Novel Timothy syndrome mutation leading to increase in *CACNA1C* window current

Nicole J. Boczek, BA,[*†] Erin M. Miller, MS, LCGC,[‡] Dan Ye, MD,[§] Vladislav V. Nesterenko, PhD,[‖] David J. Tester, BS,[§] Charles Antzelevitch, PhD, FHRS,[‖] Richard J. Czosek, MD,[‡] Michael J. Ackerman, MD, PhD,[§¶**] Stephanie M. Ware, MD, PhD[††]

From the [*]Center for Clinical and Translational Science, Mayo Clinic, Rochester, Minnesota, [†]Mayo Graduate School, Mayo Clinic, Rochester, Minnesota, [‡]The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, [§]Department Molecular Pharmacology & Experimental Therapeutics, Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, Minnesota, [‖]Department of Pharmacology, Masonic Medical Research Laboratory, Utica, New York, [¶]Department of Medicine (Division of Cardiovascular Diseases), Mayo Clinic, Rochester, Minnesota, [**]Department of Pediatrics (Division of Pediatric Cardiology), Mayo Clinic, Rochester, Minnesota, and [††]Departments of Pediatrics and Medical and Molecular Genetics, Indiana University School of Medicine, Herman B Wells Center for Pediatric Research, Indianapolis, Indiana.

BACKGROUND Timothy syndrome (TS) is a rare multisystem genetic disorder characterized by a myriad of abnormalities, including QT prolongation, syndactyly, and neurologic symptoms. The predominant genetic causes are recurrent *de novo* missense mutations in exon 8/8A of the *CACNA1C*-encoded L-type calcium channel; however, some cases remain genetically elusive.

OBJECTIVE The purpose of this study was to identify the genetic cause of TS in a patient who did not harbor a *CACNA1C* mutation in exon 8/A, and was negative for all other plausible genetic substrates.

METHODS Diagnostic exome sequencing was used to identify the genetic substrate responsible for our case of TS. The identified mutation was characterized using whole-cell patch-clamp technique, and the results of these analyses were modeled using a modified Luo–Rudy dynamic model to determine the effects on the cardiac action potential.

RESULTS Whole exome sequencing revealed a novel *CACNA1C* mutation, p.Ile1166Thr, in a young male with diagnosed TS. Functional electrophysiologic analysis identified a novel mechanism of TS-mediated disease, with an overall loss of current density and a gain-of-function shift in activation, leading to an increased window current. Modeling studies of this variant predicted prolongation of the action potential as well as the development of spontaneous early afterdepolarizations.

CONCLUSION Through expanded whole exome sequencing, we identified a novel genetic substrate for TS, p.Ile1166Thr-CACNA1C. Electrophysiologic experiments combined with modeling studies have identified a novel TS mechanism through increased window current. Therefore, expanded genetic testing in cases of TS to the entire *CACNA1C* coding region, if initial targeted testing is negative, may be warranted.

KEYWORDS Timothy syndrome; *CACNA1C*; Window current; Genetics; Whole exome sequencing

ABBREVIATIONS **AP** = action potential; **AV** = atrioventricular; **EAD** = early afterdepolarization; **IDL** = interdomain linker; **LQTS** = long QT syndrome; **LR2** = adjusted Luo-Rudy model; **PDA** = patent ductus arteriosus; **TS** = Timothy syndrome; **WES** = whole exome sequencing; **WT** = wild-type

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Introduction

Timothy syndrome (TS) is an extremely rare genetic disorder with fewer than 30 cases reported worldwide. It is characterized by a myriad of multisystem abnormalities, including QT prolongation, syndactyly, congenital heart defects, facial dysmorphisms, and neurologic symptoms including autism, seizures, and intellectual disability.[1,2] Because of extreme QT prolongation, individuals with TS can experience

ventricular fibrillation and cardiac arrest, and the overall compilation of multisystem abnormalities often leads to early death at approximately 2.5 years of age; however, in rare cases, affected individuals have survived beyond childhood.[3]

The predominant genetic cause, identified in 2004, was a recurrent *de novo* heterozygous missense mutation, p.Gly406Arg, in the alternatively spliced exon 8 (exon 8A) in the *CACNA1C*-encoded L-type calcium channel.[1] After the original genetic discovery of a mutational hotspot within exon 8A, 2 additional mutations were identified in exon 8, p.Gly406Arg and p.Gly402Ser. Exons 8 and 8A undergo alternative splicing in a mutually exclusive manner, with exon 8 being the predominantly expressed isoform.[4] It has been suggested that mutations in exon 8 result in a slightly different phenotype than the p.Gly406Arg mutation in exon 8A. For example, the 2 patients with exon 8 mutations were not reported to have syndactyly, emphasizing the variability of the phenotypic manifestation of TS. Unlike other channelopathies, in which mutational "hotspots" are rare, these 3 missense mutations make up almost all published TS cases and confer the same impaired open-state voltage-dependent inactivation. The mutation clustering has led to targeted genetic screening for suspected TS, focusing specifically on exon 8/8A and surrounding regions within the *CACNA1C* gene.

Here, we describe a novel *CACNA1C* mutation that was identified via whole exome sequencing (WES), outside of the canonical exon 8/8A region of the channel in exon 27, in a patient exhibiting a TS phenotype with QT prolongation, patent ductus arteriosus (PDA), seizures, facial dysmorphisms, joint hypermobility, hypotonia, hand anomalies (clinodactyly and short thumbs), intellectual impairment, and tooth decay. Interestingly, patch-clamp analysis identified a novel electrophysiologic phenotype, distinct from the loss of inactivation seen in the previously established TS genotypes.

Methods

Study subject

The patient was seen at Cincinnati Children's Hospital Medical Center (CCHMC). CCHMC's Institutional Review Board does not require consent for single-patient studies, and the patient is deceased. In addition, genetic testing completed on the patient was done as part of a clinical genetics evaluation and was approved by the patient's family. All other research-based questions completed in the study did not use patient materials.

HEK293 cell culture and transfection

Details regarding the constructs and HEK293 cell culture are described in the Online Supplement. Heterologous expression of Cav1.2 was accomplished by cotransfecting 1 μg *CACNA1C* wild-type (WT) or mutant (Ile1166Thr-CACNA1C) cDNA with 1 μg *CACNB2b*, 1 μg *CACNA2D1,* and 0.25 μg green fluorescent protein cDNA with the use of 9 μL Lipofectamine 2000. The media was replaced with OPTI-MEM after 4–6 hours. Transfected cells were incubated for 48 hours before electrophysiologic experiments.

Electrophysiologic measurements

Standard whole-cell patch-clamp technique was used to measure I_{CaL} WT and mutant calcium currents at room temperature (22°–24°C) using the methods provided in the Online Supplement.

Statistical analysis

All datapoints are shown as the mean value, and error bars represent the standard error of the mean. The Student *t* test was performed to determine statistical significance between 2 groups. $P < .05$ was considered significant.

Simulated L-type calcium current and ventricular action potentials

In order to simulate the possible effects of the heterozygous p.Ile1166Thr mutation in the *CACNA1C* gene on the cardiac action potential (AP), we used the dynamic Luo-Rudy model[5] with subsequent adjustments as implemented by Faber and Rudy (LR2).[6–8] Additional information regarding this model and how it was applied is available in the Online Supplement.

Results

Clinical description and genetic testing

The proband was born to a healthy 38-year-old woman and 35-year-old man, both of Caucasian descent. There was no report of consanguinity, and the family histories were noncontributory. The pregnancy was uncomplicated. The baby's birth weight was 3.5 kg (50th centile), length was 47 cm (10th centile), and head circumference measured 35 cm (35th centile). He was transferred from the birth hospital on the first day of life for further evaluation of bradycardia, which later was diagnosed as 2:1 atrioventricular (AV) conduction. The patient remained hospitalized for 6 weeks. Initial cardiac evaluation revealed 2:1 AV block (Figure 1A) and marked ventricular repolarization delay with QT-interval prolongation measurement ranging from 595 to 812 ms. Medical therapy (propranolol) was initiated, and QTc duration stabilized (550–650 ms). With beta-blockade, 2:1 AV conduction resolved, and there was consistent 1:1 AV conduction with significant T-wave alternans (Figure 1B). Initial imaging with echocardiography demonstrated a PDA and left atrial enlargement.

The baby underwent automatic implantable cardioverter-defibrillator placement, left sympathectomy, and PDA ligation at 1 month of age. On the day of device implant, he received inappropriate defibrillator discharges secondary to T-wave oversensing, and the device therapies were turned off. Defibrillator event monitoring was turned on after computerized theoretical T-wave shock avoidance was performed,[9] and therapies were reinstated after multiple days with no theoretical shock being delivered. He presented at 5 weeks of age with desaturations and increased respiratory