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

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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 regarded as qualified variants. The genes which those qualified variants were in were regarded as qualified genes. Function of each qualified gene was 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; Aurlen *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

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Commented [I1]: What about ANK2 ? this is also a potential SUDEP gene (31. Mohler PJ, Schott J-J, Gramolini AO, et al. Ankyrin-B mutation causes type 4 longQT cardiac arrhythmia and sudden cardiac death. Nature 2003;421:634-639). But probably not so important!

Commented [AS2R1]: ANK2 has been added

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 (Aurlieu *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 (Aurlen *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 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

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Figure 1 Flowchart of the study

Table 1 Baseline characteristic of included SUDEP cases

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

Note: VPA: valproic acid; PB: phenobarbital; CBZ: carbamazepine.

Table 2 Detailed death related information of included SUDEP cases

Pt. NO.	Baseline date	Date of death	Age at death (years)	Place of death	Position of death	Seizure before death	Time of Death or Found Death	Witnessed	Number of seizures within 1 year before death	Death description
1	2011/06/01	2014/03/20	35	bedroom	supine	unknown	10 a.m.	no	4	Living with his family, found dead at 10 a.m.
2	2011/02/21	2013/03/27	22	living room	latericumbent	no	11 a.m.	yes	10	Sudden loss of consciousness at 11 a.m., death confirmed at doctor's arrival
3	2011/06/28	2014/10/05	28	bedroom	prone	confirmed	NA	yes	11	Sudden death during a seizure
4	2011/04/19	2013/03/10	23	bedroom	NA	confirmed	1 a.m.	yes	NA*	Seizure during sleep, found dead at 1 a.m.
5	2011/05/21	2012/11/20	20	bedroom	latericumbent	suspected	10 a.m.	no	24	Found dead at 10 a.m. with foaming in the mouth

* seizure frequency 10/month at baseline.

Table 3 Summary of original exome sequencing data

Pt. NO.	Good Reads	Mapped Reads	Map Ratio(%)	Unique Mapped Reads	Unique Mapped Ratio(%)	Coverage (%)	Q30(%)	Mean Depth
1	62,524,884	62,271,555	99.59	55,131,488	88.18	99.88	91.72	252.6403191
2	72,158,058	71,982,191	99.76	62,556,081	86.69	99.47	92.88	252.6403191
3	79,513,206	79,304,408	99.74	67,761,286	85.22	99.51	92.48	252.6403191
4	75,268,080	74,988,853	99.63	65,103,593	86.5	99.77	92.14	252.6403191
5	80,732,872	80,445,744	99.64	68,867,451	85.3	99.8	92.13	252.6403191

Table 4 Potential candidate SNVs found in the study

gene	Chr	Pos	Ref	Alts	Alt	P1	P2	P3	P4	P5	Allele frequency in controls(in 330 living epilepsy controls / 320 ethnicity-match non-epilepsy controls)	1000G(%)	rs number	SIFT score	Polyphen2 HDIV score	Gene classification
<i>SCN5A</i>	chr3	38616877	G	A	A	0/0	0/0	0/1	0/0	0/0	0/0	0.04	rs192379242	0	1	SUDEP related
<i>CACNA1A</i>	chr19	13319766	C	T	T	0/0	0/0	0/1	0/0	0/0	6/2	0.04	rs373192655	0.03	0.997	Epilepsy related
<i>NEB</i>	chr2	152419216	A	G	G	0/0	0/0	0/0	0/1	0/0	2/3	1	rs184723737	0.04	0.998	Epilepsy related
<i>SCN9A</i>	chr2	167137045	A	G	G	0/0	0/1	0/0	0/0	0/0	1/2	0.08	rs187526567	0	1	Epilepsy related
<i>GUF1</i>	chr4	44691907	T	A	A	0/0	0/0	0/1	0/0	0/0	4/1	0.04	rs115306084	0	1	Epilepsy related
<i>TLR4</i>	chr9	120476846	G	A	A	0/0	0/1	0/0	0/0	0/0	0/3	0.04	rs55751501	0	1	Epilepsy related
<i>TRPM2</i>	chr21	45825855	C	T	T	0/0	0/0	0/0	0/0	0/1	0/1	0.24	rs77759997	0	1	Epilepsy related
<i>PLA2G6</i>	chr22	38508172	G	C	C	0/0	0/0	0/0	0/0	0/1	3/2	0.3	rs140758033	0	1	Epilepsy related
<i>TBX18</i>	chr6	85457756	C	T	T	0/0	0/0	0/0	0/1	0/0	0/0	0.02	rs200317774	0.05	1	Heart disease related
<i>KIF6</i>	chr6	39353422	G	A	A	0/1	0/0	0/0	0/0	0/0	0/0	0.04	rs139112928	0	1	Heart disease related
<i>CYSLTR2</i>	chr13	49281363	T	C	C	0/1	0/0	0/0	0/0	0/0	0/1	0.04	rs200773422	0.01	0.999	Asthma related

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 5 Literature summarize of the candidate genes identification from previous studies

Study	Sample size	Method	Candidate genes
Tu et al., 2011a	22 definite SUDEP cases & 46 possible SUDEP cases	analyze post-mortem DNA samples in variants in KCNQ1, KCNH2 (HERG) and <i>SCN5A</i>	<i>KCNH2, SCN5A</i>
Tu et al., 2011b	48 cases	analyze post-mortem DNA samples in variants in HCN1-4	<i>HCN1-4</i>
Leu et al. 2015	8 definite SUDEP cases and 10 probable SUDEP cases	analyse variants from WES data of 18 people who died of SUDEP, 87 living people with epilepsy and 1479 non-epilepsy disease controls	<i>SCN1A, LGI1, SMC4, COL6A3 and TIE1</i>
Coll et al., 2016	14 SUDEP cases	panel target resequencing	<i>SCN1A, FBN1, HCN1, SCN4A, KCNQ1, and EFHC1</i>
Bagnall et al., 2016	54 definite SUDEP cases, 5 probable SUDEP cases and 2 definite SUDEP plus cases	exome sequencing and rare variant collapsing analysis	<i>KCNH2, KCNQ1, KCNQ2, SCN5A, RYR2, HCN4, DEPDC5, GABRB3, SCN1A, SCN2A, CHRNA4, SPTAN1, and PAFAH1B1.</i>

Hata et al., 2017	9 SUDEP cases	use next generation sequencing (NGS) to examine 73 inherited heart disease-related genes	<i>LDB3, DSC2, KCNE1, MYH6, DSP and DSG2</i>
Friedman et al., 2018	8 definite/probable SUDEP cases	compare WES data of 8 SUDEP cases with 7 non-SUDEP controls	<i>ARRB2, ITPR1, GABRR2, SSTR5, GRIK1, CTNAP2, GRM8, GNAI2, GRIK5, KCNMB1, KCNIP1, DPP6, JUP, F2, and TUBA3D</i>

Supplementary File 1 Variant location and exonic function in each sample

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

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.