Genetic variants in incident SUDEP cases from a community-based prospective cohort with epilepsy

Yan Ge¹, Ding Ding², Guoxing Zhu¹, Patrick Kwan³, Wenzhi Wang⁴, Zhen Hong², Josemir W. Sander⁵,⁶

1. Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China
2. Institute of Neurology, WHO Collaborating Center for Research and Training in Neurosciences, Huashan Hospital, Fudan University, Shanghai, China
3. Department of Neuroscience, Monash University, Alfred Hospital, Melbourne, Victoria, Australia
4. Beijing Neurosurgical Institute, Capital Medical University, Beijing, China
5. NIHR University College London Hospitals Biomedical Research Centre, UCL Institute of Neurology, Queen Square, London WC1N 3BG & Chalfont Centre for Epilepsy, Chalfont St Peter SL9 0RJ, United Kingdom
6. Stichting Epilepsie Instellingen Nederland (SEIN), Achterweg 5 Heemstede 2103 SW, the Netherlands

Correspondence to:
Ding Ding. Huashan Hospital, 12 Middle Wulumuqi Rd., Shanghai 200040, China,
Phone: +86 21 52888158; FAX: +86 21 62481930; dingding@huashan.org.cn
Abstract: Sudden unexpected death in epilepsy (SUDEP) is a leading cause of epilepsy-related mortality in young adults. It has been suggested that SUDEP may kill over 20,000 people with epilepsy in China yearly. The aetiology of SUDEP is unclear. Little is known about candidate genes for SUDEP in people of Chinese origin as most studies have investigated this in Caucasians. We performed whole exome sequencing in DNA samples collected from five SUDEP cases identified in a large epilepsy cohort in rural China. Overall, 3 new rare deleterious variants in SCN5A, KIF6 and TBX18 were identified. These three genes were all previously related to heart disease, supporting the hypothesis that underlying heart disorder may be a driver of SUDEP risk.

Highlights

Keywords

Acknowledgements

Formatting of funding sources
1. Introduction

Sudden unexpected death in epilepsy (SUDEP) is a leading cause of epilepsy-related mortality in young adults in Western Countries (Devinsky et al., 2016). The incidence of all suspected (probable and possible) SUDEP in China was reported at 2.34 (95% CI 1.36-3.77) per 1,000 person-years, suggesting that yearly over 20,000 people die as result of SUDEP. (Ge et al., 2017).

The mechanism of SUDEP remains unclear. Proposed mechanisms include seizure-related respiratory, cardiac, and autonomic dysfunction, suggesting heterogeneity (Devinsky, 2011). Increased genome-wide polygenic burden in SUDEP cases provided some evidence for genetic susceptibility (Leu et al., 2015). Studies in models and humans suggest that some specific genetic background may predispose individuals to potentially fatal cardiorespiratory dysfunctions (Dlouhy et al., 2016). To date, several genetic features mainly involving ion channels co-expressed in the heart and brain have been described mainly in Caucasians. No candidate genes for SUDEP in Chinese people have been identified.

Previous reports on candidate genes for SUDEP were mainly from retrospective cases studies (Tu et al., 2011a; Tu et al., 2011b; Hata et al., 2017; Friedman et al., 2018) or from follow-up study with small sample-size (Coll et al., 2016; Coll et al., 2017). Adequately powered cohort studies are needed to corroborate these results and identify new genetic candidates. The low incidence of SUDEP and the time gap between the collection of DNA samples and the occurrence of SUDEP has made it difficult to accomplish such studies.

We performed whole exome sequencing in DNA samples collected from five probable SUDEP cases identified in a large community-based cohort in China. We filtered rare variants identified from our cases, as well as screened for SUDEP,
epilepsy, heart disease or respiratory disease-related genes from previous published reports, and compared them with publicly available data, living epilepsy controls and ethnicity-match non-epilepsy controls, to identify potential candidate genes for SUDEP in the Chinese population.

2. Material and methods

2.1 Study cohort, follow-up and confirmation of SUDEP cases

Between January 2010 and December 2011, 1,562 (median age 38 years; 58% males) individuals with epilepsy from rural areas in the Chinese provinces of Henan, Shanxi, and Ningxia, took part in the project “Validation of Clinical Assessment Tools for Population Genetic Studies of Epilepsy”. Fifty-six percent of the participants had focal seizures. Sixty percent were on anti-seizure medication (ASM) monotherapy and only 20% were in one-year remission when enrolled.

Two follow-up visits were conducted, the first during March 2013 to October 2014, and the second between October 2015 to March 2016. For those who had died, death certificates were collected from the appropriate office. A specifically designed Verbal Autopsy Questionnaire (VAQ) was used (Ge et al., 2017). All data were assessed by a multi-disciplinary expert panel. Fifteen death cases were attributed to SUDEP and 13 of them were attributed as probable SUDEP. Detailed study methods and attribution of death was previously reported (Ge et al., 2017).

2.2 Blood draw and DNA extraction at baseline

At baseline, 2ml peripheral blood of each participant was collected in EDTA anticoagulant vacutainer and all samples were sent by cold chain logistics to Huashan Hospital, Shanghai, China. Genomic DNA was extracted from whole blood. Extracted
DNA samples were stored at -80°C until use.

2.3 Selection of cases for sequencing

Inclusion criteria: 1) death previously ascertained as probable SUDEP (Ge et al., 2017); 2) age at death between 15-39 years; 3) DNA sample available and having passed an integrity assessment (on a 0.8% agarose gel).

They were 13 cases of probable SUDEP in our cohort of whom five died between ages of 15-39 years.

2.4 WES and bioinformatics analysis

The DNA library was constructed by fragmenting the genomic DNA; a Qubit®2.0 Fluorometer was used to determine the concentration of the library; the pooled capture library was quantified by Qubit (Invitrogen) and Bioanalyzer (Agilent) and sequenced on an Illumina HiSeq 2500 using a paired end, 150 nucleotides in length run mode. All sequencing processes were controlled by data collection software according to the IlluminaX User Guide. We aligned the paired-end reads to the reference human genome (hg38) using the third-party software BWA (Burrows–Wheeler Alignment, version 5.9). The average mapping ratio was as high as 99%. The Flagstat tool was utilized to assess the mapping information. We then analyzed the distribution of each sample's reads in the target region and the enrichment of reads in the genome. SNVs (single nucleotide variations) were then processed using the GATK UnifiedGenotyper (GenomeAnalysisTK-3.1-1). Lastly, we annotated the mutations using ANNOVAR software (GenomeAnalysisTK-3.1-1).

Qualified variants were identified through the following filtering process: 1) low quality reads were removed (marked “Pass” in filter and Genotype Quality >20); 2)
SNVs located out of exonic regions and splicing sites were removed; 3) synonymous SNVs were removed; 4) common SNVs (mutation frequency > 0.01 in 1000 genomes project were removed; 5) SNVs marked other than “damaging” in SIFT and PolyPhen-2 prediction were removed. Variants which passed the filtering process were regard as qualified variants. The genes which those qualified variants were in were regard as qualified genes. Function of each qualified genes were checked to screen candidate genes for SUDEP, including genes previously reported to be related to epilepsy, cardiac diseases, respiratory diseases, and SUDEP (ANK2, DEPDC5, KCNE1, KCNE2, KCNH2, KCNQ1, RYR2, SCN1A, SCN1B, SCN2A, and SCN5A) (Mohler et al., 2003; Aurlien et al., 2009; Tu et al., 2011a; Bagnall et al., 2016; Coll et al., 2016). Potential candidate variants were further genotyped in 330 living control with epilepsy alleles (age and gender matched from the original cohort) and 320 ethnicity-match healthy control alleles by restriction digestion. The flowchart is provided in Figure 1.

2.5 Standard protocol approvals, registrations, and patient consents

The original study was approved by the joint Chinese University of Hong Kong-New Territories East Cluster Research Ethics Committee and the institutional review board of the Beijing Neurosurgical Institute in China. The follow-up exercise and genetic analysis were approved by the Medical Ethics Committee of Fudan University affiliated Huashan Hospital, Shanghai, China. Written informed consent had been obtained from all participants of the original study or when applicable assent was obtained from legally acceptable guardians.

3. Results
Baseline characteristics of the five cases are summarized in Table 1. Three of them were male. All of them had convulsive seizures in the year of death. The median age of onset of epilepsy was 14 years. Four of them had had EEG recording and two had had CT/MRI scan.

Detailed death-related information is shown in Table 2. The average age of death was 25.6 years. None was in remission in the year prior to death. Three of the deaths were witnessed and two died after a witnessed seizure.

We obtained more than 86% uniquely mapped reads and more than 99% good matched reads. The average sequencing depth in the exome region was approximately more than 120 X (Table 3); Target SNV distribution and target SNV function are presented in Supplementary File 1. In summary, we obtained high quality WES data.

After the filtering process, five subjects carried 168 qualified mutations in 167 genes. Among these genetic anomalies, only one affected gene, SCN5A, was previously related to SUDEP. We also identified seven variants in genes (CACNA1A, NEB, SCN9A, GUF1, TLR4, TRPM2, and PLA2G6) associated with epilepsy and another three variants in genes (KIF6, TBX18, CYSLTR2) associated with asthma, arrhythmia or heart disease. Detailed information of these candidate genes and their function are summarized in Table 4.

Subject #1 carried qualified variants in CYSLTR2 and KIF6. The rare R64W mutation in KIF6 identified in this subject was absent in 330 living epilepsy controls and 320 ethnicity-match non-epilepsy controls. Several other variations in KIF6 was reported to be related to coronary epicardial endothelial dysfunction in male patients in a case-control study (Yoshino et al., 2016). The T410C variant in CYSLTR2 in this subject was absent in epilepsy controls and only one such variant was found in non-epilepsy control alleles. Rare variant in CYSLTR2 was previously reported in
people with asthma (Fukai et al., 2004). Subject #2 carried rare mutations in SCN9A. The T2132C variant in SCN9A was found in one of 330 epilepsy control alleles and two of 320 non-epilepsy control alleles. Rare SCN9A mutation was previously associated with febrile seizures plus and Dravet syndrome (Mulley et al., 2013). This subject had a febrile seizure when aged 2 and her sister has epilepsy.

Subject #3 died during a seizure at age 28. She carried a rare R1139W mutation in SCN5A change classified as a variant of uncertain significance and probably related to Long QT syndrome 3 or Brugada syndrome in the clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/). Her ECG showed a QT of 0.39s and a QTc of 0.4s. This variant in SCN5A was absent in epilepsy controls and in non-epilepsy controls. She also carried qualified variants in CACNA1A, GUF1.

Subject #4 had mild learning disability and carried variants in TBX18 and NEB. The G821A variant in TBX18 in this subject was absent in 330 living epilepsy controls and 320 ethnicity-match non-epilepsy controls. TBX18 had been associated with generation of cardiac pacemaker cells and sick sinus syndrome (Husse and Franz, 2016; Choudhury et al., 2018). The T13661C variant in NEB was identified in two of the epilepsy control alleles and three of the non-epilepsy control alleles. Mutation in NEB was previously reported in a Korean family with intellectual disability, epilepsy and early-childhood-onset generalized muscle weakness (Jin et al., 2014).

Subject #5 carried qualified variant in PLA2G6. This C2255G variant was identified in three of the epilepsy control alleles and two of non-epilepsy control alleles. Mutation in PLA2G6 was reported in a Chinese pedigree with familial cortical myoclonic tremor with epilepsy (Gao et al., 2016).

4. Discussion
We used WES to identify potential candidate gene in incident SUDEP cases and we found a new, rare, harmful variant in SCN5A, which was absent in epilepsy controls and non-epilepsy controls. Other variants in SCN5A were previously reported in SUDEP cases (Aurlien et al., 2009; Tu et al., 2011a), and potentially predisposing to malignant cardiac arrhythmia (Denham et al., 2018; Huang et al., 2018; Yeates et al., 2018). We also identified two rare qualified variants in KIF6 and TBX16 (previously associated with heart diseases), which was absent in epilepsy controls and in non-epilepsy controls. These findings highlighted the role of potential heart problem in Chinese victims of SUDEP.

Investigating candidate gene in SUDEP may provide elements to a better understanding of the mechanism of SUDEP. To date, several studies attempted to identify candidate genes from postmortem DNA samples. These studies identified several potential candidate variants in genes, coding for proteins with key function on cell excitability, and electrophysiology of the heart. Retrospective studies may have selection bias which may impact external validity. Genetic investigation in SUDEP cases from large cohort studies would help in verifying previous results and identifying new potential candidate variants. The incidence of SUDEP is 1.2/1000person-years (Thurman et al., 2014), implying that only very large DNA sample library can support genetic studies in incident SUDEP cases. Our DNA samples were collected prospectively at baseline in a large community-based cohort in China seven to eight years ago and thus devoid of major selection bias.

In the follow-up of the cohort, we identified 15 sudden death cases that fulfil the criteria for SUDEP (13 probable cases and 2 possible cases). We selected SUDEP cases who died in the age of 15-39, as this is the group at the highest risk. All had convulsive seizures and were not in remission, which are also markers of high risk.
In a UK study, 40% of subjects who died suddenly and might otherwise have had a diagnosis of SUDEP, were shown at postmortem to have probable cardiovascular causes of death (Novy et al., 2013). We did not have postmortem data but young age at death and events surrounding death decreased the possibility of cardiovascular or other underlying causes. With the multidisciplinary panel, paying particular attention to those in whom the reported or certified cause of death was cardiac, cerebral vascular, unknown, or sudden, we were able to identify “high-quality” probable SUDEP cases for WES.

The SCN5A gene encodes the alpha subunit of the main cardiac sodium channel Nav1.5. SCN5A variants have been causatively associated with Brugada syndrome, long QT syndrome, cardiac conduction system dysfunction, dilated cardiomyopathy, etc (Li et al., 2018). Some variants in SCN5A have been related to sudden death (Jia et al., 2018; Tan et al., 2018; Yeates et al., 2018). Ala572Asp, Pro1090Leu, Pro2006Ala variants in SCN5A (Tu et al., 2011a) and a missense mutation R523C (Aurlien et al., 2009) were found in SUDEP cases previously.

We also identified a new rare missense mutation in SCN5A in a young female SUDEP victim. This missense mutation R1193W is rare, and the frequency of this variant was 0.04% in 1000 Genomes Project, and it was absent in epilepsy control alleles from the same original cohort and non-epilepsy control alleles. It was predicted to be pathogenic by the use of the prediction program PolyPhen and SIFT and has not been previously reported. This variant may be related to Brugada syndrome, Long QT syndrome 3 in the clinvar database. The resting ECG record of this individual showed that her QT was 0.39s and QTc was 0.4s which was within the normal range, but it does not exclude potential causality. People with some mutations in SCN5A are at higher risk of sudden death, even those with a normal QT interval (Kapa et al., 2009).
Only few genetic case series studies in SUDEP were reported previously and Table 5 summarized potential rare variants identified. We also identified two new rare qualified variants, which was absent in epilepsy control alleles from the same original cohort and non-epilepsy control alleles, in genes that had not yet been reported in SUDEP cases. We identified rare variants in TBX18 and KIF6 in two of our cases. TBX18 is an attractive target for bio-pace making as it is important in sinoatrial node development and so has the potential to have a broad effect on cardiac tissue phenotype (Wiese et al., 2009). TBX18 could be important in restoring pacemaker function in human sick sinus syndrome (the commonest bradyarrhythmia in humans, and may increase the risk of sudden death) (Choudhury et al., 2018). KIF6 is ubiquitously expressed in coronary arteries and other vascular tissue and previously related with coronary heart disease. These variants in SCN5A, TBX18 and KIF6 indicated heart disorder may play a key role in the mechanism of some SUDEP cases.

We also identified rare qualified variants in CACNA1A, NEB, SCN9A, GUF1, TLR4, TRPM2, and PLA2G6 which were previously reported in people with epilepsy. CACNA1A is calcium voltage-gated channel gene mainly expressed in the brain and SCN9A was sodium voltage-gated channel which had a wide expression. We identified a variant in CYSLTR2 which was previously related to asthma. One report identified asthma as a risk factor of SUDEP (Hesdorffer and Tomson, 2013). These findings give new insights into the mechanism and etiology of SUDEP among Chinese people.

Our study has limitations. Firstly, postmortem examinations are rare in China. We used several methods to improve the diagnosis of SUDEP without postmortem examinations as previously described (Ge et al., 2017), ascertainment bias may still existed. We selected five SUDEP cases who were at a high of SUDEP for WES to
ensure a better quality. Probable SUDEP cases or other no-definite SUDEP cases were also included for testing and analysis in previous reports (Tu et al., 2011a; Labate et al., 2013; Bagnall et al., 2016; Coll et al., 2016; Coll et al., 2017; Hata et al., 2017; Friedman et al., 2018). Secondly, because we tried to screen candidate variants in gene whose function was previously associated with SUDEP, epilepsy, cardiac diseases, and respiratory diseases which was thought as the main mechanism of SUDEP (Devinsky et al., 2016), we may ignore some variants whose function was unknown or could induce sudden death via mechanisms other than heart or respiratory disorder. Thirdly, the function and pathogenicity of the candidate variants we identified in this study were still unknown, and we could only predicate the function of the variants from previous publications, SIFT and PolyPhen scores and the clinvar database. Further studies were needed to work out the effect of these variants on the function of genes. Lastly, our sample size was very small; however, the number of incident SUDEP cases are unlikely to be large in a community-based study.

5. Conclusion

SUDEP is the most tragic outcome of epilepsy. We investigated the genetic background in SUDEP cases in a Chinese cohort. We identified a rare variant in SCN5A which may have a role in the occurrence of SUDEP. We had also identified several new potential candidate genes for SUDEP. Our result could help to form a better understanding of genetic deficit and how they contribute to SUDEP, especially in Chinese population where the large data gap exists.

Disclosures

JWS has received research funding from Eisai, GSK and UCB, personal fees from
Eisai, UCB, outside the submitted work. All other authors have no disclosures to report.

Acknowledgements

This study was funded by Key Research Project of the Chinese Ministry of Science and Technology (Grant No. 2016YFC0904400), a NIH/NINDS grant (1R21NS069223-01), and National Natural Science Foundation of China (81271443). JWS is based at UCLH/UCL Biomedical Research Centre, which receives a proportion of funding from the UK Department of Health's NIHR Research Centers funding scheme. He receives support from the Dr. Marvin Weil Epilepsy Research Fund and UK Epilepsy Society. PK is supported by a Medical Research Future Fund Practitioner Fellowship.

The authors are grateful to Prof Sanjay Sisodiya for critically reviewing the manuscript.

References


Figure 1: Flowchart of the study
Table 1 Baseline characteristic of included SUDEP cases

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Seizure type</th>
<th>Age at onset (years)</th>
<th>Family history of epilepsy</th>
<th>History of febrile seizure</th>
<th>Baseline EEG</th>
<th>Baseline brain CT/MRI</th>
<th>Antiepileptic drugs</th>
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<td>1</td>
<td>Male</td>
<td>generalized</td>
<td>21</td>
<td>no</td>
<td>no</td>
<td>moderate abnormal</td>
<td>normal</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>generalized</td>
<td>14</td>
<td>sister</td>
<td>yes</td>
<td>epileptiform discharge</td>
<td>cerebral dysplasia</td>
<td>VPA &amp; PB</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>focal with secondary generalized</td>
<td>3</td>
<td>no</td>
<td>no</td>
<td>epileptiform discharge</td>
<td>NA</td>
<td>CBZ</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>focal with secondary generalized</td>
<td>4</td>
<td>no</td>
<td>no</td>
<td>normal</td>
<td>NA</td>
<td>PB</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>generalized</td>
<td>15</td>
<td>mother</td>
<td>yes</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: VPA: valproic acid; PB: phenobarbital; CBZ: carbamazepine.
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<tr>
<th>Pr. NO.</th>
<th>Baseline date</th>
<th>Date of death</th>
<th>Age at date of death (years)</th>
<th>Place of death</th>
<th>Position of death</th>
<th>Seizure before death</th>
<th>Time of Death or Found dead</th>
<th>Witnessed</th>
<th>Number of seizures within 1 year before death</th>
<th>Death description</th>
</tr>
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<td>2011/06/01</td>
<td>2014/03/20</td>
<td>35</td>
<td>bedroom</td>
<td>supine</td>
<td>unknown</td>
<td>10 a.m.</td>
<td>no</td>
<td>4</td>
<td>Living with his family, found dead at 10 a.m. sudden loss of consciousness at 11 a.m., death confirmed at doctor's arrival</td>
</tr>
<tr>
<td>2</td>
<td>2011/02/21</td>
<td>2013/03/27</td>
<td>22</td>
<td>living room</td>
<td>latericumbent</td>
<td>no</td>
<td>11 a.m.</td>
<td>yes</td>
<td>10</td>
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<td>2014/10/05</td>
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<td>bedroom</td>
<td>prone</td>
<td>confirmed</td>
<td>NA</td>
<td>yes</td>
<td>11</td>
<td>Sudden death during a seizure</td>
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<td>2013/03/10</td>
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<td>bedroom</td>
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<td>confirmed</td>
<td>1 a.m.</td>
<td>yes</td>
<td>NA*</td>
<td>Seizure during sleep, found dead at 1 a.m.</td>
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<tr>
<td>5</td>
<td>2011/05/21</td>
<td>2012/11/20</td>
<td>20</td>
<td>bedroom</td>
<td>latericumbent</td>
<td>suspected</td>
<td>10 a.m.</td>
<td>no</td>
<td>24</td>
<td>Found dead at 10 a.m. with foaming in the mouth</td>
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* seizure frequency 10/month at baseline.
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<th>Map Ratio(%)</th>
<th>Unique Mapped Reads</th>
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Note: Chr: Chromosome ID; Pos: mutation position in each chromosome; Ref: reference base; Alt: altered base; Alts: altered bases, if there is more than one altered base; P1–P5: patient 1–patient 5; 1000 G: allele frequencies in 1000 genomes project database.
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Method</th>
<th>Candidate genes</th>
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<tbody>
<tr>
<td>Tu et al., 2011a</td>
<td>22 definite SUDEP cases &amp; 46 possible SUDEP cases</td>
<td>analyze post-mortem DNA samples in variants in KCNQ1, KCNH2 (HERG) and SCN5A</td>
<td>KCNH2, SCN5A</td>
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<tr>
<td>Tu et al., 2011b</td>
<td>48 cases</td>
<td>analyze post-mortem DNA samples in variants in HCN1-4</td>
<td>HCN1-4</td>
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<tr>
<td>Leu et al. 2015</td>
<td>8 definite SUDEP cases and 10 probable SUDEP cases</td>
<td>analyse variants from WES data of 18 people who died of SUDEP, 87 living people with epilepsy and 1479 non-epilepsy disease controls</td>
<td>SCN1A, LGH1, SMC4, COL6A3 and TIE1</td>
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<tr>
<td>Coll et al., 2016</td>
<td>14 SUDEP cases</td>
<td>panel target resequencing</td>
<td>SCN1A, FBN1, HCN1, SCN4A, KCNQ1, and EFHC1</td>
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<tr>
<td>Bagnall et al., 2016</td>
<td>54 definite SUDEP cases, 5 probable SUDEP cases and 2 definite SUDEP plus cases</td>
<td>exome sequencing and rare variant collapsing analysis</td>
<td>KCNH2, KCNQ1, KCNQ2, SCN5A, RYR2, HCN4, DEPDC5, GABRB3, SCN1A, SCN2A, CHRNA4, SPTAN1, and PAFAH1B1.</td>
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<tr>
<td>Study</td>
<td>Number of SUDEP Cases</td>
<td>Methodology Description</td>
<td>Genes Identified</td>
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<tr>
<td>Hata et al., 2017</td>
<td>9 SUDEP cases</td>
<td>Use next generation sequencing (NGS) to examine 73 inherited heart disease-related genes</td>
<td>LDB3, DSC2, KCNE1, MYH6, DSP and DSG2</td>
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<tr>
<td>Friedman et al., 2018</td>
<td>8 definite/probable SUDEP cases</td>
<td>Compare WES data of 8 SUDEP cases with 7 non-SUDEP controls</td>
<td>ARRB2, ITPR1, GABRR2, SSTR5, GRIK1, CTNAP2, GRM8, GNAI2, GRIK5, KCNMB1, KCNIP1, DPP6, JUP, F2, and TUBA3D</td>
</tr>
</tbody>
</table>
Supplementary File 1 Variant location and exonic function in each sample

Note: #1-5 was patient #1-#5

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