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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 sudden 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 Thai population and establish SCN5A gene exome sequencing profile using Next-Generation sequencing.
Methods: All characteristics data of 12 SUNDS cases in Thai population were collected. Microscopic examinations were analyzed. Postmortem genetic testing of SCN5A gene exome sequencing profile in 12 SUNDS cases using Next-Generation Sequencing were performed. Pathogenicity were predicted using Polyphen-2 for missense variants and Mutationtaster for frameshift mutations. As for combined pathogenicity results, Combined Annotation Dependent Depletion (CADD) score < 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 Thai population were shown. SCN5A gene exome sequencing profile in 12 SUNDS cases in Thai population were generated. Two frameshift and six missense variants on 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 profile for SUNDS cases in Thai population were shown. Our results could be useful for forensic pathologists and medical practitioners to keep these variants as a possible risk variants with closely observed in every SUNDS in 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 was described in many nomenclatures upon countries; for example, *Bangungut* in the Philippines, *Pokkuri* in Japan and *Lai-tai* in Thailand.[1] SUNDS 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 die during sleep without any relevant medical history and family history of sudden death.

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Initial studies in Thai population show that the cause 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 a 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 were focused on this gene and its phenotype characteristics. One of the most common characteristics is presented with cardiac channelopathy, a disorders of ion channels,such as sodium and potassium ion channel mutations.[7, 9]

SCN5A gene, the voltage-gated cardiac sodium channel type 5 alpha subunit, is an interesting targeted gene for molecular testing to diagnostic SUNDS case. In previous studies that focused on *SCN5A* gene sequencing analysis, found several *SCN5A* variations that differed between populations.[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 to forensic samples which are 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 case and some novel variants were found.[13, 14] However, *SCN5A* variants in SUNDS case in Thai populations has been limited. The aim of this study is to describe phenotype of SUNDS on Thai population and analyze *SCN5A* gene exome sequencing profile using NGS.

Materials and Methods

Sample collections

Between June 2013 and July 2017, FTA bloods of 12 SUNDS cases form Forensic medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, were collected and storaged at room temperature. The inclusion criteria are as follows: 1. Age 20 – 49 years 2. Thai nationality: and, 3. Autopsy negative and toxicology negative. We excluded death from other natural deaths (can 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 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 on all exon and 5' untranslated regions of SCN5A. Upstream and downstream oligo probes were designed by DesignStudio (Illumina) with 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 bead-based normalization method using 1% spiked-in PhiX control v3 (Illumina). Cleanup PCR products were qualification on 4% Agarose gel electrophoresis.

Next-Generation Sequencing

Pool DNA libraries were sequenced by MiseqFGx (Illumina) using 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 GRCh37/hg19 reference genome (hg19_g1k_v37) using BWA-MEM. BAM files were merged and marked a duplication using Picard tool. Finally, DNA variants were called using Freebayes and annotated with SnpEff.

Variant filtering and pathogenic prediction

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

predicted pathogenicity 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 were 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 average age 38.50 years old. The situation which found death was occurred during sleep (75%, 9 cases). A case had one history of sudden death in his family members. Nine out of 12 cases were from the northeastern region of Thailand. Others were from the central and the northern regions. Average body's height and weight were 170.92 ± 3.06 cm and 62.83 ± 5.64 kg, respectively. All body mass index (BMI) were within normal range (18.5 - 24.9 kg/mm^2)[23] with average 21.51 ± 1.63 kg/mm^2. Average heart weight was 377.50 g. Five cases are in 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 myocardium in one case (8.33%) (Table 1). The microscopic finding (in case ID 8 and 9) was 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 passing filter 83.49 ± 1.24 percent. Resulting in over-cluster when compare with optimal value. Reads that passing Qscore >30 (read 1 only) were 80.95 percent of 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 were 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

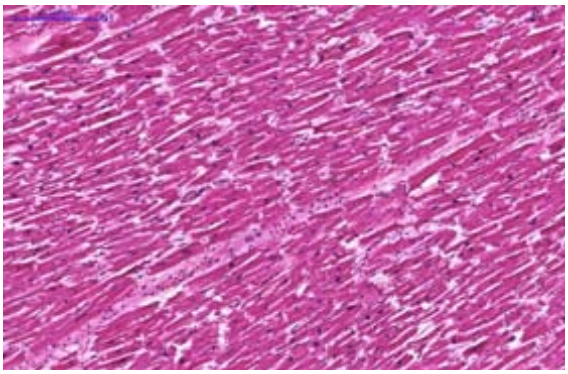

Figure 1. The microscopic finding (×50) of myocardial tissue from left ventricular free wall, there was an interstitial fibrosis observed in two cases (16.67%).

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 %

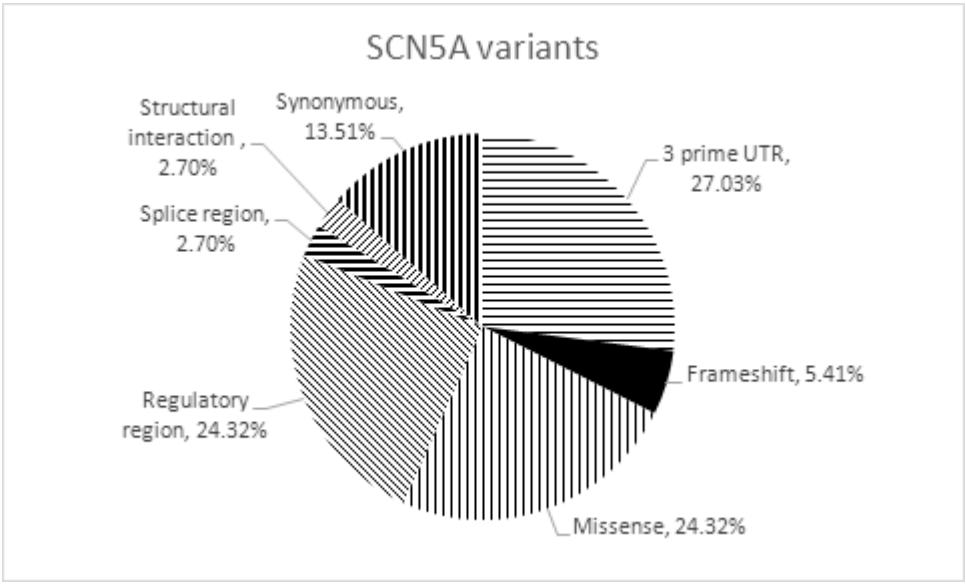

Figure 2. Summary of *SCN5A* gene variations in SUNDS cases.

Two frameshift and nine missense variants from *SCN5A* gene exome sequencing data that were compared with ExAC, 1000G, Clinvar and previous report, are described in Table 3. Two frameshift in exon 28 of *SCN5A* gene are novel variants (L1646fs and E1804fs). As for missense variant, G599R was identified a single variants that matched from ExAC without pathogenic report in ClinVar. Other six *SCN5A* missense variants are novel (T92N, E171G, A178T, Q998K, N1659S and E2013K). However, Q998K may not significant since it were predicted as benign from polyphen-2 and CADD score are less than 20. Common variants, H558R and R1193Q, were observed in Thai[24], which similar to a previous report in East Asian. 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 exon of *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 were higher than those 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 result. 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 Philippines found that SUNDS cases occurred in males 91.7% and females 8.3%.[25] The sudden death history of victim's family 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 *SCN5A, KCNJ2* and *RyR2* gene.[10] BMI of all cases are within normal range whereas heart weights were slightly increased (58.33%). These were 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, are found in two cases (16.67%). These variants might be affected protein features and cause truncated of *SCN5A* protein. For missense variants, there were 3 variants that previously reported in dbSNP and ExAC database. G599R was presented in without a supported data for pathogenicity. The other two variants, H558R and R1193Q, were commonly found in East Asian population. H558R was found to be affected sodium ion channel function when presents with other variants such as Q1077del, T512I and M1766L.[26-28] R1193Q has been reported as pathogenic since 2002 through electrophysiological study.[7] There were studies on its effect by 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 has declared that R1193Q is only a polymorphism as it commonly found in East-Asian populations. To increase the accurate incident and gene frequency, the large sample size of SUNDs is needed. The extended 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 Thai population are presented. Two frameshift and five *SCN5A* missense variants from four SUNDS cases were found which potentially significant affected SUNDS cases in Thai population. Further study should run electrophysiological analyses to confirm their effects on functional of sodium channel in SUNDS cases. Our results could be usefulness for forensic pathologists and medical practitioners to keep these variants as a possible risk factors closely observed in every SUNDS in 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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