Novel calmodulin mutations associated with congenital long QT syndrome affect calcium current in human cardiomyocytes

Daniel C. Pipilas, BS,[*] Christopher N. Johnson, PhD,[†] Gregory Webster, MD, MPH,[‡] Jurg Schlaepfer, MD,[§] Florence Fellmann, MD, PhD,[§] Nicole Sekarski, MD,[§] Lisa M. Wren, BS,[*] Kateryna V. Ogorodnik, MS,[†] Daniel M. Chazin, BA,[†] Walter J. Chazin, PhD,[†] Lia Crotti, MD, PhD,[‖] Zahurul A. Bhuiyan, MD, PhD,[§] Alfred L. George Jr, MD[*]

From the [*]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, [†]Department of Biochemistry and Center for Structural Biology, Vanderbilt University, Nashville, Tennessee, [‡]Division of Cardiology, Ann and Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, Illinois, [§]University Hospital Lausanne (CHUV), Lausanne, Switzerland, and [‖]IRCCS Istituto Auxologico Italiano, Center for Cardiac Arrhythmias of Genetic Origin and Laboratory of Cardiovascular Genetics, Milan, Italy; and Department of Molecular Medicine, University of Pavia, Pavia, Italy.

BACKGROUND Calmodulin (CaM) mutations are associated with cardiac arrhythmia susceptibility including congenital long QT syndrome (LQTS).

OBJECTIVE The purpose of this study was to determine the clinical, genetic, and functional features of 2 novel CaM mutations in children with life-threatening ventricular arrhythmias.

METHODS The clinical and genetic features of 2 congenital arrhythmia cases associated with 2 novel CaM gene mutations were ascertained. Biochemical and functional investigations were conducted on the 2 mutations.

RESULTS A novel de novo *CALM2* mutation (D132H) was discovered by candidate gene screening in a male infant with prenatal bradycardia born to healthy parents. Postnatal course was complicated by profound bradycardia, prolonged corrected QT interval (651 ms), 2:1 atrioventricular block, and cardiogenic shock. He was resuscitated and was treated with a cardiac device. A second novel de novo mutation in *CALM1* (D132V) was discovered by clinical exome sequencing in a 3-year-old boy who suffered a witnessed cardiac arrest secondary to ventricular fibrillation. Electrocardiographic recording after successful resuscitation revealed a prolonged corrected QT interval of 574 ms. The Ca^{2+} affinity of CaM-D132H and CaM-D132V revealed extremely weak binding to the C-terminal domain, with significant structural perturbations noted for D132H. Voltage-clamp recordings of human induced pluripotent stem cell–derived cardiomyocytes transiently expressing wild-type or mutant CaM demonstrated that both mutations caused impaired Ca^{2+}-dependent inactivation of voltage-gated Ca^{2+} current. Neither mutant affected voltage-dependent inactivation.

CONCLUSION Our findings implicate impaired Ca^{2+}-dependent inactivation in human cardiomyocytes as the plausible mechanism for long QT syndrome associated with 2 novel CaM mutations. The data further expand the spectrum of genotype and phenotype associated with calmodulinopathy.

KEYWORDS Arrhythmia; Calmodulin; Long QT syndrome; Calcium channel

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Introduction

Congenital arrhythmia syndromes are treatable causes of life-threatening heart rhythm disturbances that occur during childhood and early adulthood.[1] These include congenital long QT syndrome (LQTS),[2] catecholaminergic polymorphic ventricular tachycardia (CPVT),[3] Brugada syndrome,[4] and others. Sudden cardiac death may be the first manifestation of the disease. Efforts to identify at-risk persons and to screen for known genetic mutations of arrhythmia syndromes are increasingly successful, but a genetic cause cannot always be found. When a mutation is identified, the

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genotype-phenotype correlation can guide treatment and promote better understanding of the pathophysiological mechanism for arrhythmia susceptibility.[5]

Recently, mutations in genes encoding the ubiquitous Ca^{2+} sensing protein calmodulin (CaM) have been discovered as a novel genetic basis for congenital arrhythmia susceptibility.[6–10] At least 12 mostly de novo mutations have been discovered in *CALM1*, *CALM2*, and *CALM3* associated with LQTS, CPVT, or idiopathic ventricular fibrillation. In some cases, mutations have been associated with features of more than 1 arrhythmia syndrome (eg, LQTS + CPVT).[9] In the reported cases of LQTS-associated CaM mutations, infants or young children presented with life-threatening cardiac arrhythmias and a markedly prolonged QT interval. Other notable features include prenatal bradycardia, second-degree heart block, T-wave alternans, and cardiac arrest. CaM mutations associated with LQTS reduce Ca^{2+} binding affinity and impair modulation of cellular targets, particularly the L-type voltage-gated Ca^{2+} channel (LTCC).[11,12] Specifically, CaM mutants associated with LQTS weaken Ca^{2+}-dependent inactivation (CDI) and this effect has been demonstrated to prolong the plateau phase of the cardiac action potential and is predicted to promote ventricular arrhythmias.

In this article, we report 2 novel de novo mutations discovered in 2 CaM-encoding genes. Both novel CaM mutations are missense and alter the same aspartate residue at position 132. Biochemical analyses demonstrated substantial impairments in Ca^{2+} affinity for both mutations, and functional studies performed on human induced pluripotent stem cell (iPSC)–derived cardiomyocytes demonstrated impaired CDI of the LTCC. These findings further expand the spectrum of genotype-phenotype relationships among the calmodulinopathies and demonstrate functional consequences of 2 novel CaM mutations in human cardiomyocytes.

Methods

Mutation discovery

Genomic DNA was extracted from the peripheral blood lymphocytes of study participants and both sets of parents. Informed consent was obtained from the parents described in case 1 by a protocol approved by the Ethics Committee of the University Hospital Lausanne (CHUV), Lausanne, Switzerland. Approval for the retrospective review of case 2 was obtained from the Institutional Review Board of Lurie Children's Hospital of Chicago. In case 1, all coding exons and their boundaries of the *KCNQ1*, *SCN5A*, *KCNH2*, *CALM1*, and *CALM2* genes of the study participant were screened by polymerase chain reaction (PCR) and direct nucleotide sequencing. Primer sequences and PCR conditions are available on request (contact Z.A. Bhuiyan). Mutational analysis was performed by bidirectional sequencing on an ABI 3500 sequencer (Thermo Fisher Scientific, Waltham, MA). Annotation of variants in *CALM1* and *CALM2* were based on accession numbers NM_006888.4 and NM_001743.4 (National Center for Biotechnology Information), respectively.

Mutagenesis

For measurement of Ca^{2+} affinity, the CaM-D132H mutation was engineered using site-directed mutagenesis as previously reported.[7] For electrophysiological experiments, CaM mutations were generated using QuikChange (Agilent Technologies, Santa Clara CA) in the mammalian expression plasmid that enabled coexpression of CaM and enhanced green fluorescent protein (pIRES2-EGFP).

Measurement of Ca^{2+} affinity

Proteins were expressed in *Escherichia coli* BL21 (DE3) grown at 37°C in Lurie broth. Expression was induced at an optical density of 0.8 (600 nm) using 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 4 hours. Cells were harvested by centrifugation and purified as previously described.[7] ^{15}N-enriched protein was produced in the same manner, with the exception that cells were grown in minimal medium supplemented with 0.5 g/L of $^{15}NH_4Cl$ and induced with 1 mM IPTG overnight at room temperature. Sample purity and molecular weight were confirmed by sodium dodecyl sulfate gel electrophoresis and positive electrospray mass spectroscopy. Calcium ion binding was characterized using a fluorescence-based approach.[13] The heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) spectra were collected using a Bruker Avance III 600 MHz spectrometer (Bruker Corp., Billerica, MA) equipped with a cryoprobe. Sample conditions were 50 mM HEPES, 100 mM KCl, 50 μM EGTA, and pH 7.4 and data were collected at 25°C. $CaCl_2$ was titrated into the sample up to a 78:1 (Ca^{2+} to CaM) molar ratio. CaM backbone resonance assignments were determined from previously assigned conditions by tracking chemical shift changes over the course of temperature and pH titrations.

Electrophysiology on human cardiomyocytes

Human iPSC-derived cardiomyocytes were obtained from Cellular Dynamics International (Madison, WI) and cultured according to the supplier's protocol. Cells were plated on 15-mm glass coverslips coated with 0.1% gelatin solution and incubated at 37°C in 5% CO_2. Recordings were done between days 7 and 14 in culture. Transfection of cardiomyocytes was performed with TransIT-LT1 (Mirus Bio, Madison, WI) according to the manufacturer's protocol. Cells were transfected with wild-type (WT) or mutant CaM in the pIRES2-EGFP vector 40–48 hours before electrophysiological recording experiments. Transfection efficiency was estimated at 50%.

Glass electrodes were pulled using the P-1000 micropipette puller (Sutter Instruments, Novato, CA) and flame-polished for a final resistance of 1–2 MΩ. Currents carried by Ca^{2+} or Ba^{2+} were recorded at room temperature in the whole-cell configuration, filtered at 5 kHz, and leak subtracted using the P/4 method. The bath solution contained (in mM) 150 Tris, 10 glucose, 1 $MgCl_2$, and either 10 $CaCl_2$ or 10 $BaCl_2$ and adjusted to pH 7.4 with methanesulfonic acid. The osmolarity of the bath solution was 290 mOsm/L. The composition of the pipette solution was (in mM) 135 CsCl,