Even pore-localizing missense variants at highly conserved sites in *KCNQ1*-encoded $K_v7.1$ channels may have wild-type function and not cause type 1 long QT syndrome: Do not rely solely on the genetic test company's interpretation

Ashley Paquin, BA,[*] Dan Ye, MD,[†‡§] David J. Tester, BS,[†‡§] Jamie D. Kapplinger, BA,[*†] Michael T. Zimmermann, PhD,[‖] Michael J. Ackerman, MD, PhD[†‡§¶]

*From the *Mayo Clinic School of Medicine, Mayo Clinic, Rochester, Minnesota, †Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, Minnesota, ‡Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, Minnesota, §Division of Heart Rhythm Services, Department of Cardiovascular Diseases, Mayo Clinic, Rochester, Minnesota, ‖Department of Biomedical Informatics, Mayo Clinic, Rochester, Minnesota, and ¶Division of Pediatric Cardiology, Department of Pediatrics, Mayo Clinic, Rochester, Minnesota.*

Introduction

Long QT syndrome (LQTS) is an inheritable cardiac channelopathy characterized by delayed ventricular cardiomyocyte repolarization and cardiac action potential prolongation that often presents as a prolonged QT interval on a 12-lead surface electrocardiogram (ECG).[1,2] With a prevalence as high as 1:2000,[3] LQTS may manifest with episodes of syncope, seizures, or sudden cardiac arrest/sudden cardiac death typically triggered by exertion, extreme emotion, or auditory stimuli, although events during rest can also occur. However, LQTS is characterized by marked clinical heterogeneity ranging from a lifelong asymptomatic course to sudden death during infancy.[3,4] The potential for sudden cardiac arrest/sudden cardiac death without prior symptoms underscores the need for prompt and accurate diagnosis and prophylactic treatment.

KEYWORDS Arrhythmia; Cardiac arrest; Genetics; Long QT syndrome; Pediatrics
(Heart Rhythm Case Reports 2017;■:1–8)

The first 2 authors are considered co-first authors. This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program. Mr Kapplinger is supported by the National Institutes of Health (grant no. GM72474-08). Dr Ackerman is a consultant for Boston Scientific, Gilead Sciences, Invitae, Medtronic, MyoKardia, and St. Jude Medical. Dr Ackerman, Mr Tester, and Mayo Clinic have received sales-based royalties in the past from Transgenomic for their FAMILION-LQTS and FAMILION-CPVT genetic tests. Dr Ackerman and Mayo Clinic have licensed intellectual property to AliveCor but without remuneration thus far. However, none of these entities have contributed to this study in any manner. **Address reprint requests and correspondence:** Dr Michael J. Ackerman, Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Guggenheim 501, 200 First St SW, Rochester, MN 55905. E-mail address: ackerman.michael@mayo.edu.

LQTS is typically inherited in an autosomal dominant manner. About 75%–80% of patients with LQTS host mutations in 1 of 3 genes (*KCNQ1*, *KCNH2*, and *SCN5A*) that encode for ion channel α subunits responsible for maintaining proper cardiac action potential and normal heart rhythm. The *KCNQ1* gene encodes for the $K_v7.1$ pore-forming voltage-gated potassium channel α subunits responsible for the slow delayed rectifier potassium current (I_{Ks}) and is responsible for the most common LQTS subtype (LQTS type 1 [LQT1]) that accounts for 35%–40% of cases with the disorder.[1,4]

Clinical genetic testing for LQTS has been available commercially since 2004. In order to assist physicians in the interpretation of genetic findings, case-control studies demonstrated that the probability of pathogenicity of rare variants identified within the major genes can be predicted on the basis of the topological location of the variant within known structural domains.[5,6] For example, *KCNQ1* missense mutations localizing to the transmembrane region confer a relatively high (>90%) probability of pathogenicity when originating from a case of clinically probable LQTS.[5] Since the estimated pathogenicity of mutations is highly correlated with protein topology, knowledge of the mutational location within the $K_v7.1$ potassium channel can provide significant diagnostic probability for patients with mutations that have not been characterized functionally. However, despite high probabilities, extreme caution must still be exercised when diagnosing patients, especially those with a borderline or weak LQTS phenotype since a topology-derived estimate does not guarantee pathogenicity.[7]

Here, we present a case of a patient diagnosed elsewhere with LQTS but with a clinically equivocal, nondiagnostic evaluation who had a rare *KCNQ1*-A300S missense variant localizing to the pore domain. This variant was classified as

https://doi.org/10.1016/j.hrcr.2017.04.006

KEY TEACHING POINTS

- Since genetic testing for long QT syndrome exerts a substantial diagnostic, prognostic, and therapeutic impact for the index cases and their affected family members, it is of critical importance to identify the exact disease-causing mutation and to properly rule out benign variants.
- The article contains a fundamentally important message and dispels a commonly held perception, namely, that identification of a rare variant does not automatically equate to a disease diagnosis.
- Even *KCNQ1* mutations within areas of high probability of pathogenicity (ie, transmembrane spanning or pore-forming regions) or indicated by a genetic test company as "deleterious" should be interpreted with caution, especially if the variant-positive individuals have insufficient clinical evidence for a diagnosis of long QT syndrome in the first place.
- It is important that physicians analyze the veracity and concordance of the evidence underpinning both the alleged genotype and the alleged phenotype. If discordance exists, one must be critical of any genetic test company's interpretation and tread carefully with the use of the genotypic data in their clinical decision making.
- Genetic testing continues to be perceived as the ultimate diagnostic arbiter, yet the perils of testing in a poorly phenotyped family can be significant as illustrated in this case, with major implications for the family in terms of medical care, potential restrictions, insurance, and the psychological burden such a diagnosis brings.

a "predicted deleterious mutation" by the genetic testing company, which seemingly solidified the diagnosis of LQT1 with the primary heart rhythm specialist. However, after further phenotypic characterization of the patient and her family (their second opinion evaluation) as well as a functional validation assay using the patch clamp technique for this variant in question, the diagnosis of LQTS in general and LQT1 in particular was reversed and *KCNQ1*-A300S was demoted to a benign variant despite its topological location.

Methods

Pedigree

The index case is a young woman of Indian ethnicity who was noted to have a "borderline QT interval" on a routine sports physical ECG at the age of 14. Repeat ECG over the next 4 years as well as exercise and stress tests repeatedly showed "borderline" results as interpreted elsewhere. The family history was unremarkable; both parents had normal ECGs. However, a maternal first-degree male cousin died suddenly at the age of 7 while ill with a fever; an autopsy was performed, but it was inconclusive. A clinical LQTS genetic test was ordered, and a rare *KCNQ1*-A300S variant interpreted as a "predicted deleterious mutation" was identified in the patient, her 2 sisters, and their father. The study was approved by the Mayo Clinic Institutional Review Board, and all participants were consented appropriately.

In silico pathogenicity prediction of *KCNQ1*-A300S

Eight in silico tools (paralog conservation, ortholog conservation, Grantham values, SIFT, PolyPhen2, KvSNP, APPRAISE, and ConDel) were used to assess the predicted pathogenicity of A300S as previously described.[8]

Molecular modeling and molecular dynamics

The initial configuration of our 3-dimensional structural model leveraged the previous computational work of Smith et al.[9] An all-atom explicit environment composed of 173,794 atoms was generated using VMD[10] (Supplemental Figure S1). This environment included a square phosphatidylcholine (POPC) membrane patch (1.5 nm sides) with *KCNQ1* embedded in the center, transferable intermolecular potential with 3 points (TIP3) water, and 150 nm KCl. In silico mutagenesis was performed using the Mutator (version 1.3) VMD plugin. Molecular dynamics simulation (MDS) were carried out using NAMD[11] and the CHARMM27 with CMAP[12] force field. Wild-type (WT) and A300S simulations were independently energy minimized for 5000 steps, followed by heating to 300 K over 300 ps at a constant pressure via a Langevin thermostat and equilibration for 5 ns. We used a simulation time step of 1 fs and conformations were recorded every 2 ps. At a constant volume, a further 10 ns of simulation trajectory was generated. All trajectories were first aligned to the initial WT conformation using C^{α} atoms. Analysis was carried out using custom scripts, leveraging VMD and Bio3D (an R package).[13] Visualizations were performed using PyMOL[14] and VMD.

KCNQ1 and *KCNE1* mammalian expression vectors and mutagenesis

WT *KCNQ1* complementary DNA (cDNA) was subcloned into pIRES2-EGFP (Clontech, Mountain View, CA) to produce pIRES2-*KCNQ1*-WT-EGFP, and WT *KCNE1* cDNA was subcloned into pIRES2-dsRed2 (Clontech) to produce pIRES2-*KCNE1*-WT-dsRed2. The A300S variant was engineered into pIRES2-*KCNQ1*-WT-EGFP using the QuickChange XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). DNA sequencing was used to confirm the integrity of all vectors.

TSA201 cell culture and transfection

TSA201 cells were cultured in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine