activity-sensitive structure, which is from PDB 6V01 and does not contain part of the HC helix. ER indicates endoplasmic reticulum; and WT, wild-type.

function), we performed principal component analysis using 10316 variants with complete data (Figure 3A–3C).³¹ Principal component analysis separated variants by functional class (early nonsense, synonymous, severe DN, PY motif, and late nonsense) and by clinical classification (benign versus pathogenic; Figure 3A and 3B). Unsupervised *k*-means clustering (*k*=6) produced groups highly concordant with these classes, labeled DN, haploinsufficient, partial LoF, Normal 1, Normal 2, and GoF (Figure 3C and Figure S12). Gap statistic analysis showed a broad plateau from *k*=6 to 10, with a maximum at *k*=9. We selected *k*=6 because it captured the principal structure of the data while preserving interpretable cluster identities and avoiding overpartitioning. Of 26 B/LB variants, 24 clustered within the normal groups. Among 191 P/LP variants, 109 mapped to the DN cluster, and 26 and 14 were assigned to the haploinsufficient and partial LoF clusters, respectively (Figure 3A–3C and Table S6).

We next evaluated each assay's ability to distinguish P/LP from B/LB variants using receiver operating characteristic analysis (Figure 3D). The variant-only function assay performed best (area under the curve=0.94), fol-

lowed by variant-only abundance (0.89), heterozygous function (0.80), and heterozygous abundance (0.80) assays. To test whether multiassay integration improved clinical discrimination in a method-robust manner, we evaluated 3 representative supervised classifiers recommended in a recent article which examined how to combine MAVE data sets together.³¹ All 3 multiassay models (Gaussian-naïve Bayes, logistic regression, and random forest) showed similar performance (0.92–0.96), with Gaussian-naïve Bayes performing best (0.96; Figure S13). However, the performance of these combined assays was similar to the best-performing single assay (variant-only function), so we recommend the variant-only function assay for clinical use.

To calibrate the scores for clinical application, we used B/LB and P/LP variants to compute OddsPath values (recommended by the ClinGen Sequence Variant Interpretation Working Group) from binary MAVE cutoffs (Figure 3E).³² Function scores showed the strongest concordance, with OddsPath values of 23.1 for pathogenicity and 0.177 for benignity, corresponding to PS3_strong and BS3_moderate evidence (Figure 3E and Table S4). Results were robust across alternative benign/patho-

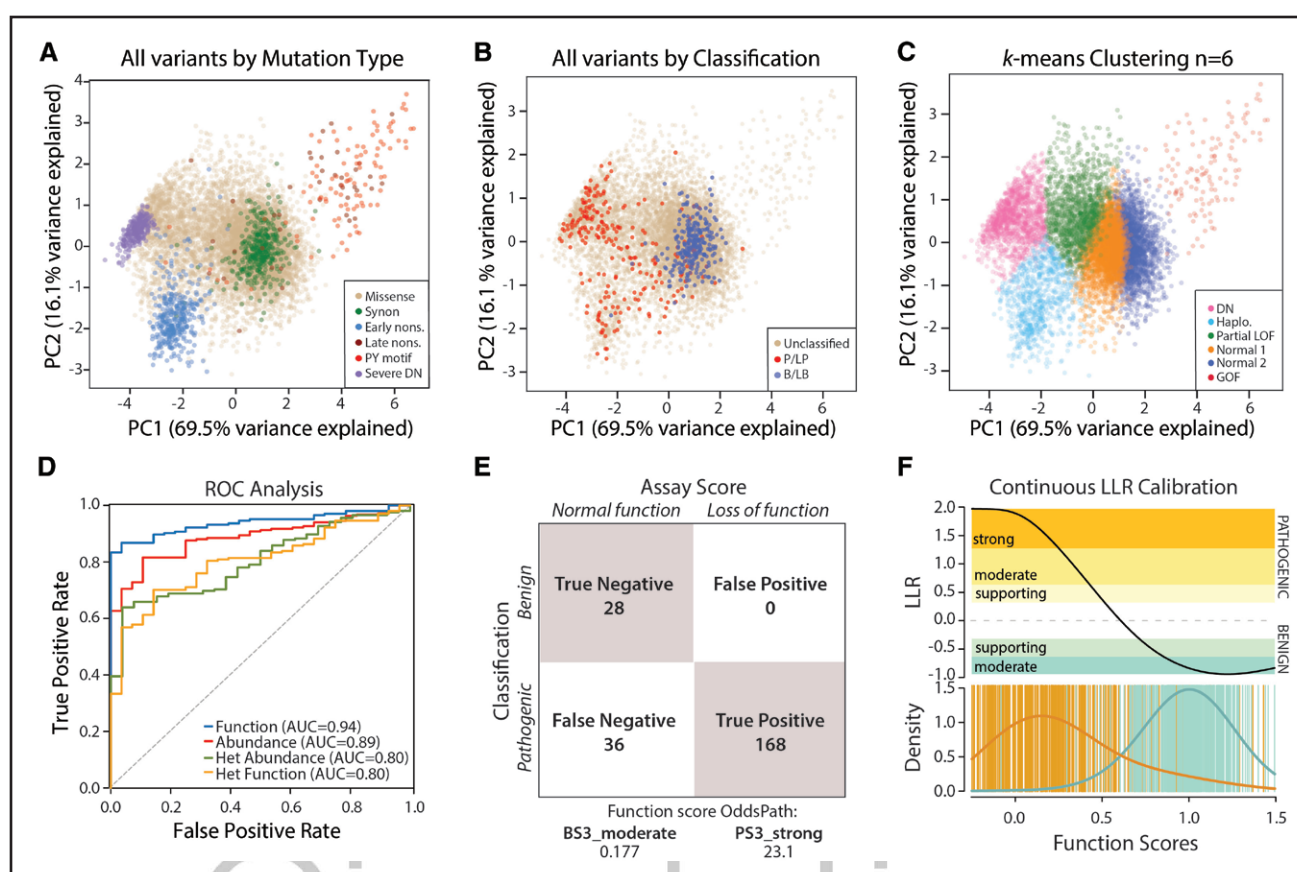


Figure 3. Combinations of assays and calibration with pathogenic and benign variants.

A through **C**, Principal components analysis of variants using all 4 scores (variant-only and heterozygous function and abundance assays). **A**, Variants color-coded by category. **B**, Variants color-coded by clinical classification. **C**, Variants clustered by *k*-means clustering (*n*=6 clusters). **D**, Receiver operating characteristic (ROC) curves for each assay's ability to predict benign versus pathogenic variants, with area under the curve (AUC) values shown for all 4 assays. **E**, OddsPath calculation for the multiplexed assay of variant effect (function assay), showing classification grids with true-positive, false-positive, true-negative, and false-negative counts aligned with clinical classifications. **F**, Calibration of multiplexed assay of variant effect function scores using log-likelihood ratios (LLRs), with the top panel showing the relationship between LLRs and function scores and the bottom panel showing the density of pathogenic and benign variants across the scoring range. B/LB indicates benign/likely benign; DN, dominant-negative; GoF, gain of function; LoF, loss of function; and P/LP, pathogenic/likely pathogenic.

genic truth sets (Figure S14). We also applied a continuous log-likelihood ratio framework, generating graded evidence strengths from PS3_strong for LoF variants to BS3_moderate for normal-function variants (Figure 3F). This approach assigned 1757 PS3_strong, 707 PS3_moderate, 333 PS3_supporting, 6660 BS3_moderate, and 1477 BS3_supporting classifications.

Applying this framework to 6 *KCNQ1* VUS from patients with LQTS, 4 variants showed abnormal function in both MAVE and automated patch clamp experiments and received PS3_strong or PS3_moderate evidence, and 2 variants with near-normal function received BS3_moderate or no evidence (Table S7). Because our assays do not capture splicing effects, we evaluated potential splicing disruption in coding regions using SpliceAI.³³ Among all possible synonymous and missense single nucleotide variants, 44 had SpliceAI scores >0.5, and 75 had suggestive scores from 0.2 to 0.5 (File S1 and Table S8). SpliceAI scores

were not incorporated into the MAVE analyses or calibrations performed in this article, but are provided to enable consideration of potential splicing effects during final variant classifications.

MAVE Scores Are Associated With QT Interval and LQTS Risk

We examined associations between *KCNQ1* MAVE scores and population allele frequencies and electrocardiographic/arrhythmia phenotypes in 711 448 participants from All of Us, BioVU, and UK Biobank.^{34–36} In gnomAD, 35 of 36 missense variants with allele frequency >3×10^{−5} had normal MAVE scores, whereas rarer or absent variants showed a broad range of functional effects (Figure 4A). The fraction of biobank participants carrying an LQTS code increased with decreasing function score, reaching 8.1% for LoF variants (Figure 4B). Among 155 401 biobank participants with ECGs, QTc (QT

corrected for heart rate) varied by function score. QTc interval was significantly longer in LoF carriers (456.7 ms [95% CI, 450.0–463.3 ms]) compared with normal-function carriers (430.9 ms [95% CI, 429.7–431.9 ms]), far exceeding the 10-ms threshold used to trigger regulatory concern for QT-prolonging drugs.³⁷

MAVE scores correlated with computational predictors (Figure S15), including AlphaMissense (Spearman $\rho = -0.49$; Figure 4C), but a subset of discordant variants had normal MAVE function scores despite pathogenic AlphaMissense scores.³⁸ In a previously published cohort of 1847 LQTS cases, variants with LoF MAVE scores showed strong enrichment relative to gnomAD controls (RR, 263 [95% CI, 241–286]; Figure 4D and Table S9).^{39,40} Variants with strong pathogenic AlphaMissense predictions were also enriched (Figure S16), with an RR of 238 (95% CI, 216–262). Variants with concordant pathogenic predictions (MAVE loss or partial LoF

and AlphaMissense strong pathogenic) had the highest risk (RR, 273 [95% CI, 254–294]), whereas concordant benign variants had minimal risk (RR, 1.8 [95% CI, 1.1–3.1]). Discordant variants (MAVE normal function but AlphaMissense strong pathogenic) showed only moderate risk (RR, 73.3 [95% CI, 59.4–90.5]), suggesting that AlphaMissense overestimates pathogenicity or identifies variants with smaller effects.

LQT1-defining clinical features, such as swimming-associated cardiac events and broad-based or slow-upstroke T waves, have been proposed as phenotype-enhanced evidence to help reclassify *KCNQ1* VUS.⁴¹ Our *KCNQ1* MAVE function scores were broadly concordant with this phenotype-based framework: variants with stronger LQT1-specific phenotypic evidence were enriched for reduced function scores, whereas variants with limited or no *KCNQ1*-defining phenotypic features generally had higher

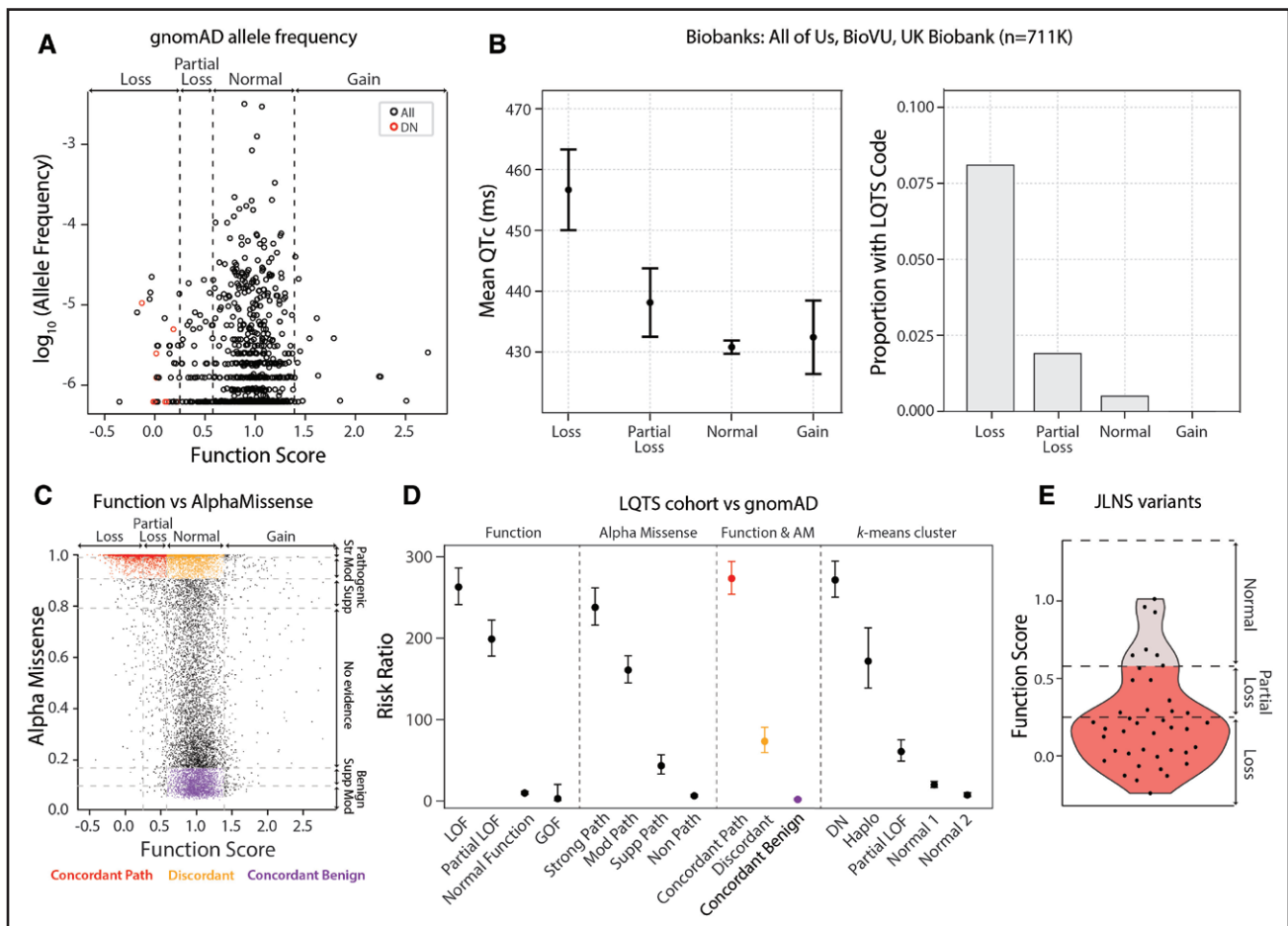


Figure 4. Multiplexed assay of variant effect scores stratify *KCNQ1* variants and are associated with arrhythmia risk.

A, Multiplexed assay of variant effect function scores versus gnomAD allele frequency for missense variants; dominant-negative (DN) variants are highlighted. **B**, Clinical associations of *KCNQ1* variant classes across biobanks (n=711 448). Left: QTc intervals (mean±95% CI). Right: long QT syndrome (LQTS) diagnostic code proportions by functional class (loss of function [LoF], partial LoF, normal, and gain of function [GoF]). **C**, Experimental function scores versus AlphaMissense pathogenicity scores. A subset of discordant variants had normal function scores and deleterious AlphaMissense predictions. **D**, Risk ratios (±95% CI) for variant classes in an LQTS cohort (n=1847) versus gnomAD controls. Left to right: multiplexed assay of variant effect-only classes, AlphaMissense-only classes, combined classes, and *k*-means clusters. This analysis is restricted to missense variants. **E**, Variants identified in patients with Jervell and Lange-Nielsen syndrome (JLNS) mostly fall within the LoF range.

function scores (Figure S18). This finding provides additional evidence that reduced KCNQ1 function measured by MAVE scores is associated with clinically distinctive LQT1 phenotypes.

When variants were grouped by *k*-means cluster, RRs followed a clear gradient: DN variants had the highest LQTS risk (RR, 272 [95% CI, 250–295]), followed by haploinsufficient (RR, 172 [95% CI, 139–213]) and partial LoF variants (RR, 60.7 [95% CI, 49.0–75.1]), whereas Normal 1 and Normal 2 clusters showed lower risk (RR, 20.0 and 7.3; Figure 4D). Similarly, using the severe/strong definition of DN variants, these variants were associated with a very high risk (Figure S16C). In an analysis restricted to individuals of European ancestry, LoF variants remained strongly enriched among LQTS cases (RR, 254 [95% CI, 230–282]), and the risk estimates across other variant classes were similar to those observed in the full cohorts (Figure S17).

Jervell and Lange-Nielsen syndrome (JLNS) is an ultrarare syndrome caused by biallelic LoF *KCNQ1* variants and characterized by severe QT prolongation and sensorineural hearing loss.⁴² Most JLNS-associated variants exhibited partial or complete LoF (34 of 41 [83%]; Figure 4E). Six JLNS-associated variants had aggregate SpliceAI scores of at least 0.10, suggesting that splicing effects not captured by our MAVE assays may contribute to their disease association. Overall, MAVE scores robustly stratify *KCNQ1* variants by risk for LQTS and outperform current computational predictors in clinical contexts.

DISCUSSION

We systematically characterized nearly all *KCNQ1* missense variants using 4 assays measuring surface abundance and channel function in variant-only and heterozygous contexts. These data sets enabled mapping of variants affecting channel trafficking, gating, or DN effects. In contrast to patch-clamp approaches, our cell fitness-based function assay provides a readout of channel activity that can scale to thousands of variants. Comparison of abundance and function scores showed that most pathogenic variants impair surface expression; however, a substantial subset of activity-sensitive variants had near-normal abundance but impaired function, including 22 known pathogenic variants. By performing MAVEs in a heterozygous context, we generated a global map of DN effects, identifying 838 DN missense variants that were associated with higher clinical risk than haploinsufficient variants. These findings provide a mechanistic framework for severe LQTS phenotypes and highlight DN variants as potential targets for intensified clinical management or future allele-specific therapies.^{43,44} Notably, DN behavior arose from disruption of a key intersubunit interface

important for function (the pore selectivity filter) and destabilizing variants in a domain not directly involved in multimerization (the voltage-sensing domain). DN effects in the voltage-sensing domain are also consistent with the cooperative gating of K_v channels, in which impaired voltage-sensor activation or coupling in 1 subunit can reduce opening of the assembled tetramer.⁴⁵ Thus, the voltage-sensor domain hotspot may reflect both impaired channel biogenesis, as supported by reduced heterozygous surface abundance, and altered cooperative gating. Although our heterozygous MAVE assays cannot fully distinguish specific *KCNQ1* variant–WT interactions from endoplasmic reticulum retention due to broader effects on cellular proteostasis, previous Förster resonance energy transfer–based studies support a model in which DN *KCNQ1* variants impair surface trafficking through direct interactions between variant and WT subunits within the tetrameric channel complex.⁴⁶ Our DN MAVE framework can be applied to other multimeric proteins in which disease severity is driven by DN effects rather than by dosage reduction alone.

The fitness defect of WT *KCNQ1* over 28 days suggests that *KCNQ1* expression imposes a small basal cost, likely from potassium leak. At the HEK293 resting membrane potential (≈ -30 mV), $K_{v7.1}$ is largely closed but retains residual activation. Channel activators increase potassium conductance at this voltage,⁴⁷ and ouabain further sensitizes cells by inhibiting Na^+/K^+ ATPase, which typically maintains sodium and potassium gradients. Assuming a 24-hour doubling time, 17% relative abundance over 28 days of triple-drug treatment could reflect an added death rate of $\approx 6.1\%$ per day or an increased doubling time of 26.4 hours. Thus, selection causes modest cumulative effects rather than acute toxicity. We observed similar behavior in our triple-drug $Na_v1.5$ assay,⁴⁸ so an assay combining a channel activator with ouabain might be generalizable to a broad set of sodium and potassium channels.

Our structure–function maps refine previous *KCNQ1* hotspot studies^{39,49,50} by resolving mutational sensitivity at single-residue resolution. In addition to validating known motifs involved in trafficking,²² calmodulin binding,²³ and ubiquitination,²⁶ we identified previously unrecognized patterns, including endoplasmic reticulum retention motifs and charge-dependent effects in unstructured regions. Disruption of cytosolic calmodulin-binding and coiled-coil domains reduced both abundance and function, consistent with their roles in channel assembly and multimerization. Periodic mutational sensitivity within HC and HD coiled-coils reflected heptad repeat architecture, and HD charged residues mediating interhelix salt bridges were particularly sensitive.^{25,51}

Most nonsense variants resulted in LoF; however, late truncations that remove the PY motif showed GoF

effects, consistent with impaired ubiquitination. *KCNQ1* late C-terminal truncating variants with increased function scores are analogous to PY motif deletions in the epithelial sodium channel that cause Liddle syndrome.⁵² The most common *KCNQ1* nonsense variant, Y662X, falls into this category and has been reported in patients with early-onset atrial fibrillation (reported with *KCNQ1* GoF variants),⁵³ syncope, or sudden death^{29,54}; however, Y662X's clinical classification currently ranges from VUS to benign in ClinVar. Of the 126 missense GoF variants in the GoF *k*-means cluster, only 6 were present in gnomAD. The most common was E663K (allele frequency=3.8×10⁻⁶), and the 6 variants had a combined allele frequency of 1.02×10⁻⁵. We did not identify clinically relevant phenotypic associations, such as shorter QT intervals, for GoF variants in our biobank analyses. These analyses may be underpowered because of the small number of GoF variant carriers. Future work will be needed to further clarify the clinical associations of these variants.

k-means clustering identified variant groups associated with disease risk, with DN variants showing a higher RR than haploinsufficient variants. These clusters should be interpreted as a summary of functional patterns rather than a definitive mechanistic taxonomy. Although gap-statistic analysis supported several plausible cluster numbers, we selected *k*=6 as a parsimonious solution; the distinction between the Normal 1 and Normal 2 clusters was modest and driven primarily by the relatively noisy heterozygous functional assay score.

We evaluated assay performance and calibrated functional scores for clinical use. The variant-only function assay showed the strongest discrimination between benign and pathogenic variants, and calibration using either binary OddsPath or continuous log-likelihood frameworks yielded clinically interpretable functional evidence. We generated new pathogenic or benign functional evidence for >10 000 variants, including 1757 variants with PS3_strong and 6660 variants with BS3_moderate evidence, which will substantially reduce the burden of VUS in *KCNQ1*. The current study complements our previous high-throughput study of *KCNH2* variants,⁵⁵ which combined automated patch clamp measurements for >500 variants with a MAVE surface abundance assay. Together, these studies provide large-scale functional data sets for the 2 major genetic causes of long QT syndrome. Whereas the *KCNH2* study provided substantially broader direct electrophysiologic characterization, the current *KCNQ1* study extends the MAVE framework beyond surface abundance by adding scalable channel function assays and heterozygous assays to identify DN effects.

MAVE function scores were strongly associated with QTc prolongation and LQTS diagnoses in a case-

control analysis and across 3 large biobanks. Whereas MAVE scores correlated with computational predictors, discordant variants showed only moderate LQTS risk, suggesting that current predictors may overestimate pathogenicity in *KCNQ1*. The monotonic relationship between experimentally measured channel dysfunction and QT prolongation at population scale provides rare empirical support for long-standing assumptions in clinical electrophysiology. Intriguingly, the 838 DN variants measured in this study had a higher aggregate LQTS RR than haploinsufficient variants. This observation is consistent with a previous report of higher event rates among patients with DN LQT1 variants compared with haploinsufficient variants.⁵⁶ Future work will be needed to confirm these observations and further determine whether MAVE-identified DN variants confer greater LQT1 disease risk or more severe clinical outcomes than haploinsufficient variants in clinical cohorts.

A large majority of JLNS-associated variants showed LoF in our MAVE assays, although a small subset had partial or near-normal measured protein function. Discordant JLNS variants may reflect assay measurement error, differences between HEK293 cells and native cardiac or inner-ear contexts, or disease mechanisms not captured by our coding-variant assays, including RNA splicing effects suggested by elevated SpliceAI scores in several discordant variants. Biallelic variants classified as LoF in our variant-only function assay are the strongest candidates to cause JLNS (File S1).

Limitations

Limitations of this study include the inability to assess splicing effects, which could be addressed using high-throughput splicing assays or endogenous gene-editing approaches.^{57,58} KCNE1 was not included because its coexpression interferes with *KCNQ1* myc-tag labeling and interferes with *KCNQ1* activator drug binding.⁴⁷ Although KCNE1 coexpression generally has only modest effects on variation in *KCNQ1* surface abundance and channel function,^{2,30} some variants may specifically disrupt KCNE1-dependent gating or *KCNQ1*-KCNE1 coupling. Previous studies have identified KCNE1-dependent effects for *KCNQ1* variants near the extracellular S1/S1-S2 region and residues in S4 and S5,⁵⁹⁻⁶¹ suggesting that our maps may be less accurate in these regions. Future work could evaluate discordant P/LP variants with normal MAVE scores by patch clamp in the presence of KCNE1 or develop KCNE1-inclusive MAVE assays to capture mechanisms missed here.

The assays were performed in heterologous cells rather than cardiomyocytes and therefore do not capture all aspects of native cardiac electrophysiology, such as cardiomyocyte-specific trafficking, binding partners, and adrenergic modulation. Nonetheless, the strong

performance of the assays against established benign and pathogenic control variants suggests that the effects of most *KCNQ1* coding variants are preserved across cellular contexts. The minority of pathogenic variants with normal MAVE scores may reflect mechanisms not captured by these assays. Future scalable studies in induced pluripotent stem cell-derived cardiomyocytes using emerging technologies^{62–64} may help identify these additional mechanisms and further refine variant interpretation.

The 8.1% prevalence of LQTS diagnostic codes among LoF carriers is lower than that observed in family-based penetrance studies,^{65,66} and should be interpreted as a minimum estimate of electronic health record–based diagnostic enrichment rather than true clinical penetrance, given limitations of biobank phenotyping, including undercoding and incomplete ascertainment.

We note that the benign–pathogenic validation and LQTS case–control enrichment analyses are not fully independent, because some criteria used to classify variants as benign or pathogenic also influence their representation in case–control data sets, including allele frequency and previous observation in LQTS cases.

CONCLUSION

This comprehensive *KCNQ1* atlas demonstrates that high-throughput functional genomics can elucidate ion channel biology, identify DN effects at scale, and translate molecular measurements into population-level disease risk. For clinicians evaluating patients with suspected LQT1, these data provide variant-level functional evidence that can be integrated with phenotype, segregation, family history, and population-frequency data during clinical variant interpretation. The variant-only functional assay results are most immediately applicable to variant classification and genetic counseling, whereas using DN status to guide changes in clinical management or predict arrhythmia risk will require further validation in deeply phenotyped LQT1 cohorts. As sequencing uncovers additional rare variants in actionable disease genes, similar approaches will be essential for converting genetic data into mechanistic insight and clinical action.

ARTICLE INFORMATION

Received April 10, 2026; accepted August 20, 2026.

Affiliations

Department of Medicine (M.L.H., M.C.L., J.E.S., T.A.A., A.M., R.K., T.Y., M.E.C., D.J.B., J.L.A., M.R.F., M.A.B., E.S., G.D., B.M.K., B.C.K., D.M.R., A.M.G.), Vanderbilt Genetics Institute (D.M.R., A.M.G.), and Departments of Biomedical Informatics (D.M.R.) and Pharmacology (D.M.R.), Vanderbilt University Medical Center, Nashville, TN. Vanderbilt Human Genetics Program, Vanderbilt University, Nashville, TN (E.O., R.E.D.). Mark Cowley Lidwill Research Program in Cardiac Electrophysiology, Victor Chang Cardiac Research Institute, Darlinghurst, Australia (Q.S., J.I.V.). Stanford Center for Inherited Cardiovascular Disease, Division of Cardiovascular Medicine, Department of Medicine, Stanford School of Medicine, Palo Alto, CA (R.C., J.W.O.,

V.N.P., E.A.A.). Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA (C.A.M.). Department of Computational and Systems Biology, University of Pittsburgh School of Medicine, PA (F.P.R.). School of Clinical Medicine, University of New South Wales, Sydney, Australia (J.I.V., C.-A.N.).

Acknowledgments

The authors thank Ashli Chew, Zerubabell Daniel, and Lynn Hall for assistance with experiments; Kayla Carmichael and Jeff Calhoun for assistance with computational analyses; Doug Fowler and Kenneth Matreyek for providing the HEK293 landing pad cells; and Al George, Chuck Sanders, Henrike Heyne, Francesca Rissom, and the CardioVar consortium for discussions.

Author Contributions

Conceptualization: Drs Harvey, Roden, and Glazer. Formal analysis: Drs Harvey, Lancaster, Abe, Cai, Muhammad, Blackwell, O'Sullivan, Vandenberg, Ng, Roden, and Glazer, and E. Osborn, J.E. Smith, Q. Shen, and R. Kannankeril. Funding acquisition: Drs Kroncke, MacRae, Roth, Parikh, Ashley, Roden, and Glazer. Investigation: Drs Harvey, Yang, Fleming, Blackwell, Aldridge, Ng, and Glazer, and Q. Shen, R.E. Dolder, M.A. Blair, M.E. Calandranis, R. Kannankeril, and E. Soria. Supervision: Drs Davogustto, Kroncke, MacRae, Knollmann, Roth, Parikh, Ashley, Vandenberg, Ng, Roden, and Glazer. Writing—original draft: Drs Harvey, Lancaster, Roden, and Glazer, and E. Osborn. Writing—review and editing: all authors.

Sources of Funding

This project was supported by National Institutes of Health grants R01 HL164675, R00 HG010904, R35 GM150465, R01 HL160863, K12CA090625, and K08HG014001; American Heart Association fellowships 25PRE1360565 and 23CDA1042141; and a Medical Research Future Fund (Genomics Health Futures Mission grant MRF2016760).

Disclosures

Dr MacRae: founder and shareholder of Atman Health and Tanaist, scientific advisor and shareholder for TMA Precision Health, scientific advisor for Everyone Medicines, and nonexecutive director and shareholder of LifeMD. Dr Parikh: consultant or scientific advisory board member for Lexeo Therapeutics, Solid Biosciences, and Constantium Biosciences. Dr Ashley: founder of Personalis, Deepcell, Svexa, Saturnus Bio, and Swift Bio; founder advisor for Candela and Parameter Health; advisor for Pacific Biosciences; nonexecutive director for AstraZeneca and Dexcom; publicly traded stock: Personalis, Pacific Biosciences, and AstraZeneca; collaborative support in kind from Illumina, Pacific Biosciences, Oxford Nanopore, Cache, and Cellsonics. Dr Roth: advisor and shareholder with Constantium Biosciences; shareholder in SeqWell. No other author declares any conflicts of interest. During the preparation of this work, the authors used ChatGPT, Gemini, and Grammarly for computer programming assistance or to improve the readability and language of the manuscript. After using these tools, the authors reviewed and edited the content as needed, and they take full responsibility for the content of the published article.

Supplemental Material

Methods
Figures S1–S19
Tables S1–S14
Files S1–S3
References 67–101

REFERENCES

- Kim J-B. Channelopathies. *Korean J Pediatr*. 2014;57:1–18. doi: 10.3345/kjp.2014.57.1.1
- Huang H, Kuenze G, Smith JA, Taylor KC, Duran AM, Hadziselimovic A, Meiler J, Vanoye CG, George AL, Sanders CR. Mechanisms of KCNQ1 channel dysfunction in long QT syndrome involving voltage sensor domain mutations. *Sci Adv*. 2018;4:eaar2631. doi: 10.1126/sciadv.aar2631
- Shalaby FY, Levesque PC, Yang WP, Little WA, Conder ML, Jenkins-West T, Blannar MA. Dominant-negative KvLQT1 mutations underlie the LQT1 form of long QT syndrome. *Circulation*. 1997;96:1733–1736. doi: 10.1161/01.cir.96.6.1733
- Sanguinetti MC, Curran ME, Zou A, Shen J, Spector PS, Atkinson DL, Keating MT. Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I(Ks) potassium channel. *Nature*. 1996;384:80–83. doi: 10.1038/384080a0
- Sun J, MacKinnon R. Cryo-EM structure of a KCNQ1/CaM complex reveals insights into congenital long QT syndrome. *Cell*. 2017;169:1042–1050.e9. doi: 10.1016/j.cell.2017.05.019

6. Sachyani D, Dvir M, Strulovich R, Tria G, Tobelaim W, Peretz A, Pongs O, Svergun D, Attali B, Hirsch JA. Structural basis of a Kv7.1 potassium channel gating module: studies of the intracellular c-terminal domain in complex with calmodulin. *Structure*. 2014;22:1582–1594. doi: 10.1016/j.str.2014.07.016
7. Mandala VS, MacKinnon R. The membrane electric field regulates the PIP₂-binding site to gate the KCNQ1 channel. *Proc Natl Acad Sci U S A*. 2023;120:e2301985120. doi: 10.1073/pnas.2301985120
8. Wang Q, Curran ME, Splawski I, Burn TC, Millholland JM, VanRaay TJ, Shen J, Timothy KW, Vincent GM, de Jager T, et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat Genet*. 1996;12:17–23. doi: 10.1038/ng0196-17
9. Schwartz PJ, Crotti L. Long QT syndrome. *N Engl J Med*. 2025;393:2023–2034. doi: 10.1056/NEJMra2400853
10. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, Gollob MH, Gordon AS, Harrison SM, Hershberger RE, et al; ACMG Secondary Findings Working Group. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. 2023;25:100866. doi: 10.1016/j.jgim.2023.100866
11. Wilde AAM, Semsarian C, Márquez MF, Sepehri Shamloo A, Ackerman MJ, Ashley EA, Sternick Eduardo B, Barajas-Martinez H, Behr ER, Bezzina CR, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) expert consensus statement on the state of genetic testing for cardiac diseases. *J Arrhythm*. 2022;38:491–553. doi: 10.1002/joa3.12717
12. Glazer AM, Tabet DR, Parikh VN, Kroncke BM, Cote AG, Yamamoto Y, Wang Q, Muhammad A, Lancaster MC, O'Neill MJ, et al. Creating an atlas of variant effects to resolve variants of uncertain significance and guide cardiovascular medicine. *Nat Rev Cardiol*. 2026;23:149–163. doi: 10.1038/s41569-025-01201-7
13. Boulet IR, Raes AL, Ottischtych N, Snyders DJ. Functional effects of a KCNQ1 mutation associated with the long QT syndrome. *Cardiovasc Res*. 2006;70:466–474. doi: 10.1016/j.cardiores.2006.02.006
14. Lu H, Ding W, Xiao H, Dai M, Xue Y, Jia Z, Guo J, Wu M, Shen B, Zhao R. Association of the P441L KCNQ1 variant with severity of long QT syndrome and risk of cardiac events. *Front Cardiovasc Med*. 2022;9:922335. doi: 10.3389/fcvm.2022.922335
15. Vanoye CG, Desai RR, Fabre KL, Gallagher SL, Potet F, DeKeyser J-M, Macaya D, Meiler J, Sanders CR, George AL. High-throughput functional evaluation of KCNQ1 decrypts variants of unknown significance. *Circ Genom Precis Med*. 2018;11:e002345. doi: 10.1161/CIRCGEN.118.002345
16. Anderson CL, Kuznicki CE, Childs RR, Hintz CJ, Delisle BP, January CT. Large-scale mutational analysis of Kv11.1 reveals molecular insights into type 2 long QT syndrome. *Nat Commun*. 2014;5:5535. doi: 10.1038/ncomms6535
17. Matreyek KA, Stephany JJ, Chiasson MA, Hasle N, Fowler DM. An improved platform for functional assessment of large protein libraries in mammalian cells. *Nucleic Acids Res*. 2020;48:e1. doi: 10.1093/nar/gkz910
18. Kanki H, Kupersmidt S, Yang T, Wells S, Roden DM. A structural requirement for processing the cardiac K⁺ channel KCNQ1. *J Biol Chem*. 2004;279:33976–33983. doi: 10.1074/jbc.M404539200
19. Huang H, Chamness LM, Vanoye CG, Kuenze G, Meiler J, George AL Jr, Schleich JP, Sanders CR. Disease-linked supertrafficking of a potassium channel. *J Biol Chem*. 2021;296:100423. doi: 10.1016/j.jbc.2021.100423
20. Brewer KR, Vanoye CG, Huang H, Clowes Mosier KR, Desai RR, Hayes JB, Burnette DT, George AL, Sanders CR. Integrative analysis of KCNQ1 variants reveals molecular mechanisms of type 1 long QT syndrome pathogenesis. *Proc Natl Acad Sci U S A*. 2025;122:e2412971122. doi: 10.1073/pnas.2412971122
21. Sun J, MacKinnon R. Structural basis of human KCNQ1 modulation and gating. *Cell*. 2020;180:340–347.e9. doi: 10.1016/j.cell.2019.12.003
22. Jespersen T, Rasmussen HB, Grunnet M, Jensen HS, Angelo K, Dupuis DS, Vogel LK, Jorgensen NK, Klaerke DA, Olesen S-P. Basolateral localisation of KCNQ1 potassium channels in MDCK cells: molecular identification of an N-terminal targeting motif. *J Cell Sci*. 2004;117:4517–4526. doi: 10.1242/jcs.01318
23. Yus-Najera E, Santana-Castro I, Villarroel A. The identification and characterization of a noncontinuous calmodulin-binding site in nonactivating voltage-dependent KCNQ potassium channels. *J Biol Chem*. 2002;277:28545–28553. doi: 10.1074/jbc.M204130200
24. Howard RJ, Clark KA, Holton JM, Minor DL Jr. Structural insight into KCNQ (Kv7) channel assembly and channelopathy. *Neuron*. 2007;53:663–675. doi: 10.1016/j.neuron.2007.02.010
25. Xu Q, Minor DL Jr. Crystal structure of a trimeric form of the K(V)7.1 (KCNQ1) A-domain tail coiled-coil reveals structural plasticity and context dependent changes in a putative coiled-coil trimerization motif. *Protein Sci*. 2009;18:2100–2114. doi: 10.1002/pro.224
26. Jespersen T, Membrez M, Nicolas CS, Pitard B, Staub O, Olesen S-P, Baró I, Abriel H. The KCNQ1 potassium channel is down-regulated by ubiquitylating enzymes of the Nedd4/Nedd4-like family. *Cardiovasc Res*. 2007;74:64–74. doi: 10.1016/j.cardiores.2007.01.008
27. Shamgar L, Ma L, Schmitt N, Haitin Y, Peretz A, Wiener R, Hirsch J, Pongs O, Attali B. Calmodulin is essential for cardiac IKS channel gating and assembly: impaired function in long-QT mutations. *Circ Res*. 2006;98:1055–1063. doi: 10.1161/01.RES.0000218979.40770.69
28. Schwartz PJ, Moreno C, Kotta M-C, Pedrazzini M, Crotti L, Dagradi F, Castelletti S, Haugaa KH, Denjoy I, Shkolnikova MA, et al. Mutation location and IKs regulation in the arrhythmic risk of long QT syndrome type 1: the importance of the KCNQ1 S6 region. *Eur Heart J*. 2021;42:4743–4755. doi: 10.1093/eurheartj/ehab582
29. Glazer AM, Davogusto G, Shaffer CM, Vanoye CG, Desai RR, Farber-Eger EH, Dikilitas O, Shang N, Pacheco JA, Yang T, et al; eMERGE Network. Arrhythmia variant associations and reclassifications in the eMERGE-III sequencing study. *Circulation*. 2022;145:877–891. doi: 10.1161/CIRCULATIONAHA.121.055562
30. Rinné S, Oertli A, Nagel C, Tomsits P, Jenewein T, Käb S, Kaufenstein S, Loewe A, Beckmann BM, Decher N. Functional characterization of a spectrum of novel Romano-Ward syndrome KCNQ1 variants. *Int J Mol Sci*. 2023;24:1350. doi: 10.3390/ijms24021350
31. Calhoun JD, Dawood M, Rowlands CF, Fayer S, Radford EJ, McEwen AE, Tejura M, Turnbull C, Spurdle AB, Starita LM, et al. Combining multiplexed functional data to improve variant classification. *Genome Med*. Published July 11, 2026. doi: 10.1186/s13073-026-01715-w
32. Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, Kanavy DM, Luo X, McNulty SM, Starita LM, et al; Clinical Genome Resource Sequence Variant Interpretation Working Group. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Med*. 2020;12:3. doi: 10.1186/s13073-019-0690-2
33. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, Darbandi SF, Knowles D, Li YI, Kosmicki JA, Arbelaez J, Cui W, Schwartz GB, et al. Predicting splicing from primary sequence with deep learning. *Cell*. 2019;176:535–548.e24. doi: 10.1016/j.cell.2018.12.015
34. Denny JC, Rutter JL, Goldstein DB, Philippakis A, Smoller JW, Jenkins G, Dishman E; All of Us Research Program Investigators. The “All of Us” research program. *N Engl J Med*. 2019;381:668–676. doi: 10.1056/NEJMs1809937
35. Avery C, Babanejad M, Baker J, Bledsoe X, Blostein F, Corty RW, Ellis K, Hung AM, Lake A, Shelley J, et al; Vanderbilt University Medical Center and Alliance for Genomic Discovery Investigators. Genome sequencing of 35,024 predominantly African ancestry persons addresses gaps in genomics and healthcare. *medRxiv*. 2025;2025.10.30.25338549. doi: 10.1101/2025.10.30.25338549
36. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779
37. Turner JR, Rodriguez I, Mantovani E, Gintant G, Kowey PR, Klotzbaugh RJ, Prasad K, Sager PT, Stockbridge N, Strnadova C; Cardiac Safety Research Consortium. Drug-induced proarrhythmia and torsade de pointes: a primer for students and practitioners of medicine and pharmacy. *J Clin Pharmacol*. 2018;58:997–1012. doi: 10.1002/jcph.1129
38. Cheng J, Novati G, Pan J, Bycroft C, Žemgulytė A, Applebaum T, Pritzel A, Wong LH, Zielinski M, Sargeant T, et al. Accurate proteome-wide missense variant effect prediction with AlphaMissense. *Science*. 2023;381:eadg7492. doi: 10.1126/science.adg7492
39. Walsh R, Lahrouchi N, Tados R, Kyndt F, Glinge C, Postema PG, Amin AS, Nannenberg EA, Ware JS, Whiffin N, et al; Nantes Referral Center for Inherited Cardiac Arrhythmia. Enhancing rare variant interpretation in inherited arrhythmias through quantitative analysis of consortium disease cohorts and population controls. *Genet Med*. 2021;23:47–58. doi: 10.1038/s41436-020-00946-5
40. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al; Genome Aggregation Database Consortium. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581:434–443. doi: 10.1038/s41586-020-2308-7

41. Bains S, Dotzler SM, Krijger C, Giudicessi JR, Ye D, Bikker H, Rohatgi RK, Tester DJ, Bos JM, Wilde AAM, et al. A phenotype-enhanced variant classification framework to decrease the burden of missense variants of uncertain significance in type 1 long QT syndrome. *Heart Rhythm*. 2022;19:435–442. doi: 10.1016/j.hrthm.2021.11.017
42. Winbo A, Stattin E-L, Diamant U-B, Persson J, Jensen SM, Rydberg A. Prevalence, mutation spectrum, and cardiac phenotype of the Jervell and Lange-Nielsen syndrome in Sweden. *Europace*. 2012;14:1799–1806. doi: 10.1093/europace/eus111
43. Dotzler SM, Kim CSJ, Gendron WAC, Zhou W, Ye D, Bos JM, Tester DJ, Barry MA, Ackerman MJ. Suppression-replacement KCNQ1 gene therapy for type 1 long QT syndrome. *Circulation*. 2021;143:1411–1425. doi: 10.1161/CIRCULATIONAHA.120.051836
44. Cócera-Ortega L, Wilders R, Kamps SC, Fabrizi B, Huber I, van der Made I, van den Bout A, de Vries DK, Gepstein L, Verkerk AO, et al. shRNAs targeting a common KCNQ1 variant could alleviate long-QT1 disease severity by inhibiting a mutant allele. *Int J Mol Sci*. 2022;23:4053. doi: 10.3390/ijms23074053
45. Pathak M, Kurtz L, Tombola F, Isacoff E. The cooperative voltage sensor motion that gates a potassium channel. *J Gen Physiol*. 2005;125:57–69. doi: 10.1085/jgp.200409197
46. Wilson AJ, Quinn KV, Graves FM, Bitner-Glindzic M, Tinker A. Abnormal KCNQ1 trafficking influences disease pathogenesis in hereditary long QT syndromes (LQT1). *Cardiovasc Res*. 2005;67:476–486. doi: 10.1016/j.cardiores.2005.04.036
47. Zou X, Wu X, Sampson KJ, Colecraft HM, Larsson HP, Kass RS. Pharmacological rescue of specific long QT variants of KCNQ1/KCNE1 channels. *Front Physiol*. 2022;13:902224. doi: 10.3389/fphys.2022.902224
48. Glazer AM, Kroncke BM, Matreyek KA, Yang T, Wada Y, Shields T, Salem J-E, Fowler DM, Roden DM. Deep mutational scan of an SCN5A voltage sensor. *Circ Genom Precis Med*. 2020;13:e002786. doi: 10.1161/CIRCGEN.119.002786
49. Kapa S, Tester DJ, Salisbury BA, Harris-Kerr C, Pungliya MS, Alders M, Wilde AAM, Ackerman MJ. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation*. 2009;120:1752–1760. doi: 10.1161/CIRCULATIONAHA.109.863076
50. Novelli V, Faultless T, Cerrone M, Care M, Manzoni M, Bober SL, Adler A, De-Giorgio F, Spears D, Gollob MH. Enhancing the interpretation of genetic observations in KCNQ1 in unselected populations: relevance to secondary findings. *Europace*. 2023;25:eua4317. doi: 10.1093/europace/ead317
51. Wiener R, Haitin Y, Shanger L, Fernández-Alonso MC, Martos A, Chomsky-Hecht O, Rivas G, Attali B, Hirsch JA. The KCNQ1 (Kv7.1) COOH terminus, a multitiered scaffold for subunit assembly and protein interaction. *J Biol Chem*. 2008;283:5815–5830. doi: 10.1074/jbc.M707541200
52. Schild L, Lu Y, Gautschi I, Schneeberger E, Lifton RP, Rossier BC. Identification of a PY motif in the epithelial Na channel subunits as a target sequence for mutations causing channel activation found in Liddle syndrome. *EMBO J*. 1996;15:2381–2387.
53. Mehta NJ, Khan IA, Mehta RN, Burgonio B, Lakhanpal G. Effect of thrombolytic therapy on QT dispersion in elderly versus younger patients with acute myocardial infarction. *Am J Ther*. 2003;10:7–11. doi: 10.1097/00045391-200301000-00004
54. Yoneda ZT, Anderson KC, Quintana JA, O'Neill MJ, Sims RA, Glazer AM, Shaffer CM, Crawford DM, Stricker T, Ye F, et al. Early-onset atrial fibrillation and the prevalence of rare variants in cardiomyopathy and arrhythmia genes. *JAMA Cardiol*. 2021;6:1371–1379. doi: 10.1001/jamacardio.2021.3370
55. O'Neill MJ, Ng C-A, Aizawa T, Sala L, Bains S, Winbo A, Ullah R, Shen Q, Tan C-Y, Kozek K, et al. Multiplexed assays of variant effect and automated patch clamping improve KCNH2-LQTS variant classification and cardiac event risk stratification. *Circulation*. 2024;150:1869–1881. doi: 10.1161/CIRCULATIONAHA.124.069828
56. Moss AJ, Shimizu W, Wilde AAM, Towbin JA, Zareba W, Robinson JL, Qi M, Vincent GM, Ackerman MJ, Kaufman ES, et al. Clinical aspects of type-1 long-QT syndrome by location, coding type, and biophysical function of mutations involving the KCNQ1 gene. *Circulation*. 2007;115:2481–2489. doi: 10.1161/CIRCULATIONAHA.106.665406
57. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, Janizek JD, Huang X, Starita LM, Shendure J. Accurate classification of BRCA1 variants with saturation genome editing. *Nature*. 2018;562:217–222. doi: 10.1038/s41586-018-0461-z
58. O'Neill MJ, Yang T, Laudeman J, Calandranis ME, Harvey ML, Solus JF, Roden DM, Glazer AM. ParSE-seq: a calibrated multiplexed assay to facilitate the clinical classification of putative splice-altering variants. *Nat Commun*. 2024;15:8320. doi: 10.1038/s41467-024-52474-4
59. Chan PJ, Osteen JD, Xiong D, Bohnen MS, Doshi D, Sampson KJ, Marx SO, Karlin A, Kass RS. Characterization of KCNQ1 atrial fibrillation mutations reveals distinct dependence on KCNE1. *J Gen Physiol*. 2012;139:135–144. doi: 10.1085/jgp.201110672
60. Lundby A, Ravn LS, Svendsen JH, Olesen S-P, Schmitt N. KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT interval. *Heart Rhythm*. 2007;4:1532–1541. doi: 10.1016/j.hrthm.2007.07.022
61. Nakajo K, Kubo Y. Steric hindrance between S4 and S5 of the KCNQ1/KCNE1 channel hampers pore opening. *Nat Commun*. 2014;5:4100. doi: 10.1038/ncomms5100
62. Friedman CE, Fayer S, Pendyala S, Chien W-M, Loiben A, Tran L, Chao LS, McKinstry A, Ahmed D, Farris SD, et al. Multiplexed functional assessments of MYH7 variants in human cardiomyocytes. *Circ Genom Precis Med*. 2024;17:e004377. doi: 10.1161/CIRCGEN.123.004377
63. Fayer S, Garge RK, Hopkins M, Friedman CE, McGee AV, Rico J, Powell RL, McDermot E, Smith NT, Pendyala S, et al. Editing stem cell genomes at scale to measure variant effects in diverse cell and genetic contexts. *medRxiv*. 2025;2025.11.12.25340127. doi: 10.1101/2025.11.12.25340127
64. Yamamoto Y, Chua K, Staudt D, Ferrasse A, Kirillova A, De Jong HN, Floyd BJ, Cadisch C, Wiel L, Wang Q, et al. Scaled multidimensional assays of variant effect identify sequence-function relationships in hypertrophic cardiomyopathy. *Circulation*. 2026;154:733–749. doi: 10.1161/CIRCULATIONAHA.125.075535
65. Shimizu W, Noda T, Takaki H, Kurita T, Nagaya N, Satomi K, Suyama K, Aihara N, Kamakura S, Sunagawa K, et al. Epinephrine unmasks latent mutation carriers with LQT1 form of congenital long-QT syndrome. *J Am Coll Cardiol*. 2003;41:633–642. doi: 10.1016/s0735-1097(02)02850-4
66. Napolitano C, Priori SG, Schwartz PJ, Bloise R, Ronchetti E, Nastoli J, Bottelli G, Cerrone M, Leonardi S. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA*. 2005;294:2975–2980. doi: 10.1001/jama.294.23.2975
67. Jain PC, Varadarajan R. A rapid, efficient, and economical inverse polymerase chain reaction-based method for generating a site saturation mutant library. *Anal Biochem*. 2014;449:90–98. doi: 10.1016/j.ab.2013.12.002
68. Matreyek KA, Stephany JJ, Fowler DM. A platform for functional assessment of large variant libraries in mammalian cells. *Nucleic Acids Res*. 2017;45:e102. doi: 10.1093/nar/gkx183
69. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25:1754–1760. doi: 10.1093/bioinformatics/btp324
70. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25:2078–2079. doi: 10.1093/bioinformatics/btp352
71. Kowarz E, Löscher D, Marschalek R. Optimized Sleeping Beauty transposons rapidly generate stable transgenic cell lines. *Biotechnol J*. 2015;10:647–653. doi: 10.1002/biot.201400821
72. Mátés L, Chuah MKL, Belay E, Jerchow B, Manoj N, Acosta-Sanchez A, Grzela DP, Schmitt A, Becker K, Matrai J, et al. Molecular evolution of a novel hyperactive Sleeping Beauty transposase enables robust stable gene transfer in vertebrates. *Nat Genet*. 2009;41:753–761. doi: 10.1038/ng.343
73. Hermann M, Stillhard P, Wildner H, Seruggia D, Kapp V, Sánchez-Iranzo H, Mercader N, Montoliu L, Zeilhofer HU, Pelczar P. Binary recombinase systems for high-resolution conditional mutagenesis. *Nucleic Acids Res*. 2014;42:3894–3907. doi: 10.1093/nar/gkt1361
74. Muhammad A, Calandranis ME, Li B, Yang T, Blackwell DJ, Harvey ML, Smith JE, Daniel ZA, Chew AE, Capra JA, et al. High-throughput functional mapping of variants in an arrhythmia gene, KCNE1, reveals novel biology. *Genom Med*. 2024;16:73. doi: 10.1186/s13073-024-01340-5
75. Mattmann ME, Yu H, Lin Z, Xu K, Huang X, Long S, Wu M, McManus OB, Engers DW, Le UM, et al. Identification of (R)-N-(4-(4-methoxyphenyl)thiazol-2-yl)-1-tosylpiperidine-2-carboxamide, ML277, as a novel, potent and selective Kv7.1 (KCNQ1) potassium channel activator. *Bioorg Med Chem Lett*. 2012;22:5936–5941. doi: 10.1016/j.bmcl.2012.07.060
76. Salata JJ, Jurkiewicz NK, Wang J, Evans BE, Orme HT, Sanguinetti MC. A novel benzodiazepine that activates cardiac slow delayed rectifier K⁺ currents. *Mol Pharmacol*. 1998;54:220–230. doi: 10.1124/mol.54.1.220
77. Ma D, Zhong L, Yan Z, Yao J, Zhang Y, Ye F, Huang Y, Lai D, Yang W, Hou P, et al. Structural mechanisms for the activation of human cardiac KCNQ1 channel by electro-mechanical coupling enhancers. *Proc Natl Acad Sci U S A*. 2022;119:e2207067119. doi: 10.1073/pnas.2207067119

78. Chen L, Peng G, Comollo TW, Zou X, Sampson KJ, Larsson HP, Kass RS. Two small-molecule activators share similar effector sites in the KCNQ1 channel pore but have distinct effects on voltage sensor movements. *Front Physiol*. 2022;13:903050. doi: 10.3389/fphys.2022.903050
79. Xu Y, Wang Y, Zhang M, Jiang M, Rosenhouse-Dantsker A, Wassenaar T, Tseng G-N. Probing binding sites and mechanisms of action of an I(Ks) activator by computations and experiments. *Biophys J*. 2015;108:62–75. doi: 10.1016/j.bpj.2014.10.059
80. Hou P, Kang PW, Kongmeneck AD, Yang N-D, Liu Y, Shi J, Xu X, White KM, Zaydman MA, Kasimova MA, et al. Two-stage electro-mechanical coupling of a KV channel in voltage-dependent activation. *Nat Commun*. 2020;11:676. doi: 10.1038/s41467-020-14406-w
81. Seeböhm G, Pusch M, Chen J, Sanguinetti MC. Pharmacological activation of normal and arrhythmia-associated mutant KCNQ1 potassium channels. *Circ Res*. 2003;93:941–947. doi: 10.1161/01.RES.0000102866.67863.2B
82. Jensen O, Ansari S, Gebauer L, Müller SF, Lowjaga KAAT, Geyer J, Tzvetkov MV, Brockmüller J. A double-Flip-in method for stable overexpression of two genes. *Sci Rep*. 2020;10:14018. doi: 10.1038/s41598-020-71051-5
83. Ng C-A, Farr J, Young P, Windley MJ, Perry MD, Hill AP, Vandenberg JI. Heterozygous KCNH2 variant phenotyping using Flip-In HEK293 and high-throughput automated patch clamp electrophysiology. *Biol Methods Protoc*. 2021;6:bpab003. doi: 10.1093/biomethods/bpab003
84. Jiang C, Richardson E, Farr J, Hill AP, Ullah R, Kroncke BM, Harrison SM, Thomson KL, Ingles J, Vandenberg JI, et al. A calibrated functional patch-clamp assay to enhance clinical variant interpretation in KCNH2-related long QT syndrome. *Am J Hum Genet*. 2022;109:1199–1207. doi: 10.1016/j.ajhg.2022.05.002
85. Kozek KA, Glazer AM, Ng C-A, Blackwell D, Egly CL, Vanags LR, Blair M, Mitchell D, Matreyek KA, Fowler DM, et al. High-throughput discovery of trafficking-deficient variants in the cardiac potassium channel KV11.1. *Heart Rhythm*. 2020;17:2180–2189. doi: 10.1016/j.hrthm.2020.05.041
86. Valenzuela-Palomo A, Bueno-Martínez E, Sanoguera-Miralles L, Lorca V, Fraile-Bethencourt E, Esteban-Sánchez A, Gómez-Barrero S, Carvalho S, Allen J, García-Álvarez A, et al. Splicing predictions, minigene analyses, and ACMG-AMP clinical classification of 42 germline PALB2 splice-site variants. *J Pathol*. 2022;256:321–334. doi: 10.1002/path.5839
87. O'Neill MJ, Sala L, Denjoy I, Wada Y, Kozek K, Crotti L, Dagradi F, Kotta M-C, Spazzolini C, Leenhardt A, et al. Continuous Bayesian variant interpretation accounts for incomplete penetrance among Mendelian cardiac channelopathies. *Genet Med*. 2023;25:100355. doi: 10.1016/j.gim.2022.12.002
88. Whiffin N, Minikel E, Walsh R, O'Donnell-Luria AH, Karczewski K, Ing AY, Barton PJR, Funke B, Cook SA, MacArthur D, et al. Using high-resolution variant frequencies to empower clinical genome interpretation. *Genet Med*. 2017;19:1151–1158. doi: 10.1038/gim.2017.26
89. Fayer S, Horton C, Dines JN, Rubin AF, Richardson ME, McGoldrick K, Hernandez F, Pesaran T, Karam R, Shirts BH, et al. Closing the gap: systematic integration of multiplexed functional data resolves variants of uncertain significance in BRCA1, TP53, and PTEN. *Am J Hum Genet*. 2021;108:2248–2258. doi: 10.1016/j.ajhg.2021.11.001
90. van Loggerenberg W, Sowlati-Hashjin S, Weile J, Hamilton R, Chawla A, Sheykhkarimli D, Gebbia M, Kishore N, Frésard L, Mustajoki S, et al. Systematically testing human HMBS missense variants to reveal mechanism and pathogenic variation. *Am J Hum Genet*. 2023;110:1769–1786. doi: 10.1016/j.ajhg.2023.08.012
91. Li H. Tabix: fast retrieval of sequence features from generic TAB-delimited files. *Bioinformatics*. 2011;27:718–719. doi: 10.1093/bioinformatics/btq671
92. Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M. ColabFold: making protein folding accessible to all. *Nat Methods*. 2022;19:679–682. doi: 10.1038/s41592-022-01488-1
93. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Židek A, Potapenko A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596:583–589. doi: 10.1038/s41586-021-03819-2
94. Steinegger M, Söding J. MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. *Nat Biotechnol*. 2017;35:1026–1028. doi: 10.1038/nbt.3988
95. O'Neill MJ, Ma JG, Aldridge JL, Solus JF, Harvey GR, Roberson PH, Barc J, Bezzina CR, Roden DM, Walsh R, et al. Automated patch clamp data improve variant classification and penetrance stratification for SCN5A-Brugada syndrome. *Eur Heart J*. 2026;47:4679–4693. doi: 10.1093/eurheartj/ehaf874
96. Ashby D. Book review: *Practical Statistics for Medical Research* (Douglas G. Altman, Chapman and Hall, London, 1991). *Stat Med*. 1991;10:1635–1636. doi: 10.1002/sim.4780101015
97. Jagota M, Ye C, Albers C, Rastogi R, Koehl A, Ioannidis N, Song YS. Cross-protein transfer learning substantially improves disease variant prediction. *Genome Biol*. 2023;24:182. doi: 10.1186/s13059-023-03024-6
98. Wu Y, Li R, Sun S, Weile J, Roth FP. Improved pathogenicity prediction for rare human missense variants. *Am J Hum Genet*. 2021;108:1891–1906. doi: 10.1016/j.ajhg.2021.08.012
99. Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, Musolf A, Li Q, Holzinger E, Karyadi D, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet*. 2016;99:877–885. doi: 10.1016/j.ajhg.2016.08.016
100. Roden DM, Denny JC. Integrating electronic health record genotype and phenotype data sets to transform patient care. *Clin Pharmacol Ther*. 2016;99:298–305. doi: 10.1002/cpt.321
101. Tabet DR, Kuang D, Lancaster MC, Li R, Liu K, Weile J, Coté AG, Wu Y, Hegele RA, Roden DM, et al. Benchmarking computational variant effect predictors by their ability to infer human traits. *Genome Biol*. 2024;25:172. doi: 10.1186/s13059-024-03314-7