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Original article

SCN5A gene exome sequencing profile in sudden unexplained nocturnal death syndrome in Thai population

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Background: Sudden unexplained nocturnal death syndrome (SUNDS) or Lai-tai is a sudden death that often occurs in Southeast Asia. The characteristics of SUNDS cases are healthy young males, 20 – 49 yrs., that suddenly die during sleep with no relevant medical history. Previous studies found that SUNDS is similar to Brugada syndrome (BrS) which correlates to SCN5A mutations. Moreover, SCN5A variations affect sodium ion channel functions are different from one populations to anothers.
Objective: The aim of this study is to describe the phenotype of SUNDS cases in the Thai population and establish SCN5A gene exome sequencing profiles using Next-Generation sequencing.
Methods: All characteristics data of 12 SUNDS cases in the Thai population were collected. Microscopic examinations were analyzed. Postmortem genetic testing of SCN5A gene exome sequencing profiles in 12 SUNDS cases using Next-Generation Sequencing were performed. Pathogenicities were predicted using Polyphen-2 for missense variants and Mutationtaster for frameshift mutations. As for combined pathogenicity results, Combined Annotation Dependent Depletion (CADD) scores < 20 were filtered out. After that, SCN5A variants were compared with ExAC, 1000G, ClinVar and previous reports. Descriptive analysis in SUNDS cases characteristics were analyzed using Microsoft Excel 2013.
Results: Characteristics of SUNDS cases with microscopic examination in the Thai population were shown. SCN5A gene exome sequencing profile in 12 SUNDS cases in the Thai population were generated. Two frameshift and six missense variants on the SCN5A gene from four SUNDS cases (33.34%) were found that potentially significantly affect sodium channel function. Only G599R has been previously reported. Other variants are novel (T92N, E171G, A178T, L1646fs, N1659S, E1804fs and E2013K).
Conclusion: Phenotype and SCN5A gene exome sequencing profiles for SUNDS cases in the Thai population were shown. Our results could be useful for forensic pathologists and medical practitioners to keep these variants as a possible risk variants and closely observed in every SUNDS in the Thai population.

Keywords: Sudden unexplained nocturnal death syndrome (SUNDS), SCN5A gene, Lai-tai, Channelopathies, Next-Generation sequencing (NGS).

Sudden unexplained nocturnal death syndrome (SUNDS) is defined as death in young adult men that suddenly occurred during sleep. This has been described in many nomenclatures in different countries; for example, *Bangungut* in the Philippines, *Pokkuri* in Japan and *Lai-tai* in Thailand.[1] SUNDS is often found in North-eastern Thailand. In 1992 - 93, the incident rate of SUNDS ranges for 25.9 to 38 in 10,000 individuals.[2, 3] The common characteristics are healthy young males, suddenly dieing during sleep without any relevant medical history and family history of sudden death.

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Initial studies in the Thai population show that the causes of SUNDS are environment factors including stress[4] and hypokalemia.[5] In 2001, prior genetic studies in Thailand conducted, linkage analysis of *SCN5A* exon 5, 12, 17, 18, 23 and 28 in survivors from SUNDS and families, which should indicate as Brugada syndrome (BrS) patient rather than deceases from SUNDS. The polymorphisms were reported for the first genetic study in Thai patients.[6] After 2002, the finding showed that SUNDS had epidemiological and clinical characteristics similar to BrS, related to *SCN5A* gene mutations.[7] The most common BrS genotype was found in 20 - 25 percent of BrS patients.[8] Recently, many studies have focused on this gene and its phenotype characteristics. One of the most common characteristics is presenting with cardiac channelopathy, a disorders of ion channels such as sodium and potassium ion channel mutations.[7, 9]

The *SCN5A* gene, the voltage-gated cardiac sodium channel type 5 alpha subunit, is an interesting targeted gene for molecular testing to diagnose SUNDS cases. In previous studies that focused on the *SCN5A* gene sequencing analysis, several *SCN5A* variations that differed between populations were found.[7, 10, 11]

Nowadays, Next-Generation Sequencing (NGS) plays an important role in molecular testing. Multiple samples and target genes can be performed in a single run with more sensitivity than Sanger sequencing. This technique suits forensic samples which have degraded and have a small amount of DNA.[12] Several researches shifted to use this technology to perform postmortem genetic testing of many genes in SUNDS cases and some novel variants were found.[13, 14] However, *SCN5A* variants in SUNDS cases in the Thai populations has been limited. The aim of this study is to describe phenotypes of SUNDS in the Thai population and analyze *SCN5A* gene exome sequencing profiles using NGS.

Materials and Methods

Sample collections

Between June 2013 and July 2017, FTA bloods of 12 SUNDS cases from Forensic Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, were collected and stored at room temperature. The inclusion criteria were as follows: 1. Age 20 – 49 years 2. Thai nationality: and, 3. Autopsy negative and toxicology negative. We excluded death from other natural deaths (indicated cause of death) and decomposition (a postmortem period more than 24 hours). The study has been approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. (IRB no. 447/59)

DNA Extraction and Quantification

Ten punches of FTA blood 1.2 mm^2 were purified following a manufacturer's protocol. DNA was extracted by DNA IQ™ System-Database (Promega) and quantified using Quantifiler™ Human DNA Quantification Kit (Thermofisher) on Applied Biosystems® 7500 Real-Time PCR system following the manufacturer's protocols.

Design oligo probe for target gene

This study targeted all exon and 5' untranslated regions of SCN5A. Upstream and downstream oligo probes were designed by DesignStudio (Illumina) with a target amplicon size 175 bp, single-end sequencing.

Library preparation

DNA libraries were prepared by Truseq Custom Amplicon Low Input Library Prep Kit (Illumina). Library preparations were performed following the manufacturer's protocols with a bead-based normalization method using 1% spiked-in PhiX control v3 (Illumina). Cleanup PCR products were qualified by 4% agarose gel electrophoresis.

Next-Generation Sequencing

Pool DNA libraries were sequenced by MiseqFGx (Illumina) using a MiSeq Reagent Micro Kit v2 (300-cycle) with a 2×150 bps read length.

Data analysis

All data analyses were processed in Galaxy (https://usegalaxy.org/).[15] Data quality was evaluated using FASTQC. Then, sequences were trimmed using Trimmomatic (per base quality <20). The sequences were aligned to the GRCh37/hg19 reference genome (hg19_g1k_v37) using BWA-MEM. BAM files were merged and marked a duplication using the Picard tool. Finally, DNA variants were called using Freebayes and annotated with SnpEff.

Variant filtering and pathogenic prediction

VCF files for *SCN5A* gene variations in SUNDS cases were visualized in GenomeBrowse (golden helix).[16] Variants with read depth ≥ 100 were

predicted pathogenic by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) for missense variants[17] and Mutationtaster (http://www.mutationtaster.org/index.html) for frameshift mutations.[18] Combined Annotation Dependent Depletion (CADD) scores (http://cadd.gs.washington.edu/) were used to exclude variants with scaled C-score< 20.[19] Each variant was compared with the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)[20], the 1000 Genomes Project Consortium (1000G) (http://www.internationalgenome.org/)[21] database, Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/)[22] and published reports for disease association. Descriptive analysis in SUNDS cases characteristics were analyzed using Microsoft Excel 2013.

Results

Characteristics of SUNDS cases

The characteristics for 12 SUNDS cases are shown in Table 1. All cases were male with an average age of 38.50 years old. The situation in which death occurred mainly during sleep (75%, 9 cases). One case history of sudden death in his family. Nine out of 12 cases were from the northeastern region of Thailand. Others were from the central and the northern regions. Average bodys' height and weight were 170.92 ± 3.06 cm and 62.83 ± 5.64 kg, respectively. All body mass indexes (BMI) were within the normal range (18.5 - 24.9 kg/mm^2)[23] with an average of 21.51 ± 1.63 kg/mm^2. The average heart weight was 377.50 g. Five cases are in the normal heart weight range (270 - 360 g).

Macroscopic and microscopic examination

Heart tissue samples from all cases were examined. For macroscopic examination of the hearts, no gross significance was observed in 5 cases (41.67%). Subepicardial hemorrhage were described in four cases (33.33%), and discoloration of the myocardium in one case (8.33%) (Table 1). The microscopic findings (in case ID 8 and 9) were described an interstitial fibrosis of myocardial tissue found in the left ventricle free wall as well as the septal wall (Figure 1).

Run quality and data quality

In this study, the total read was 1.82 Gbps, with 0.05 - 13.30 Gbps read per sample. Cluster Density was 1,318 ± 34 K/mm^2 with a passing filter 83.49 ± 1.24 percent resulting in over-cluster when compared with the optimal value. Reads that passed Qscore >30 (only 1 read) were 80.95 percent of the total read. Data quality for each sample was evaluated by FASTQC from Galaxy. Per base quality is 90.46 percent.

SCN5A gene variations in SUNDS cases

SCN5A gene variations were categorized in 6 types as follows: 3 prime UTR, frameshift, missense, regulatory region, splice region, structural interaction and synonymous variant. In total, 37 *SCN5A* variants were found with two frameshift mutations (5.41%) and nine missense variants (24.32%). The summary of *SCN5A* variants in SUNDS cases are shown as a pie chart in Figure 2.

Table 1. Characteristic of SUNDS cases.

ID	Gender	Age at death (Years)	Situation at death	Family history	Domicile	Height (cm)	Weight (kg)	BMI	Heart weight (g)	Macroscopic examination
1	M	41	Sleep	N	NE	171	72	24.62	410	Hemorrhage
2	M	49	NA	N	NE	181	76	23.20	390	Normal
3	M	34	Sleep	N	C	163	55	20.70	300	Normal
4	M	35	NA	N	C	170	68	23.53	425	Hemorrhage
5	M	30	Sleep	N	NE	170	62	21.45	360	Hemorrhage
6	M	49	Sleep	N	N	174	60	19.82	390	Hemorrhage
7	M	31	Sleep	N	NE	176	50	16.14	395	Normal
8	M	45	Sleep	Y	NE	166	60	21.77	450	Fibrosis
9	M	37	Sleep	N	NE	170	68	23.53	345	Fibrosis
10	M	36	NA	N	NE	170	59	20.42	315	Normal
11	M	36	Sleep	N	NE	170	60	20.76	425	Discoloration
12	M	39	Sleep	N	NE	170	64	22.15	325	Normal

M: male; NE: Northeast, C: Bangkok and central, N: North; BMI: Body mass index (normal range) = 18.5-24.9 kg/mm^2; Normal heart weight (g): male 270-360 g. NA: data not available

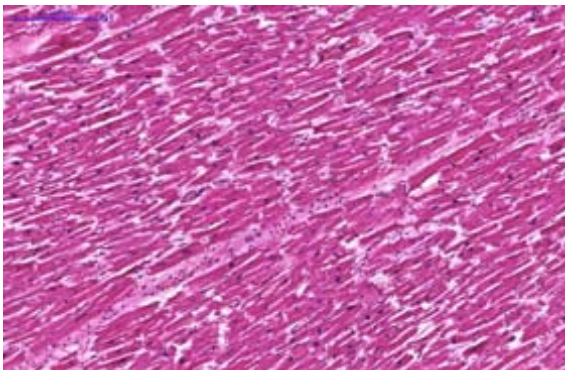

Figure 1. The microscopic finding (×50) of myocardial tissue from left ventricular free wall. There was an interstitial fibrosis observed in two cases (16.67%).

Table 2. Run quality results.

	Preliminary Optimal	**Result**
Yield (Gbp)	1.2	1.82
Cluster density (k/mm^2)	1,000 – 1,200	1,318 ± 34
Cluster PF (%)	80	83.49 ± 1.24
Qscore (read 1)	> q30	80.95 %

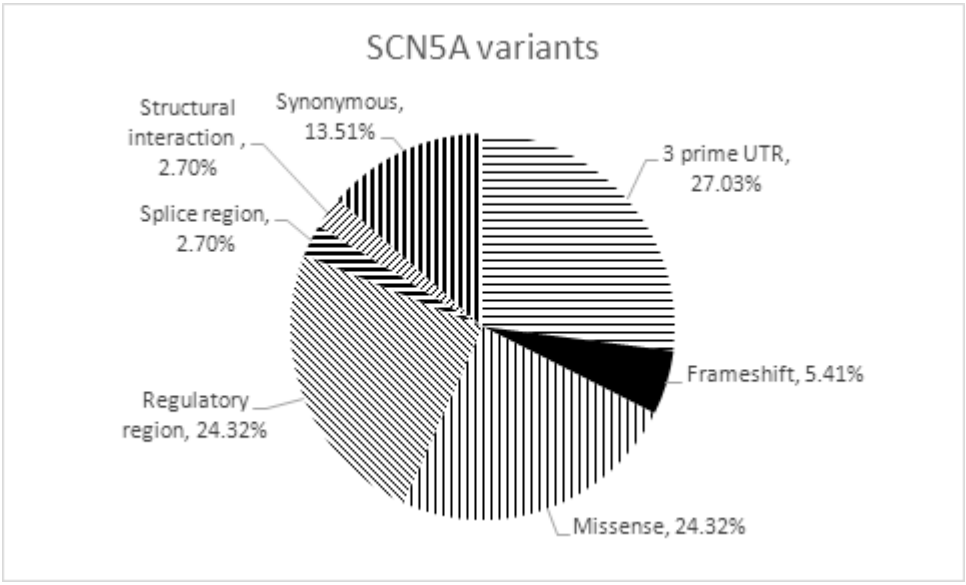

Figure 2. Summary of *SCN5A* gene variations in SUNDS cases.

Two frameshift and nine missense variants from *SCN5A* gene exome sequencing data that were compared with ExAC, 1000G, Clinvar and a previous report, are described in Table 3. Two frameshifts in exon 28 of *SCN5A* gene are novel variants (L1646fs and E1804fs). As for missense variants, G599R was identified as a single variants that matched from ExAC without pathogenic report in ClinVar. The other six *SCN5A* missense variants are novel (T92N, E171G, A178T, Q998K, N1659S and E2013K). However, Q998K may not be significant since it was predicted as benign from polyphen-2 and CADD scores of less than 20. Common variants, H558R and R1193Q, were observed in the Thai population[24], which is similar to a previous report in East Asians. The present study found H558R in three cases (minor allele frequency = 0.13), while only one case has R1193Q (minor allele frequency = 0.04).

Discussion

In this study, the whole exons of *the SCN5A* gene in SUNDS cases were studied. From 12 SUNDS cases, potentially significant *SCN5A* variants were identified in 4 cases (33.34%). The yield of *SCN5A* gene variations in our study was higher than those in earlier studies in China which reported as 6.5 - 8%.[1, 11] The increase incident of our study would be from our strict inclusion criteria that finally filtered only a death with normal structural heart or negative autopsy results. The characteristics of SUNDS were observed for each case. All cases were young male victims. Rare female victims were reported; for example, the studies of autopsy finding in SUNDS cases from the Philippines found that SUNDS cases occurred in males 91.7% and females 8.3%.[25] The sudden death history of victims' families were closely observed to determine an inherit pattern of this disease. In genetic analysis of SUNDS cases from Germany, they performed genetic screening in first-degree relatives and found that mutations were inherited in an autosomal-dominant trait of the *SCN5A, KCNJ2* and *RyR2* genes.[10] BMI of all cases are within the normal range whereas heart weights were slightly increased (58.33%). It was previously suggested that SUNDS victims had a transitory stage of cardiomyopathy.[1]

We, hereby, reported *SCN5A* variants in SUNDS cases. Two novel frameshift mutations, L1646fs and E1804fs, were found in two cases (16.67%). These variants might affect protein features and cause truncation of SCN5A protein. For missense variants, there were 3 variants that were previously reported in the dbSNP and ExAC database. G599R was presented without supporting data for pathogenicity. The other two variants, H558R and R1193Q, were commonly found in East Asian populations. H558R was found to affect sodium ion channel function when present with other variants such as Q1077del, T512I and M1766L.[26-28] R1193Q has been reported as pathogenic since 2002 through an electrophysiological study.[7] There were studies on its effect by an electrophysiological study in many researches and revealed that it caused a persistent sodium current resulting in prolong QTc, as in LQTs and BrS.[29] However, several studies after that have declared that R1193Q is only a polymorphism as it is commonly found in East-Asian populations. To increase the accurate incident and gene frequency, a large sample size of SUNDs is needed. Extension to other channelopathy genes should be considered to observe the association between genes and phenotype as well.

Conclusion

In this study, *SCN5A* gene exome sequencing profiles in sudden unexplained nocturnal death syndrome in the Thai population are presented. Two frameshift and five *SCN5A* missense variants from four SUNDS cases were found which potentially significantly affected SUNDS cases in the Thai population. Further studies should run electrophysiological analyses to confirm their effects on the function of sodium channels in SUNDS cases. Our results could be useful for forensic pathologists and medical practitioners to keep these variants as a possible risk factors closely observed in every SUNDS in the Thai population.

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Conflict of interest

None of the authors has any potential conflict of interest to disclose.

Table 3. Missense variants from SCN5A gene in SUNDS cases.
Mutation CDS and Amino acid were called from ENST00000333535 using SnpEff. Protein region based on Uniprot database.
MAF: minor allele frequency, NA: data not available

ID	Exon	Type of variant	Mutation CDS	Mutation AA	Protein region	dbSNP	MAF	ExAC (All/EAS)	Polyphen-2/ Mutationtaster	CADD	Clinvar	Thais	Other population
5	5	Missense	c.512A>G	E171G	DIS2	Novel	0.04	NA	Probably damaging	29.5	NA	NA	NA
5	12	Missense	c.1795G>A	G599R	DI-DII	rs779691420	0.05	$8.79972E^{-06}$	Probably damaging	31	NA	NA	NA
5	28	Missense	c.4976A>G	N1659S	DIVS5	Novel	0.08	NA	Probably damaging	24.1	NA	NA	NA
6,7,9	12	Missense	c.1673A>G	H558R	DI-DII	rs1805124	0.13	0.10	Benign	0.01	Benign	Suktitipat *et al.*, 2017	Kauferstein *et al.*, 2013 Liu *et al.*, 2014
6	28	Frameshift	c.5409delA	E1804fs	C-terminus	Novel	0.01	NA	Disease causing	23.4	NA	NA	NA
8	20	Missense	c.3578G>A	R1193Q	DII-DIII	rs41261344	0.04	0.07	Benign	22.8	Conflicting interpretations of pathogenicity, risk factor	Suktitipat *et al.*, 2017	Vatta *et al.*, 2002 Liu *et al.*, 2014
9	3	Missense	c.275C>A	T92N	N-terminus	Novel	0.04	NA	Probably damaging	27	NA	NA	NA
9	28	Frameshift	c.4935delG	L1646fs	DIVS4-DIVS5	Novel	0.01	NA	Disease causing	22.8	NA	NA	NA
9	28	Missense	c.6037G>A	E2013K	C-terminus	Novel	0.01	NA	Probably damaging	27.4	NA	NA	NA
12	5	Missense	c.532G>A	A178T	DIS2	Novel	0.04	NA	Probably damaging	34	NA	NA	NA
12	17	Missense	c.2992C>A	Q998K	DII-DIII	Novel	0.05	NA	Benign	0.002	NA	NA	NA

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