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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*

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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,

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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

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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.

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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