DOI: 10.1161/CIRCGEN.120.003032

Prevalence and Phenotypic Correlations of Calmodulinopathy-Causative *CALM1-3* Variants Detected in a Multi-Center Molecular Autopsy Cohort of Sudden Unexplained Death Victims

Running title: *Clemens et al.; Prevalence of CALM Variants in SIDS/SUDY*

Daniel J. Clemens, BS[1]; Belinda Gray, BSc(Med), MBBS, PhD[2-5]; Richard D. Bagnall, PhD[5,6]; David J. Tester, BS[1,7]; John R. Giudicessi, MD, PhD[7]; Joseph J. Maleszewski, MD[7,8]; Lia Crotti, MD, PhD[9-11]; Peter J. Schwartz, MD[9]; Emma Matthews, MRCP[12]; Christopher Semsarian, MBBS, PhD, MPH[4-6]; Elijah R. Behr, MD, PhD, MPH[2,3]; Michael J. Ackerman MD, PhD[1,7,13]

[1]Dept of Molecular Pharmacology & Experimental Therapeutics (Windland Smith Rice Sudden Death Genomics Laboratory); [7]Dept of Cardiovascular Medicine (Division of Heart Rhythm Services); [8]Dept of Laboratory Medicine & Pathology; [13]Dept of Pediatric & Adolescent Medicine (Division of Pediatric Cardiology), Mayo Clinic, Rochester, MN; [2]Molecular & Clinical Sciences Rsrch Inst, St George's Univ of London; [3]Cardiology Clinical Academic Group, St George's Univ Hospitals' National Health Service (NHS) Foundation Trust, London, UK; [4]Sydney Medical School Faculty of Medicine & Health, [6]Agnes Ginges Centre for Molecular Cardiology at Centenary Inst, The Univ of Sydney; [5]Dept of Cardiology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia; [9]Istituto Auxologico Italiano, IRCCS, Center for Cardiac Arrhythmias of Genetic Origin & Laboratory of Cardiovascular Genetics; [10]Istituto Auxologico Italiano, IRCCS Dept of Cardiovascular, Neural & Metabolic Sciences, San Luca Hospital; [11]Dept of Medicine & Surgery Univ of Milano-Bicocca, Milan, Italy; [12]Queen Square Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery & Univ College London Inst of Neurology, London, UK

Correspondence:

Michael J. Ackerman, MD, PhD

Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory

Guggenheim 501, Mayo Clinic

200 First Street SW, Rochester, MN 55905

Tel: 507-284-0101

E-mail: ackerman.michael@mayo.edu

Journal Subject Terms: Arrhythmias; Genetics; Pediatrics; Sudden Cardiac Death

Key words: arrhythmia (heart rhythm disorders); genetics; pediatrics; sudden death; calmodulin

DOI: 10.1161/CIRCGEN.120.003032

Nonstandard Abbreviations and Acronyms

sudden unexplained death (SUD)

sudden infant death (SID)

interquartile range (IQR)

Sudden unexplained death (SUD) is a profoundly tragic event for families and their communities. These cases can be categorized into two main groups including sudden infant death syndrome (SIDS, < 1year of age), and SUD in the young (SUDY, 1-35 years). In the US alone, SIDS accounts for approximately 3000 sudden deaths each year, while SUDY occurs in up to 5000 individuals annually.[1] Approximately 10% of SIDS and 25% of SUDY may be caused by pathogenic variants in cardiac channelopathy-susceptibility genes.[2]

Three different calmodulin genes, *CALM1* (chr14q21), *CALM2* (chr2p21), and *CALM3* (chr19q13), each of which has a distinct genomic locus and unique nucleotide sequence, all encode for an identical 149 amino acid calmodulin protein (CaM).[3] Since 2012, pathogenic variants in *CALM1-3*-encoded CaM, approximately 90% of which are de novo, have been implicated as the underlying cause of multiple arrhythmic phenotypes including long QT syndrome (CaM-LQTS), catecholaminergic polymorphic ventricular tachycardia (CaM-CPVT), and idiopathic ventricular fibrillation (CaM-IVF), collectively termed the calmodulinopathies.[4]

Data from the recently published International Calmodulinopathy Registry found that 68% of 74 calmodulinopathic patients have suffered at least one major arrhythmic event at an average onset age of 4 years, and 27% experienced sudden cardiac death (SCD).[4] However,

DOI: 10.1161/CIRCGEN.120.003032

despite its malignant and potentially lethal phenotype, the prevalence of calmodulinopathic variants in cases of SIDS and SUDY remains unknown.

Here, we determined the spectrum and prevalence of *CALM1-3* pathogenic variants in a large multi-center cohort of 599 SIDS and 258 SUDY cases contributed from three international medical centers in the United States, United Kingdom, and Australia. In order to prevent the re-identification of individuals included in this study, individual patient data will not be made available to other researchers. This study complies with the Declaration of Helsinki; locally appointed ethics committees including Mayo Clinic's Institutional Review Board have approved the research protocol.

Of the SIDS cases, 362 (60%) were male and 237 (40%) were female. The median age at death was 2 months (interquartile range (IQR) 1-4 months), and 349 (58%) of these cases were white. In the SUDY cohort, 176 (68%) cases were male and 82 (32%) were female. The median age at death was 21 years (IQR 16-29 years) with 88% dying between the ages of 1-35 years and 196 (76%) were white.

Postmortem genomic DNA, derived from each decedent, underwent either whole exome or targeted gene panel sequencing followed by a gene-specific analysis of *CALM1*, *CALM2*, and *CALM3*, using Ingenuity Variant Analysis software. Only rare (minor allele frequency ≤ 0.005% in gnomAD) nonsynonymous variants with a call quality score of ≥ 20 and a read depth of ≥ 10 were considered. Identified variants were classified according to the American College of Medical Genetics (ACMG) guidelines.

Overall, we identified a pathogenic *CALM1-3* variant in 3 out of 857 SUD cases (0.035%). Interestingly, none of these variants were present in our SIDS cohort (0/599, 0%), but all 3 were identified in cases of SUDY (3/258, 1.2%; p=0.027; Figure 1A). The yield of

DOI: 10.1161/CIRCGEN.120.003032

pathogenic *CALM 1-3* variants was significantly higher in SUDY cases dying between the ages of 1 and 10 years (3/32, 9.4%) compared to those older than 10 years at age of death (0/226, 0%; p=0.002; Figure 1B). In comparison, approximately 7% of our SIDS cohort and 26% of our SUDY cohort hosted a rare nonsynonymous variant in one of the four major channelopathy genes (*KCNQ1*, *KCNH2*, *SCN5A*, or *RYR2*).

A CaM-p.Asn54Ile variant (*CALM1*, c.161A>T) was identified in a 9-year-old female who died suddenly following extreme emotion. This variant is located in the inter-EF-hand I-II linker domain and is known to cause CaM-CPVT (Figure 1C).[4] The second variant, CaM-p.Phe90Leu (*CALM2*, c.268T>C), which resides in the inter-EF-hand II-III linker domain was found in a 5-year-old male who experienced sudden death during physical exertion. While this variant has not been identified before in *CALM2*, CALM1-p.Phe90Leu has been associated previously with CaM-IVF previously (Figure 1C).[4] The CaM-p.Asn98Ser variant (*CALM2*, c.293A>G) was identified in a 2-year-old male who died suddenly while engaging in toddler play. This variant is located in the EF-hand III domain and has been associated with both LQTS and CPVT in *CALM1*- and *CALM2*-encoded calmodulin (Figure 1C).[4] However, patients with the p.Asn98Ser variant typically do not express an overt LQTS phenotype.[5]

Although CaM-LQTS patients exhibit a more malignant phenotype and have a higher rate of SCD than other calmodulinopathy phenotypes, it is not surprising that the *CALM* variants identified in our SUDY cases have been associated with CaM-CPVT or CaM-IVF and not CaM-LQTS. Typically, CaM-LQTS manifests with severe and readily detectable clinical features (QTc > 550 ms, bradycardia, 2:1 AV block, T-wave alternans) often occurring during infancy and is therefore likely detected prior to the occurrence of sudden death. Thus, the absence of CaM-LQTS variants in SIDS and SUDY may be explained by their high penetrance and marked

expressivity. In contrast, CaM-CPVT and CaM–IVF patients do not display the same readily detectable clinical feature and may elude detection until a sentinel event of SCD after the first year of life. Additionally, channelopathies are responsible for a greater percentage of SUDY than SIDS and therefore larger number of cases may be needed to identify *CALM*-related SIDS cases.

While pathogenic variants in *CALM1-3* do not contribute meaningfully to SIDS, about 1% of SUDY overall stems from pathogenic *CALM* variants. Additionally, *CALM* variants may account for up to 10% of the SUD cases occurring during childhood. Therefore, the *CALM1*, *CALM2*, and *CALM3* genes should be included in postmorterm genetic testing (aka, the molecular autopsy), especially in children who have died between the ages of 1-10 years.

Sources of Funding: This work was supported by the Mayo Clinic Windland Smith Rice Comprehensive Sudden Cardiac Death Program. JRG thanks the Mayo Clinic Clinician-Investigator Training Program and Department of Cardiovascular Medicine for fostering an outstanding environment for physician-scientist training. CS is the recipient of a National Health and Medical Research Council Practitioner Fellowship (#1154992). BG is the recipient of a National Health and Medical Research Council Early Career Fellowship (#1122330). EM is the recipient of a Wellcome Clinical Research Career Development Fellowship (#209583/Z/17/Z).

Disclosures: Dr. Ackerman is a consultant for Abbott, ARMGO Pharma, Audentes Therapeutics, Biotronik, Boston Scientific, Daiichi Sankyo, Invitae, LQT Therapeutics, Medtronic, MyoKardia, and UpToDate. Dr. Ackerman and Mayo Clinic are involved in an equity/royalty relationship with AliveCor, Blue Ox Health Corporation, and Stemonix. These relationships are all modest, and none of these entities have contributed to this study in any manner. The other authors report no conflicts.

References:

1. Kung H-C, Hoyert DL, Xu J, Murphy SL. Deaths: final data for 2005. *Natl Vital Stat Rep*. 2008;56:1-120.

DOI: 10.1161/CIRCGEN.120.003032

2. Semsarian C, Ingles J, Wilde AA. Sudden cardiac death in the young: the molecular autopsy and a practical approach to surviving relatives. *Eur Heart J*. 2015;36:1290-1296.

3. Fischer R, Koller M, Flura M, Mathews S, Strehler-Page M, Krebs J, Penniston J, Carafoli E, Strehler E. Multiple divergent mRNAs code for a single human calmodulin. *J Biol Chem*. 1998;263:17055-17062.

4. Crotti L, Spazzolini C, Tester DJ, Ghidoni A, Baruteau A-E, Beckmann B-M, Behr ER, Bennett JS, Bezzina CR, Bhuiyan ZA, et al. Calmodulin mutations and life-threatening cardiac arrhythmias: insights from the International Calmodulinopathy Registry. *Eur Heart J*. 2019;40:2964-2975.

5. Makita N, Yagihara N, Crotti L, Johnson CN, Beckmann BM, Roh MS, Shigemizu D, Lichtner P, Ishikawa T, Aiba T, et al. Novel calmodulin mutations associated with congenital arrhythmia susceptibility. *Circ Cardiovasc Genet*. 2014;7:466-474.

Figure Legend:

Figure 1. Yield and Location of *CALM* Variants Identified in SIDS and SUDY. **A.** A table showing the yield of *CALM* variants in the SIDS and SUDY cohorts and in SUDY cases between the ages of 1-10 years and those > 10 years of age. **B.** A schematic of the CaM protein showing the N- and C-domains, each with two EF hands (EF-I through EF-IV) with calcium (red) bound. Blue circles represent WT residues, and white circles represent variants identified in our SUDY cohort. Combined Annotation Dependent Depletion (CADD) score > 20 is considered an in silico threshold for possible pathogenicity.

A

	SIDS	SUDY	P-Value
Yield of CALM1-3 Variant Positive Cases	0/599 (0%)	3/258 (1.2%)	0.027
	1-10 Years	**>10 Years**	**P-Value**
Yield of CALM1-3 Variant Positive SUDY Cases	3/32 (9.4%)	0/226 (0%)	0.002

B