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DOI: https://doi.org/10.1016/j.seizure.2023.11.002

Seizure: European Journal of Epilepsy 113 (2023) 66–75

Contents lists available at ScienceDirect

Seizure: European Journal of Epilepsy

journal homepage: www.elsevier.com/locate/seizure

# Exome analysis focusing on epilepsy-related genes in children and adults with sudden unexplained death

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ARTICLE INFO

*Keywords:*
Epilepsy
Genetics
Sudden infant death syndrome (SIDS)
Sudden unexplained death (SUD)
Sudden unexpected death in epilepsy (SUDEP)

ABSTRACT

*Purpose:* Genetic studies in sudden infant death syndrome (SIDS) and sudden unexplained death (SUD) cohorts have indicated that cardiovascular diseases might have contributed to sudden unexpected death in 20–35 % of autopsy-negative cases. Sudden unexpected death can also occur in people with epilepsy, termed as sudden unexpected death in epilepsy (SUDEP). The pathophysiological mechanisms of SUDEP are not well understood, but are likely multifactorial, including seizure-induced hypoventilation and arrhythmias as well as genetic risk factors. The sudden death of some of the SIDS/SUD victims might also be explained by genetic epilepsy, therefore this study aimed to expand the post-mortem genetic analysis of SIDS/SUD cases to epilepsy-related genes.
*Methods:* Existing whole-exome sequencing data from our 155 SIDS and 45 SUD cases were analyzed, with a focus on 365 epilepsy-related genes. Nine of the SUD victims had a known medical history of epilepsy, seizures or other underlying neurological conditions and were therefore classified as SUDEP cases.
*Results:* In our SIDS and SUD cohorts, we found epilepsy-related pathogenic/likely pathogenic variants in the genes *OPA1, RAI1, SCN3A, SCN5A* and *TSC2*.
*Conclusion:* Post-mortem analysis of epilepsy-related genes identified potentially disease-causing variants that might have contributed to the sudden death events in our SIDS/SUD cases. However, the interpretation of identified variants remains challenging and often changes over time as more data is gathered. Overall, this study contributes insight in potentially pathophysiological epilepsy-related mechanisms in SIDS, SUD and SUDEP victims and underlines the importance of sensible counselling on the risk and preventive measures in genetic epilepsy.

## 1. Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant under one year of age, which remains unexplained after a thorough case investigation, including a complete autopsy, examination of the death scene, and review of the clinical history [1]. The 'back to sleep' campaign in the 1980ties, which recommended a supine sleeping position and a safe sleeping environment, contributed significantly to the decline of SIDS incidence [2]. However, the current incidence still adds up to 0.1–0.8 deaths per 1000 live births and the underlying pathophysiological mechanisms of SIDS are not well understood [3]. SIDS is best described by a triple risk model, including 1) a critical developmental period, 2) exogenous stress factors and 3) a vulnerable infant [4]. Genetic predisposition may also play a role in SIDS and several genetic studies in large SIDS cohorts have indicated that genetic cardiovascular diseases may have contributed to sudden unexpected death in 20–35 % of the cases [5–7].

Sudden unexplained death (SUD) is described as the sudden, unexpected death of a young individual between 1 and 49 years of age, where the cause of death remains unexplained after a thorough medico-legal investigation, including a complete autopsy, review of the circumstances of death, and the clinical history [8]. The incidence of SUD comprises 1.3 to 8.5 per 100,000 deaths per year [9]. Up to 30 % of the SUD cases might be explained by genetic diseases such as cardiac disorders affecting the heart rhythm [10–13].

According to the International League Against Epilepsy (ILAE), sudden unexpected death in epilepsy (SUDEP) is defined as sudden, unexpected, witnessed or unwitnessed, non-traumatic and non-drowning death in patients with epilepsy, with or without evidence for a seizure, in which post-mortem examination does not reveal an

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https://doi.org/10.1016/j.seizure.2023.11.002
Received 23 August 2023; Received in revised form 31 October 2023; Accepted 1 November 2023
Available online 2 November 2023

anatomical or toxicological cause of death [14–16]. SUDEP is the most common cause of premature mortality in individuals with epilepsy and most often affects patients with drug-resistant epilepsy. The incidence of SUDEP is similar in children and adults, with approximately 1.2 to 1.45 deaths per 1000 patient-years [17,18]. Several risk factors for SUDEP have been identified, the most important being high frequency generalized tonic-clonic seizures, nocturnal seizures and living alone [19,20]. 60 % of SUDEP fatalities occur during sleep, whereof most incidents are unwitnessed, implicating possible undiagnosed sleep apnoea and cardiac arrhythmia as possible contributors [21]. The pathophysiological mechanisms of SUDEP are not fully understood but are likely multifactorial [22]. In general, SUDEP can occur in any genetic disorder that phenotypically manifests with tonic-clonic seizures. One recently published study proposed that an interaction between adenosine-mediated respiratory depression and serotonergic enhancement of respiration could play a crucial role in SUDEP [23]. In recent years, massively parallel sequencing (MPS) -based tools have identified many novel genes involved in epilepsy phenotypes, whereas most of the epilepsies are caused by *de novo* variants in dominant genes [24]. The diagnostic yield of molecular testing in patients with epilepsy and in SUDEP cohorts varies widely, ranging from 20 to 60 % [25–30].

Many ion channel- or arrhythmia-related genes are co-expressed in the heart and in the brain, providing a link between genetically predisposed epilepsy and cardiac arrhythmias [31–34]. Well-known cardiac disease-related genes were associated with SUDEP, like *SCN5A, KCNH2* and *SCN1A*. One study identified sequence alterations in the two genes *SCN5A* and *KCNH2,* for which variants previously have been associated with long QT syndrome (LQTS), in six (13 %) of 68 SUDEP cases [35]. Another paper discussed the coexistence of epilepsy and Brugada syndrome (BrS) in a family with a mutation in the *SCN5A* gene, implying that the same mutation may predispose to both epilepsy and cardiac arrhythmia, probably at a different age in the same individual [36]. Another study reported a family with idiopathic epilepsy and LQTS, and genetic testing identified a pathogenic *KCNH2* variant in affected family members [37]. More than 80 % of patients with Dravet syndrome (epileptic encephalopathy of childhood) carry a pathogenic variant in *SCN1A*, which encodes a neuronal voltage-gated sodium channel alpha subunit 1, expressed in both, brain and heart [38]. Patients with Dravet syndrome have an elevated mortality rate and SUDEP is the most common cause of death. In a group of over 500 patients with developmental and epileptic encephalopathies (DEEs), four genes were associated with SUDEP: *SCN1A, SCN2A, SCN8A*, and *STXBP1* [39].

Genetic studies in large SIDS and SUD cohorts have focused on possible underlying cardiovascular mechanisms only. This retrospective study aimed to bridge the gap of knowledge regarding possible correlation of SIDS/SUD including SUDEP by analysing the available whole-exome sequencing (WES) data of a large cohort of 155 SIDS and 45 SUD cases with a focus on 365 epilepsy-related genes.

## 2. Material and methods

### 2.1. SIDS study population

All SIDS cases had been collected between 1985 and 2014 at the Zurich Institute of Forensic Medicine (ZIFM) in Switzerland. Most cases were examined by the same forensic pathologist, ensuring a high level of consistency in autopsy procedures and case reporting. The SIDS cases have been classified according to the latest accepted international definitions of SIDS, including a complete autopsy, review of the circumstances of death, and examination of the clinical history [40]. The SIDS cohort included 155 SIDS cases with a median age at death of 17.4 ± 10.67 weeks (range 0.6–48.1 weeks). 62.2 % of the deceased were boys (94 males/61 females) and all of them were of European origin.

### 2.2. SUD study population

The SUD cohort consisted of 45 SUD cases that had been autopsied at the ZIFM between 2012 and 2019. The examination of the sudden death victims was performed according to the respective European and Swiss guidelines for the management of young SUD cases [41–43]. These guidelines include a thorough death scene investigation, a complete autopsy with pharmacological-toxicological and histopathological screening, and a review of the clinical history. The mean age at death of the 45 SUD cases was 30.2 ± 14.5 years (range: 1–63 years) and 75 % of them were males. Most of the cases had a European origin (88.9 %). The remaining five cases (11.1 %) were of African (three cases), Indian (one case) and Chinese (one case) origin. Sudden unexpected death has occurred in 33 % of the cases at rest, in 31 % during sleep, in 31 % during physical activity (e.g. swimming, hiking, walking, playing football), in 2 % while bathing, and for one SUD case, the activity at death is unknown. Among the 45 SUD cases, nine cases had a known medical history of epilepsy and were therefore classified as definite SUDEP, according to the definition by Nashef et al. [14] (Fig. 1).

### 2.3. Exome data analysis

Whole exome sequencing of our SIDS and SUD cohorts has been performed in previous studies [5,13,44]. The existing exome data of the 155 SIDS and 45 SUD cases was used to filter for variants within 365 genes associated with epilepsy (Table S1). The Alamut Batch Software version 1.11 (Interactive Biosoftware, Rouen, France) was used for the annotation of variants. Output results were reported in an Excel-sheet for data analysis. Variants were filtered according to our in-house filter strategy [13]. Filter criteria were 1) a global minor allele frequency (MAF) of ≤0.005 % (1:20,000 alleles) to evaluate ultra-rare variants based on recent sudden death studies [6,45], 2) focus on exonic and splice site variants, and 3) the exclusion of synonymous variants. The MAF was obtained from the Genome Aggregation Database (gnomAD) [46], the largest available human database containing exomes and whole genomes from unrelated individuals of different ethnicities, sequenced in various population genetics studies [47]. Integrative genomics viewer (IGV) version 2.9.2 was used to visualize the coverage of identified variants. The pathogenicity of variants was assessed twice within a two-year interval based on the ACMG recommendations [48], obtained from the Varsome database versions v.10.0 and v.11.7.5 (Saphetor, Lausanne, Switzerland) [49]. In addition, all identified variants were checked in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Only variants predicted to be pathogenic or likely pathogenic were further evaluated. Identified variants have been submitted to the Leiden Open Variation Database (LOVD) (https://databases.lovd.nl/shared/individuals?search_created_by=01602).

### 2.4. Variant confirmation

Variants with <20x bidirectional coverage and/or a minor allele frequency ratio <0.3 were confirmed by conventional Sanger sequencing (data available on request).

## 3. Results

### 3.1. Exome analysis with a focus on epilepsy-associated genes

We analyzed the exome data of 155 SIDS and 45 SUD cases by focusing on 365 genes associated with epilepsy. Following the ACMG guidelines, 39 pathogenic or likely pathogenic heterozygous rare variants were identified in 34 (21.9 %) out of the 155 SIDS cases (Table 1). 11 out of the 39 identified variants were predicted as pathogenic, located in the genes *ARV1, CENPJ, DPM1, L2HGDH, NPC1, PANK2, PDSS2, POMT2, RAI1, SAMHD* and *TMTC31*. In the SUD cohort 18 pathogenic or likely pathogenic heterozygous rare variants were

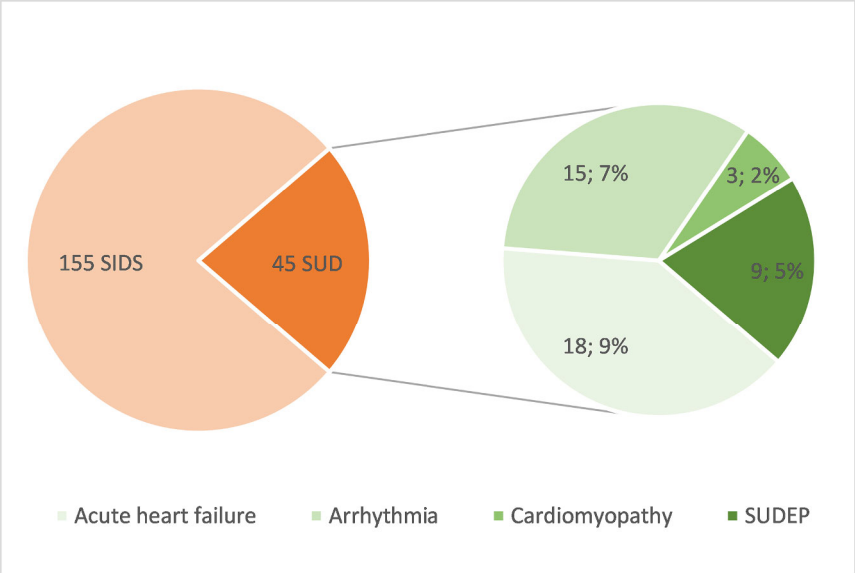


**Fig. 1.** Presumed causes of death in the SUD cohort (45 cases) based on the autopsy findings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

identified in 15 (33.3 %) out of the 45 SUD cases (Table 2). Six out of the 18 detected sequence alterations were predicted as pathogenic, located in the genes *DYNC1H1, NPRL2, POMT2, SCN3A, SCN5A* and *TBC1D24*. Sequence alterations that were evaluated as variants of uncertain significance (VUS) were not considered further.

### 3.2. Variant re-classification

The classification of variants can change over time as more data is gathered, such as functional studies or co-segregation of a variant with a clinical phenotype [50]. Therefore, all 57 identified variants predicted as pathogenic or likely pathogenic in the two cohorts were re-classified after a two-year period. In the SIDS cohort, 20 of the initially 39 variants predicted as pathogenic/likely pathogenic remained in the same classification category (in 19 (12.2 %) of the 155 SIDS cases) (Table 1). In contrast, 16 sequence alterations were re-classified from pathogenic/likely pathogenic to VUS and four variants to benign or likely benign. Eight pathogenic variants were found in the genes *CENPJ, DPM1, L2HGDH, NDUFS3, NPC1, PANK2, POMT2*, and *SAMHD1*, all inherited in an autosomal recessive manner. Three likely pathogenic variants in the genes *OPA1, RAI1* and *TSC2* are inherited in an autosomal dominant manner. In the SUD cohort, six of the 18 variants initially classified as pathogenic/likely pathogenic remained in the same category (in six (13.3 %) of the 45 SUD cases), whereas the classification of the remaining variants was down-graded from pathogenic/likely pathogenic to VUS (Table 2). One variant predicted as pathogenic was found in the gene *SCN5A* and five likely pathogenic sequence alterations in the genes *BTD, ITPA, POMT1, RMND1* and *SCN3A*. Only the *SCN5A* and *SCN3A* variants are inherited in an autosomal dominant manner.

### 3.3. SUDEP cases with pathogenic/likely pathogenic variants

In the SUD cohort, five cases had a known history of epilepsy (SUDS006, SUDS008, SUDS037, SUDS057 and SUDS059). In addition, in four cases (SUDS032, SUDS050, SUDS066 and SUDS077) SUDEP was assumed to be the most likely cause of death in absence of any previously reported epileptic events (Table 3). Pathogenic or likely pathogenic variants were detected in two of the cases with known epilepsy and in two of the SUDEP cases. In a 27 years-old man (SUDS008), who was found dead on the floor of his apartment, one pathogenic heterozygous variant was detected in the gene *SCN5A* (NM_001099404.1, c.2204C>A, p.(Ala735Glu)). He had a known medical history of familial hypertension, which was treated with medication. In addition, he experienced one unique epileptic seizure at the age of 21 years, but the neurological counselling was inconspicuous. Four years later, he had a one-time spontaneous event of an atrial fibrillation and the afterwards performed ECG showed some abnormalities. However, the cardiac follow-up examination was inconspicuous, and no further measures were considered at that time point. In a 20 years-old man (SUDS037) a likely pathogenic heterozygous sequence alteration was identified in the gene *BTD* (NM_001281723.3, c.425C>T, p.(Ala142Val)). According to his medical history, he had several episodes of epileptic seizures, bronchial asthma and a spherocytosis. A 21 year-old woman (SUDS032) with intellectual deficits carried a chromosomal rearrangement causing an isodicentric chromosome 15 duplication (47XX, +inv dup (15) (pter-))pter (160). In addition, she had a craniocerebral trauma two years before she died. In a 32 years-old man (SUDS050) a likely pathogenic heterozygous variant was detected in the gene *RMND1* (NM_017909.4, c.829G>C, p.(Glu277Gln)). One day before the sudden death event, he was not feeling well and experienced an unconscious phase after a fall. The next morning, he was found dead in his bed. It is not known, whether he ever had epileptic seizures in his life.

## 4. Discussion

High-throughput sequencing has been demonstrated to be a great diagnostic tool in clinical practice in a variety of genetic conditions. In heterogeneous study populations, such as sudden infant death syndrome (SIDS) and sudden unexplained death (SUD), it is a useful strategy to identify rare DNA sequence variants in order to discover possible underlying genetic diseases and a probable cause of death. To our knowledge, this is the first study to look into genetic causes of epilepsy in a large cohort of both SIDS and SUD cases. We analyzed the exome sequencing data from 155 SIDS and 45 SUD cases by focusing on 365 genes associated with epilepsy. Variant classification is a dynamic process and may change due to improvements in available genetic data, functional studies, and co-segregation with a clinical phenotype [50–52]. Therefore, the pathogenicity of the variants identified in this study was re-assessed after a two-year interval.

**Table 1**
Pathogenic and likely pathogenic variants identified in the 155 SIDS cases.

| Case No. | Gene | HGVS RefSeq-Nr. | rsId | Coding Effect | cDNA | Protein change | Allele frequency (gnomAD) | Inheritance | Pathogenicity evaluated with Varsome v 10.0 assessed in 2021** | Pathogenicity evaluated with Varsome v.11.7.5 assessed in 2023** | ACMG categories | ClinVar (assessed in 2023) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SIDS006 | *DPM1* | NM_001317034.1 | rs121908583 | missense | c.274C>G | p.(Arg92Gly) | 0.000021 | AR | Pathogenic | Pathogenic | PVS1, PP5, PM2 | Likely pathogenic |
| SIDS007 | *POMT2* | NM_013382.7 | rs886042401 | frameshift | c.1123_1124dup | p.(Tyr376Profs*23) | 0.000004 | AR | Pathogenic | Pathogenic | PVS1, PP5, PM2 | Pathogenic |
| SIDS012 | *OPA1* | NM_130837.3 | rs147077380 | missense | c.1934G>A | p.(Arg645Gln) | 0.000035 | AD | Likely Pathogenic | Likely pathogenic | PM5, PP3, PM2, PP5 | VUS |
| SIDS013 | *RARS2* | NM_001350505.1 | rs765088174 | missense | c.1544A>G | p.(Asp515Gly) | 0.000028 | AR | Likely Pathogenic | Likely pathogenic | PP5, PM2, PP3, BP1 | Likely pathogenic |
| SIDS014 | *TPP1* | NM_000391.4 | - | missense | c.629A>G | p.(Asn210Ser) | 0.000000 | AR | Likely Pathogenic | Likely pathogenic | PM1,PM2, PP3 | VUS |
| SIDS016 | *PCCB* | NM_001178014.1 | - | missense | c.332G>T | p.(Arg111Leu) | 0.000000 | AR | Likely Pathogenic | VUS | PM1, PM2 | Not reported in ClinVar |
| SIDS022 | *COQ8A* | NM_020247.5 | - | missense | c.1288G>A | p.(Val430Met) | 0.000000 | AR | Likely Pathogenic | VUS | PP3, PM2 | Not reported in ClinVar |
| SIDS023 | *SCN5A* | NM_001099404.1 | rs199473233 | missense | c.4057G>A | p.(Val1353Met) | 0.000028 | AD | Likely Pathogenic | Benign | PM1, PP3, BS2, BS3 | Conflicting interpretation |
| SIDS027 | *ACY1* | NM_000666.3 | rs747746548 | missense | c.461G>A | p.(Gly154Asp) | 0.000004 | AR | Likely Pathogenic | VUS | PP3, PM2 | Not reported in ClinVar |
| SIDS030 | *ALDH7A1* | NM_001182.5 | - | missense | c.394G>T | p.(Val132Leu) | 0.000000 | AR | Likely Pathogenic | VUS | PP3, PM2 | Not reported in ClinVar |
| SIDS030 | *COL4A1* | NM_001845.6 | - | missense | c.2759C>T | p.(Pro920Leu) | 0.000000 | AD | Likely Pathogenic | VUS | PM1, PM2 | no |
| SIDS032 | *NPC1* | NM_000271.5 | rs1428599096 | missense | c.2833G>A | p.(Asp945Asn) | 0.000008 | AR | Likely Pathogenic | Likely pathogenic | PP3, PM1, PM2, PP5 | Conflicting interpretation |
| SIDS038 | *ADSL* | NM_000026.4 | rs756210458 | missense | c.421C>T | p.(Arg141Trp) | 0.000012 | AR | Likely Pathogenic | Likely pathogenic | PM5, PM2, PP3 | Pathogenic/likely pathogenic |
| SIDS040 | *ARV1* | NM_001346992.1 | - | start loss | c.1A>G | p.(?) | 0.000000 | AR | Pathogenic | Likely pathogenic | PVS1, PM2 | Not reported in ClinVar |
| SIDS044 | *ETFDH* | NM_004453.4 | rs376263577 | missense | c.358G>C | p.(Asp120His) | 0.000020 | AR | Likely Pathogenic | VUS | PM1, PM2 | VUS |
| SIDS044 | *SAMHD1* | NM_015474.4 | rs121434517 | stop gain | c.433C>T | p.(Arg145*) | 0.000008 | AR | Pathogenic | Pathogenic | PVS1, PP5, PM2 | Pathogenic |
| SIDS044 | *TSC2* | NM_000548.5 | - | missense | c.1600G>A | p.(Val534Met) | 0.000000 | AD | Likely Pathogenic | Likely pathogenic | PP3, PM5, PM2 | VUS |
| SIDS056 | *OPA1* | NM_130837.3 | rs1157991384 | missense | c.1846G>C | p.(Glu616Gln) | 0.000000 | AD | Likely Pathogenic | VUS | PM1,PM2, PP3 | Not reported in ClinVar |
| SIDS057 | *PDSS2* | NM_020381.4 | rs17853951 | missense | c.1003G>A | p.(Gly335Arg) | 0.000050 | AR | Pathogenic | VUS | PVS1, PM2 | VUS |
| SIDS066 | *RAI1* | NM_030665.4 | - | frameshift | c.845_857del | p.(Gln282Argfs*78) | 0.000000 | AD | Pathogenic | Likely pathogenic | - | Not reported in ClinVar |
| SIDS072 | *ARHGEF9* | NM_001353923.1 | rs782237174 | missense | c.466G>A | p.(Glu156Lys) | 0.000005 | x-linked | Likely Pathogenic | Likely benign | PM2, BP6, BP1 | Likely benign |
| SIDS083 | *RARS2* | NM_001350505.1 | rs755448382 | missense | c.403A>G | p.(Asn135Asp) | 0.000008 | AR | Likely Pathogenic | Likely pathogenic | PP3, PM1, PM2 | VUS |
| SIDS091 | *AMT* | NM_000481.4 | rs150079386 | missense | c.655C>T | p.(Arg219Cys) | 0.000042 | AR | Likely Pathogenic | VUS | PM1, PP3, PM2 | VUS |
| SIDS107 | *PANK2* | NM_153638.3 | - | stop gain | c.570_571del | p.(Tyr190*) | 0.000000 | AR | Pathogenic | Pathogenic | - | Pathogenic |
| SIDS120 | *COL4A1* | NM_001845.6 | - | missense | c.2270A>G | p.(Lys757Arg) | 0.000000 | AD | Likely Pathogenic | VUS | PM1,PM2, PP3 | Not reported in ClinVar |
| SIDS122 | *FOXRED1* | NM_017547.4 | rs545703077 | missense | c.733C>T | p.(Arg245Cys) | 0.000008 | AR | Likely Pathogenic | VUS | PP3, PM2, BP1 | Not reported in ClinVar |
| SIDS123 | *NPC1* | NM_000271.5 | rs372445155 | missense | c.1421C>T | p.(Pro474Leu) | 0.000016 | AR | Pathogenic | Pathogenic | PP5, PP3, PM1, PM5, PM2 | Pathogenic |
| SIDS140 | *MTHFR* | NM_005957.5 | rs769953411 | missense | c.1163G>A | p.(Arg388His) | 0.000020 | AR | Likely Pathogenic | VUS | PP3, PM2, PP5, BP1 | Conflicting interpretation |
| SIDS156 | *NDUFS3* | NM_004551.3 | rs104894270 | missense | c.595C>T | p.(Arg199Trp) | 0.000016 | AR | Likely Pathogenic | Pathogenic | PP3, PP5, PM5, PM2 | Likely pathogenic |
| SIDS156 | *TBC1D24* | NM_001199107.2 | rs1387751971 | missense | c.1188C>G | p.(Ile396Met) | 0.000011 | AR | Likely Pathogenic | VUS | PM5, PM2 | Not reported in ClinVar |
| SIDS161 | *TMTC3* | NM_181783.4 | rs1263921941 | stop gain | c.22G>T | p.(Glu8*) | 0.000004 | AR | Pathogenic | Likely pathogenic | PVS1,PM2 | Not reported in ClinVar |
| SIDS167 | *L2HGDH* | NM_024884.3 | rs766538932 | frameshift | c.530del | p.(Pro177Hisfs*6) | 0.000035 | AR/AD | Pathogenic | Pathogenic | PVS1, PP5, PM2 | Pathogenic |
| SIDS176 | *GCH1* | NM_000161.3 | rs527416949 | missense | c.299C>T | p.(Ser100Leu) | 0.000000 | AR/AD | Likely Pathogenic | VUS | PM1, PM2 | VUS |
| SIDS220 | *CACNA1E* | NM_001205293.3 | - | missense | c.2311C>T | p.(Arg771Cys) | 0.000000 | AD | Likely Pathogenic | Likely benign | PM2, BP4, BP1 | Not reported in ClinVar |
| SIDS225 | *SUOX* | NM_001032387.1 | - | missense | c.913G>A | p.(Ala305Thr) | 0.000004 | AR | Likely Pathogenic | VUS | PP3, PM2 | VUS |
| SIDS226 | *NPC1* | NM_000271.5 | rs752728865 | missense | c.2933G>A | p.(Arg978His) | 0.000018 | AR | Likely Pathogenic | Likely pathogenic | PM5, PP5, PM1, PM2, BP4 | Likely pathogenic |
| SIDS226 | *RARS2* | NM_001350505.1 | rs377591456 | missense | c.422A>G | p.(His141Arg) | 0.000014 | AR | Likely Pathogenic | Likely pathogenic | PP3, PP5, PM1, PM2 | Pathogenic |
| SIDS235 | *CENPJ* | NM_018451.5 | rs201822162 | stop gain | c.40C>T | p.(Gln14*) | 0.000008 | AR | Pathogenic | Pathogenic | PVS1, PP5, PM2 | Pathogenic/likely pathogenic |
| SIDS236 | *PIGO* | NM_032634.4 | - | missense | c.941A>G | p.(Glu314Gly) | 0.000000 | AR | Likely Pathogenic | Likely benign | PM2, BP4, BP1 | Not reported in ClinVar |

**according to ACMG criteria [48].
ACMG = American College of Medical Genetics guidelines for the interpretation of sequence variants, AD = autosomal dominant, AR = autosomal recessive, VUS = variant of uncertain significance.
color description for ACMG categories: red = strong evidence of pathogenicity, dark orange = moderate evidence of pathogenicity, orange = supporting evidence of pathogenicity, green = supporting evidence of benign impact (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Pathogenic and likely pathogenic variants identified in the 45 SUD cases.

| Case No. | Gene | HGVS RefSeq-Nr. | rsId | Coding Effect | cDNA | Protein change | Allele frequency (gnomAD) | Inheritance | Pathogenicity evaluated with Varsome v 10.0 assessed in 2021** | Pathogenicity evaluated with Varsome v.11.7.5 assessed in 2023** | ACMG categories | ClinVar (assessed in 2023) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUDS003 | *GCK* | NM_033507.3 | - | in-frame | c.452_454del | p.(Phe151del) | 0.000000 | AD | Likely Pathogenic | VUS | - | Not reported in ClinVar |
| SUDS006* | *PIGN* | NM_012327.5 | - | frameshift | c.2766_2767del | p.(Cys923Trpfs*21] | 0.000000 | AR | Likely Pathogenic | VUS | - | Not reported in ClinVar |
| SUDS008* | *POMT2* | NM_013382.7 | - | missense | c.334A>G | p.(Met112Val) | 0.000000 | AR | Pathogenic | VUS | PP3, PM2, BP1 | Not reported in ClinVar |
| SUDS008* | *SCN5A* | NM_001099404.1 | rs137854611 | missense | c.2204C>A | p.(Ala735Glu) | 0.000000 | AD | Pathogenic | Pathogenic | PP3, PP5, PM5, PM2 | Likely pathogenic |
| SUDS013 | *SCN3A* | NM_006922.4 | rs749133387 | missense | c.1579G>A | p.(Glu527Lys) | 0.000028 | AD | Pathogenic | Likely pathogenic | PVS1, PM2 | VUS |
| SUDS028 | *POMT1* | NM_001353193.1 | rs778373035 | frameshift | c.1790_1791del | p.(Ile597Serfs*30) | 0.000008 | AR | Likely Pathogenic | Likely pathogenic | PVS1, PP5, PM2 | Not reported in ClinVar |
| SUDS030 | *GFM1* | NM_001308164.1 | rs375512235 | missense | c.833A>G | p.(Asn278Ser) | 0.000014 | AR | Likely Pathogenic | VUS | PP5, PM2, BP1 | Conflicting interpretations |
| SUDS032* | *KCNT1* | NM_020822.3 | - | frameshift | c.1387_1399del | p.(Asp463Glnfs*12) | 0.000000 | AD/AR | Likely Pathogenic | VUS | - | Not reported in ClinVar |
| SUDS037* | *BTD* | NM_001281723.3 | rs397514364 | missense | c.425C>T | p.(Ala142Val) | 0.000004 | AR | Likely Pathogenic | Likely pathogenic | PM1, PM5, PP3, PP5, PM2 | Conflicting interpretations |
| SUDS037* | *COL4A1* | NM_001845.6 | rs368900861 | missense | c.823G>A | p.(Gly275Arg) | 0.000016 | AD | Likely Pathogenic | VUS | PP5, PM1, PM2 | VUS |
| SUDS039 | *PCCB* | NM_001178014.1 | - | missense | c.1477T>C | p.(Phe493Leu) | 0.000000 | AR | Likely Pathogenic | VUS | PP5, PM1, PM2 | Not reported in ClinVar |
| SUDS040 | *PHGDH* | NM_006623.4 | rs143217390 | missense | c.138G>C | p.(Gln46His) | 0.000050 | AR | Likely Pathogenic | VUS | PP3, PM2 | VUS |
| SUDS050* | *RMND1* | NM_017909.4 | rs143508229 | missense | c.829G>C | p.(Glu277Gln) | 0.000004 | AR | Likely Pathogenic | Likely pathogenic | PP3, PP5, PM2 | Not reported in ClinVar |
| SUDS068 | *ITPA* | NM_033453.4 | rs760868571 | missense | c.190G>T | p.(Val64Leu) | 0.000046 | AR | Likely Pathogenic | Likely pathogenic | PP3, PM2, BP1 | VUS |
| SUDS068 | *TBC1D24* | NM_001199107.2 | - | missense | c.418C>G | p.(Leu140Val) | 0.000000 | AR | Pathogenic | VUS | PP5, PM2, BP4 | VUS |
| SUDS080 | *DYNC1H1* | NM_001376.5 | rs1406149790 | missense | c.2002G>A | p.(Val668Ile) | 0.000004 | AD | Pathogenic | VUS | PM5, PM2 | VUS |
| SUDS084 | *NPRL2* | NM_006545.5 | - | missense | c.334C>G | p.(Leu112Val) | 0.000000 | AD | Pathogenic | VUS | PP5, PM1, PM2, PP3 | Not reported in ClinVar |
| SUDS112 | *MCCC2* | NM_022132.5 | - | missense | c.1652C>T | p.(Ala551Val) | 0.000000 | AR | Likely Pathogenic | VUS | PP5, PM1, PM2 | Not reported in ClinVar |

*cases with known history of epilepsy or cases in which SUDEP was assumed to be the most likely cause of death in absence of a terminal seizure.
**according to ACMG criteria [48].
ACMG = American College of Medical Genetics guidelines for the interpretation of sequence variants, AD = autosomal dominant, AR = autosomal recessive, VUS = variant of uncertain significance. color description for ACMG categories: red = strong evidence of pathogenicity, dark orange = moderate evidence of pathogenicity, orange = supporting evidence of pathogenicity, green = supporting evidence of benign impact (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 4.1. SIDS cases with pathogenic/likely pathogenic variants

In our SIDS cohort, none of the 155 SIDS victims had a reported history of epilepsy or seizures. However, the incidence rate of epilepsy is particularly high in the first year of life, and infantile apneic seizures are unexpected life-threatening events [53]. Three SIDS cases (SIDS012, SIDS044, SIDS066) carried likely pathogenic variants in the genes *OPA1, RAI1* and *TSC2* in which the mode of inheritance occurs in an autosomal dominant manner. *OPA1* encodes for a large multimeric dynamin-like GTPase protein, and plays a crucial role in mediating mitochondrial fusion, oxidative phosphorylation and mitochondrial DNA maintenance. Pathogenic *OPA1* mutations can lead to autosomal dominant optical atrophy, but additional neurological features including seizures have also been reported in some patients [54]. The retinoic acid-induced 1 gene (*RAI1*) acts as a transactional factor and is expressed at high levels in the brain [55]. *RAI1* micro-deletions are causing Smith-Magenis syndrome, which is a complex genetic disorder characterized by developmental delay, intellectual disability, and seizures in up to 30 % of the patients [56]. *TSC2* is involved in mTORopathies characterized by excessive mechanistic target of rapamycin pathway activation. mTORopathies refer to a group of neurodevelopmental disorders caused by a dysregulation of the mammalian target of rapamycin (mTOR) signaling pathway, which serves as a ubiquitous regulator of cell metabolism [57]. This is of interest, because post-mortem analysis of certain brain regions showed significantly increased mTOR pathway activation in SUDEP [58]. In addition, variants in *TSC2* have been identified in patients with tuberous sclerosis complex, a condition characterized by developmental problems and seizures [59]. The remaining pathogenic or likely pathogenic variants identified in the SIDS cohort are located in genes with an autosomal recessive mode of inheritance, and therefore the here reported heterozygous variants most probably have not a severe effect on the pathophysiological mechanisms leading to the sudden death. Still they could act as risk modifiers, such as *SAMHD1* (c.433C>T, p.(Arg145*)) that was detected in SIDS044. *SAMHD1* was found to play a role in Aicardi-Goutières syndrome, a genetically driven very rare genetic encephalopathy that often mimics a congenital infection [60]. It might suit the pathophysiological mechanisms leading to SIDS, although it has not been described in the context of SIDS so far. Due to the suspected multifactorial etiologies in SIDS, other pathogenic mechanisms or other risk factors (e.g. intrinsic susceptibility or environmental risk factors) might have contributed to the early death. Prone sleeping position is a risk factor for both, SIDS and SUDEP, possibly explained by impaired $CO_2$-induced arousals [61]. However, in our SIDS cohort we did not find indications for genetic involvement of respiratory chemoreception [62].

### 4.2. SUD cases with pathogenic/likely pathogenic variants

Within the SUD cohort, six pathogenic or likely pathogenic variants were detected in six of the 45 cases, two of which are inherited in an autosomal dominant manner. The pathogenic *SCN5A* variant (c.2204C>A, p.(Ala735Glu)) was found in SUDS008, one of our SUDEP cases, that is discussed below. The likely pathogenic variant in the gene *SCN3A* (c.1579G>A, p.(Glu527Lys)) was detected in SUDS013. Variants in both genes are associated with channelopathies, which result from the dysfunction of ion channels and have been implicated in a wide variety of diseases including epilepsy and cardiac arrhythmias. It is therefore plausible that this genotype plays an important role in determining predisposition to SUDEP. While *SCN3A*-related disorders encompass a spectrum of clinical variety associated with epilepsy and brain malformations, *SCN5A* variants have been reported in patients with epilepsy, mostly co-occurring with other *SCN5A*-related cardiac disorders [63, 64].

**Table 3**
Detailed demographic characteristics of the nine SUDEP cases.

| Case No. | Sex | Age (years) | Ethnicity | BMI | Medical history | Symptoms prior to death | Event at death | Macroscopic anomalies in the brain | Microscopic anomalies in the brain | Post-mortem toxicology | Family history of SD | Identified variants of interest |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUDS006 | F | 7 | European | NA | Rolandic epilepsy | None | Playing with other children | None | Unspecific discrete swelling | Negative | No | - |
| SUDS008 | M | 27 | European | 30.64 | Familial hypertension, one unique epileptic seizure at the age of 21 years and a one-time spontaneous event of an atrial fibrillation at the age of 25 years | n.s. | Resting | Increased brain weight (1660 g) | Signs of hypoxia, discrete swelling | Hypotensive drugs | No | *SCN5A* p. (Ala735Glu) |
| SUDS032 | F | 21 | European | 27.34 | Mental retardation Isodicentric chromosome 15 syndrome | n.s. | Sleeping | Slight frontal surface depression (secondary to trauma) | Posttraumatic gliosis, bilateral hippocampal atrophy, microdysgenesis, heterotopia | Antipsychotics | No | inv dup (15) |
| SUDS037 | M | 20 | European | 17.51 | Asthma, epilepsy, spherocytosis | n.s. | Sleeping | None | NA | Cannabis, amphetamine | NA | *BTD* c.425C>T |
| SUDS050 | M | 32 | African | 24.86 | Seizure | Seizures | At rest | None | Few hippocampal cellular apoptosis | Negative | NA | *RMND1* p. (Glu277Gln) |
| SUDS057 | M | 36 | European | 26.29 | Epilepsy | n.s. | Sleeping | None | None | Alcohol (0.24‰), antiepileptic drugs | NA | - |
| SUDS059 | M | 21 | European | 21.63 | Epilepsy | None | Bathing | None | NA | Antiepileptic drugs | NA | - |
| SUDS066 | F | 8 | European | NA | Unconfirmed suspicion of Marfan-syndrome | Fever, seizures | Resting | None | Unspecific microglial changes (inflammation) | Negative | No | - |
| SUDS077 | F | 28 | European | 19.84 | Seizure | n.s. | Resting | None | Swelling, signs for increased intracranial pressure and abnormal migration | Negative | No | - |

*NA* not available, *n.s.* not specified, *SD* sudden death

### 4.3. SUDEP cases with pathogenic/likely pathogenic variants

In our SUD cohort, nine cases were classified as SUDEP, in four of which pathogenic or likely pathogenic variants were detected. In one of the SUDEP victims (SUDS008), a genetic variant was found in *SCN5A* (c.2204C>A, p.(Ala735Glu)). After complete autopsy, acute cardiac failure due to cardiac arrhythmia was considered the most probable cause of death. It is likely that the detected *SCN5A* variant had a major impact, as it was very recently found to have a negative effect on the sodium voltage-gated channel function and might lead to BrS [65]. The second case (SUDS032) had a known isodicentric chromosome 15 syndrome. SUDEP is a common cause of death in children and young adults with isodicentric chromosome 15q11.2q13 duplications or supernumerary isodicentric chromosome 15 (idic(15)) (formerly known as inverted duplication 15) [66]. The exact pathophysiological mechanism is not known, but the retractable epilepsy and frequent seizures, leading to postictal suppression of brain activity, may be an important contribution. An additional influence of administered drugs, such as GABA agonists, cannot be excluded. In this context, it is of interest, that the measurement of transcript levels in the cortex for three genes encoding subunits of $GABA_A$ receptors that lie within the duplicated region in idic15 syndrome revealed partly altered expression of the genes [67]. In addition, SUDS032 had a frameshift *KCNT1* variant (c.1387_1399del, p.(Asp463Glnfs*12)), that is inherited in an autosomal dominant manner, but that was recently down-classified as VUS. *KCNT1* encodes a ligand-gated potassium channel with several functions, including an important role in the neuronal response to hypoxia [68]. Mutations in *KCNT1* cause a spectrum of focal epilepsies, SUDEP and cardiac disorders [69]. The third SUDEP case (SUDS037) already had a diagnosis of epilepsy with two reported epileptic seizures. The post-mortem toxicological analysis disclosed cannabis and amphetamine consumption, which are both risk factors in the context of an epilepsy-related death event. He carried a heterozygous genetic variant in the gene *BTD* (c.425C>T, p.(Ala142Val)). The impact of the identified variant in the *BTD* gene is unclear in this context as biotinidase deficiency is inherited in an autosomal recessive manner and is usually excluded by newborn screening [70]. In our fourth SUDEP case (SUDS050), who according to autopsy records suffered from a seizure prior to death, the spectrum of possible underlying genetic aetiologies was further expanded with the finding of a missense variant in *RMND1* (c.829G>C, p.(Glu277Gln)). Pathogenic variants in *RMND1* (required for meiotic nuclear division 1 homolog) are known to cause combined oxidative phosphorylation deficiency (COXPD11), a severe multisystem disorder, and compound heterozygous variants can cause severe epilepsy in addition to cardiomyopathies [71].

None of our SIDS, SUD and SUDEP victims carried a pathogenic variant in *SCN1A*, which is somewhat surprising, as these are usually the largest proportion of variants detected in SUDEP cohorts. A recent comprehensive systematic review of genetics and sudden unexpected death in epilepsy included eight studies with 161 individuals and all types of epilepsy [34]. The study showed that the main epilepsy associated genes (*SCN1A, SCN8A, SCN1B, KCNA1, PRRT2, HCN2* and *MECP2)*, and pre-autopsy known cases of Dravet syndrome (*SCN1A* mutations and autism with duplication of chromosome 15) were associated with 11 % and 9 % of cases, respectively. Chahal et al. examined 96 SUDEP cases, five of which had a diagnosis of Dravet syndrome and carried pathogenic variants in the *SCN1A* gene [35].

It is not yet clear why specifically early onset epileptic syndromes caused by variants in voltage-gated sodium channels should have a high risk for SUDEP, but due to the tissue-specific expression including the heart, a context with their effects on cardiorespiratory function is likely. From a clinical point of view, additional risk factors such as underlying genetic diseases have to be considered in sensible counselling on SUDEP. Previous studies from the last decades have identified risk-factors, such as high frequency of tonic-clonic seizures, nocturnal seizures, and lack of night time supervision, but not specifically the underlying cause of the disease [72–74]. A recent review by Trivisano et al. pointed out that SUDEP has been reported not only in the context of channelopathies but in further patients with genetic epilepsies due to mutations in genes such as *DEPDC5, TBC1D24, FHF1,* or 5q14.3 deletion [75]. For individuals with known underlying pathogenic variants, clinicians may advise selected patients and families, if being in line with their individualized epilepsy phenotype, about the risk of SUDEP.

### 4.4. Other aspects

There are some important aspects to consider when performing genetic studies in SIDS, SUD and SUDEP cases. Most of the relevant background information on the deceased is based primarily on autopsy results, whereas details about possible medical histories (such as ECGs and EEGs) and family investigations are in many cases only partially available. In addition, post-mortem evaluation of SUDEP is challenging as the deaths are often unwitnessed and anatomical findings are in most of the cases not specific enough to conclude that epilepsy was a contributor to death. In genetic studies, accurate interpretation of variants is challenging and often many of the identified variants are classified as VUS with unclear effect on protein function, nevertheless they still could act as genetic modifiers involved in the sudden death event [76]. In addition, the classification of identified variants constantly evolves requiring a periodic re-analysis of variants of interest [50]. Consequently, an experienced multidisciplinary team is required for the proper interpretation of genetic results and adequate family counselling.

There are some limitations in our current study. Firstly, functional studies would have been required to further investigate the here reported pathogenic and likely pathogenic variants to verify their potential pathogenic role. Secondly, the exome data from our SIDS and SUD cases were analyzed focusing on 365 epilepsy-related genes, based on current knowledge on genetics in epilepsy. However, this list is not conclusive and novel genes associated with epilepsy may emerge, that could have contributed to the sudden death event. Thirdly, family members were not available for co-segregation studies. This would be necessary to determine the mode of inheritance and to identify other genetic carriers at risk for sudden death. In addition, we re-evaluated the identified variants predicted as pathogenic or likely pathogenic in the two cohorts after a two-year period, but not the VUS variants. However, some of these variants might have been re-classified as pathogenic or likely pathogenic in the meantime. Most of our identified variants are associated with autosomal recessive inherited disorders. Further analysis such as whole genome sequencing might have revealed further contributing changes like chromosomal rearrangements leading to additional functional impacts in the same gene.

## 5. Conclusions

In conclusion, post-mortem molecular autopsy investigation in our SIDS and SUD victims has been demonstrated to be helpful in unrevealing possible underlying pathogenic mechanisms involving epilepsy-associated genes. Genes co-expressed in the brain and heart could independently induce epilepsy and/or arrhythmia. Mutant genes in the brain could affect cardiac rhythm through central or peripheral regulation, but could also affect cerebral electrical activity by changing the haemodynamics or the internal environment. However, more effort is needed to better understand the pathophysiology leading to sudden death. Sensible counselling on SUDEP remains challenging and should focus not only on identifying the epilepsy phenotype but also on the genetic cause, and lead to reconsideration of enhancing night-time or sleeping supervision with the aim of reducing the risk of SUDEP.

## Funding

The study was funded by the Emma Louise Kessler Foundation.

## Compliance with ethical standards

This study was conducted in full conformance with Swiss laws and regulations. Ethical approval was provided by the local ethics committee (KEK-ZH-No. 2013-0086). SIDS cases are irreversibly anonymized. For the SUD cases, the requirements of the local ethic committee included written informed consent of family members. If no family members were available, SUD cases had been irreversibly anonymized. Family members of the SIDS and SUD cases were not available for co-segregation analyses.

## CRediT authorship contribution statement

**Sarah E. Buerki:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Cordula Haas:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Jacqueline Neubauer:** Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

We thank Corinne Moser for technical assistance and Agnes Geher for help in data analysis. We thank the Emma Louise Kessler Foundation for funding this study. The exome analysis was supported by the Swiss National Science Foundation (SNF, project-no. 320030-149456, 2013–2017).

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.seizure.2023.11.002.

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