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SPECIAL ARTICLE

The Promise and Peril of Precision Medicine: Phenotyping Still Matters Most

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Abstract

We illustrate the work necessary to reverse course after identification of a *KCNQ1* variant interpreted erroneously as causing long QT syndrome (LQTS) and to identify the true cause of a case of sudden death in the young. Surrogate genetic testing of a decedent's living brother identified a rare *KCNQ1*-V133I variant, which prompted an implantable cardioverter defibrillator and subsequent diagnosis of LQTS in other family members. Subsequently, this presumed LQT1 family came to our institution for further clinical evaluation and research-based investigations, including *KCNQ1*-V133I variant—specific analysis of the decedent, heterologous expression studies of *KCNQ1*-V133I, and a whole-exome molecular autopsy along with genomic triangulation using his unaffected parents' DNA. After evaluating several V133I-positive family members, clinical doubt was cast on the veracity of the previously levied diagnosis of LQT1, resulting in a re-opening of the case and an intense pursuit of the lethal substrate. Furthermore, the decedent tested negative for V133I, and heterologous expression studies demonstrated a normal cellular phenotype for V133I-containing Kv7.1 channels. Instead, after whole-exome molecular autopsy, a de novo pathogenic variant (p.R454W) in *DES*-encoded desmin was identified. As detailed herein, the forensic evaluation of sudden death in the young requires meticulous focus on the decedent followed by a careful and deliberate assessment of the decedent's relatives. Surrogate genetic testing can have disastrous consequences and should be avoided. Genetic test results require careful scrutiny to avoid unintended and potentially devastating repercussions. Although the root cause of the decedent's tragic death would have remained a mystery, the unintended consequences for the living relatives described herein might have been avoided based on clinical grounds alone. All family members had electrocardiograms with normal QT intervals, making the diagnosis of familial LQTS unlikely. As such, if the clinicians caring for these patients had focused solely on clinical data from the survivors, there might have been no reason to embark on a path of inappropriate treatment based on genetic testing.

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Sudden cardiac death is a major worldwide public health burden with an estimated annual incidence ranging from 180,000 to 450,000[1] in the United States and as many as 3.7 million deaths globally.[2] Among these sudden deaths in the United States, approximately 2000 to 5000 young people aged 1 to 35 years die suddenly.[3] For many of these sudden deaths in the young (SDYs), comprehensive medicolegal investigations that include a conventional autopsy examination elucidate a clear cause of death. However, in up to 50% of these cases, gross and microscopic inspection of the heart does not reveal a definite cardiac etiology.[4] These deaths are often termed autopsy-negative sudden unexplained death (SUD).[5]

Potentially lethal and heritable cardiac channelopathies, such as long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome, are associated typically with grossly and histologically normal hearts and may account for a significant portion of SUDs. In addition, heritable cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy, and arrhythmogenic cardiomyopathy, can display minimal structural abnormalities deemed inconclusive.

Recently, guidelines for autopsy investigations of SDY cases stipulate procurement and retention of tissue suitable for DNA extraction as a class I recommendation and advise that postmortem genetic testing (ie, the molecular autopsy) be considered the new standard of care in the decedent's evaluation.[6-8] Herein, we illustrate how antemortem surrogate

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genetic testing can have devastating consequences and how the whole-exome molecular autopsy (WEMA) with genomic triangulation provided closure and clarity for an SDY family. In addition, the miscues in phenotypic assessment of the living and the dead along with the erroneous interpretation of the genetic test results showcase some of the challenges in making the promise of precision medicine a reality and serve as a vivid reminder that phenotyping still matters most.

MATERIALS AND METHODS

Study Participants

A Hispanic family with a previously rendered diagnosis of autosomal dominant LQT1 came to Mayo Clinic in Rochester, Minnesota, for a second opinion evaluation after the sudden death of their 13-year-old son. Importantly, a genetic evaluation of the deceased son's sample (ie, postmortem genetic testing, also known as the molecular autopsy) was not performed before the family's second opinion evaluation. Instead, "surrogate" genetic testing of the decedent's unaffected living brother revealed *KCNQ1*-V133I, which was interpreted elsewhere as an LQT1-causative mutation. After receiving written informed consent for this Mayo Clinic Institutional Review Board–approved study, peripheral blood samples from the 3 living family members (father, mother, and brother) and a blood spot card taken at autopsy from the boy with SDY were collected for genomic DNA isolation and further genetic interrogation. This study was conducted from September 2012 through May 2016.

KCNQ1-V133I Variant–Specific Analysis of the Decedent's Sample

After genomic DNA isolation from the decedent's autopsy specimen, *KCNQ1*-V133I variant–specific analysis was performed using standard DNA dye terminator cycle sequencing protocols as described previously.[9]

Heterologous Expression Studies of *KCNQ1*-V133I

The *KCNQ1*-V133I variant was engineered into wild-type (WT)-*KCNQ1* (Kv7.1) complementary DNA as previously described.[10] The integrity of the construct was verified by DNA sequencing (Advanced Genetic Technologies Center, University of Kentucky).

Human embryonic kidney (HEK293) cells were transfected transiently with WT (3 μg), V133I (3 μg), or WT (1.5 μg) plus V133I (1.5 μg) plasmid DNA using the SuperFect reagent (Qiagen) as previously described.[10] *KCNE1* (3 μg) and green fluorescent protein (0.3 μg) plasmid DNA were co-transfected for all experiments. *KCNE1* is required to generate I_{Ks}-like current in heterologous expression systems.[11] The cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum at 37°C and were analyzed 24 to 30 hours after transfection.

The standard whole-cell patch clamp procedure was performed on green fluorescent protein–positive HEK293 cells as previously described.[10]

Statistics for Cellular Electrophysiology Studies

Electrophysiologic data are reported as the mean ± SE. A paired or unpaired *t* test was performed when appropriate to determine whether values were different from one another. For comparison of 3 or more groups, a 1-way analysis of variance was performed. If the analysis of variance showed a $P<.05$, then a post hoc Tukey multiple comparison test was used to identify which data sets were significantly different at $P<.05$.

WEMA and Genomic Triangulation

Whole-exome sequencing (WES) and subsequent variant annotation (Advance Genomics Technology Center and Bioinformatics Core Facilities, Mayo Clinic) were performed on genomic DNA derived from the deceased child, the unaffected father, and the unaffected mother as previously described.[12]

After WES, single nucleotide variants and insertion/deletions (INDELs) were filtered to identify variants that followed a sporadic, autosomal dominant, or autosomal recessive inheritance pattern using VarSeq software (Golden Helix Inc). All variants were first filtered for a call quality score of at least 20 and a read depth of at least 10. To be considered a candidate pathogenic variant, the variant identified in the child had to be nonsynonymous (ie, amino acid altering: missense,

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