Egyptian Journal of Forensic Sciences

ORIGINAL ARTICLE Open Access

Association between *SCN5A* and sudden unexplained nocturnal death syndrome in Thai decedents: a case–control study

Supawon Srettabunjong[1*], Duangkamon Eakkunnathum[1], Wanna Thongnoppakhun[2] and Orapan Sripichai[3]

Abstract

Background: Sudden unexplained nocturnal death syndrome (SUNDS) is a genetic disorder that can cause sudden death in young and healthy adults during sleep at night. Cardiac arrhythmia has been proposed as the factor responsible for such deaths. This study was thus aimed to investigate the association between *SCN5A*, the gene that has been extensively studied and thought to be associated with cardiac conduction system abnormality causing sudden cardiac death, and SUNDS in Thai decedents.

Results: Genomic DNA was extracted using blood samples from 12 unrelated SUNDS subjects and 151 unrelated non-SUNDS control decedents. Subjects were divided into three subgroups as follows: subjects who resided in regions other than Northeastern Thailand ($n = 99$), age-matched subjects who resided in Northeastern Thailand ($n = 28$), and subjects older than 40 years who resided in Northeastern Thailand ($n = 24$). Genomic DNA from SUNDS cases was screened for genetic variations for the entire 28 coding exons of *SCN5A*. The identified variants were also genotyped in control subjects using high-resolution melting analysis and sequencing.

Conclusions: Twelve variants of *SCN5A* were identified, including six polymorphisms and another six variants previously reported to be related with cardiac conduction defects. Two identified variants (rs1805126 and rs7429945) deserved further study because of their strong odd ratios.

Keywords: Cardiac sodium ion channel, Death during sleep, Lai-tai, Molecular autopsy, Postmortem genetic testing, Sudden cardiac death

Background

Sudden death is a major concern in forensic medicine, especially when the decedent is young and healthy and the postmortem examination fails to reveal any significant findings. Sudden unexplained nocturnal death syndrome (SUNDS) is a genetic disorder that can cause sudden death in young, healthy adults during sleep at night. Autopsy findings in cases of SUNDS reveal no evident histopathological changes that explain the cause of death. The clinical and pathologic features of these cases of sudden death, especially the characteristic lack of overt structural heart disease, are similar among cases. SUNDS is highly prevalent in healthy young Southeast Asian males (Munger and Booton 1998; Gotoh 1976; Cheng et al. 2011; Srettabunjong 2019), and it may have devastating effects on families in many different ways.

SUNDS has been given different local names, including "bangungut" in the Philippines (Munger and Booton 1998), "pokkuri" in Japan (Gotoh 1976), "sudden manhood death syndrome" in China (Cheng et al. 2011), and "lai-tai" in Thailand (Srettabunjong 2019). The annual incidence of SUNDS has been reported to be as high as 43 per 100,000 people, all being men aged 20–40 years in the Philippines (Gervacio-Domingo et al. 2007); 26–38 per 100,000 people, all being men aged 20–49 years in Northeast Thailand (Tatsanavivat et al. 1992; Tungsanga and Sriboonlue 1993); and as low as 1–2 per 100,000 people predominantly in men aged 17–55 years in Southern China (Cheng et al. 2011; Zheng et al. 2015). However, the true incidence of SUNDS is unknown.

* Correspondence: subunjong@gmail.com
[1]Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Rd, Bangkoknoi District, Bangkok 10700, Thailand
Full list of author information is available at the end of the article

Cardiac arrhythmia has been proposed as the factor responsible for death in individuals who experience SUNDS (Nademanee et al. 1997). Cardiac arrhythmia has also been assumed to be associated with the pathogenesis of sudden cardiac death in the young, such as congenital long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia, arrhythmogenic right ventricular cardiomyopathy, and Brugada syndrome (BrS) (Elger et al. 2010). Previous studies reported that these conditions are allelic disorders, and some sporadic SUNDS cases may be caused by inherited cardiac arrhythmia, such as LQTS and BrS (Cheng et al. 2011; Liu et al. 2013). Additionally, both SUNDS and BrS represent the same autosomal dominant familial disorder, and they can result from mutations in the cardiac sodium channel *SCN5A*, thus causing loss of channel function (Sangwatanaroj et al. 2002). Some previous studies also reported that some SUNDS cases are likely caused by the same allelic disorder that causes BrS (Vatta et al. 2002; Liu et al. 2014; Huang et al. 2016). However, mutations have only been identified in a small proportion of probands and the clinical markers that would identify victims at high risk for SUNDS have not yet been identified. Moreover, the etiology of SUNDS remains unknown.

SCN5A is located on chromosome 3p21 and encodes the type 5 α-subunit of the cardiac voltage-gated sodium channel ($Na_V1.5$) (Chen et al. 1998). The $Na_V1.5$ protein, which is 80 kb in size and consists of 2016 amino acids, is expressed only in the heart (Daimi et al. 2015). It is a pore-forming transmembrane protein consisting of four internal homologous domains (DI–DIV). Each domain consists of six transmembrane segments (S1–S6). The fourth transmembrane segment (S4) of each domain acts as a "voltage sensor." Na^+ ions can pass through this channel according to their electrochemical gradient. As such, mutations that inhibit the normal functions of *SCN5A* gene can lead to abnormal heart rhythms that may result in sudden death. Previous studies identified more than 250 *SCN5A* mutations linked to type 1 BrS, most of which are missense mutations (Brunklaus et al. 2014). Functional studies of *SCN5A* mutations uncovered loss-of-function in the sodium channel (Watanabe and Minamino 2016). Molecular mechanisms that may have an influence on this syndrome have been proposed, such as decreased protein expression from trafficking defects or the expression of non-functional channels, and decreased sodium influx by changes in channel gating properties via the process of activation and/or inactivation (Brunklaus et al. 2014; Watanabe and Minamino 2016; Amin et al. 2010). Van der berg et al. (2002) described a large family presenting with an *SCN5A*-linked disorder with features of premature nocturnal sudden death, LQTS, BrS, and familial conduction system disease. Several variants of *SCN5A* have been implicated as the underlying cause of LQTS type 3, BrS, and progressive cardiac arrhythmias (Vatta et al. 2002; Chen et al. 1998; Benito et al. 2008; Antzelevitch 2007). Mutations in this gene have also been reported as the cause of SUNDS in approximately 6–20% of cases (Vatta et al. 2002; Liu et al. 2014).

Based on our review of the literature, no previous studies investigated the relationship between *SCN5A* and SUNDS in Thai decedents. The aim of this study was to identify genetic variants of *SCN5A* in Thai SUNDS decedents and investigate whether any identified variations are significantly associated with SUNDS. Any findings of *SCN5A* genetic variations may benefit the relatives of SUNDS victims that may also be at risk of experiencing SUNDS.

Methods

This case–control study evaluated blood samples obtained from femoral veins of unrelated Thai decedents within 24 h after death. All decedent subjects underwent postmortem examination under the jurisdiction of the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, which is located in Bangkok, Central Thailand. The study protocol was ethically approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University. The subjects were divided into SUNDS and control groups. SUNDS cases were unrelated subjects who died during sleep at night with previous healthy history and a negative comprehensive postmortem investigation including histological and toxicological examination that can explain the cause of death. Control cases were unrelated subjects who died of any cause of death excluding SUNDS. To minimize the effect of genetic backgrounds that could lead to false-positive or false-negative results (Hu and Ziv 2008), control subjects were divided into three subgroups: subjects who resided in regions other than Northeastern Thailand (DR group, 99 subjects), age-matched subjects who resided in Northeastern Thailand (NE group, 28 subjects), and subjects older than 40 years who resided in Northeastern Thailand (NE^+ group, 24 subjects). All control subjects were healthy and aged 15 years or older with no history of cardiac arrhythmic diseases, radiotherapy, chemotherapy, bone marrow transplantation, blood transfusion within 2 years, other *SCN5A*-related conditions (e.g., certain cardiovascular diseases), and genetic diseases. Subjects who developed some degree of decomposition or who were embalmed were excluded from the study. Subject information, including age, race, medical history, family history, autopsy findings, and cause of death, was documented.

All blood samples were stored at 4 °C shortly after being drawn from the femoral veins of the decedents.

Genomic DNA for each subject was extracted from blood samples within 72 h after collection and quantified using a Nanodrop® ND-1000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Genetic testing

Genetic screening of all coding regions of *SCN5A* was performed in all SUNDS cases using polymerase chain reaction (PCR) and direct Sanger sequencing. A total of 40 pairs of PCR primers spanning all 28 exons of *SCN5A* were designed and employed for genetic screening (primer sequences available on request). PCR reactions were performed in a PTC-100™ Programmable Thermal Controller (MJ Research, Inc., St. Bruno, Quebec, Canada). Sanger sequencing was performed in an ABI 3730XL DNA Analyzer (Applied Biosystems, Inc. Foster City, CA, USA), using BigDye® Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems). The same primers were used for direct sequencing and PCR. Sequence chromatograms were then analyzed using Sequencing Analysis 5.2 software (Applied Biosystems).

The obtained sequencing data were then compared with the corresponding referenced cDNA sequence of *SCN5A* retrieved from GenBank® (NG_008934.1) using Mutation Surveyor® (Demo) (SoftGenetics, PA, USA). To identify the association between *SCN5A* variants and SUNDS, variants were selected by focusing on SNP information for CDX (Chinese Dai in Xishuangbanna, China), CHS (Southern Han Chinese, China), KHV (Kinh in Ho Chi Minh City, Vietnam), and JPT (Japanese in Tokyo, Japan) populations, including allele frequency, linkage disequilibrium (LD), and single-nucleotide polymorphism (SNP) locations. SNPs with a minor allele frequency of approximately 5% from the Exome Aggregation Consortium (ExAC), 1000 Genomes Project (http://www.1000genomes.org/ensembl-browser), and NHLBI GO Exome Sequencing Project (GO-ESP, http://snp.gs.washington.edu/EVS) databases were preferable.

These identified variants were also screened in the controls using high-resolution melting (HRM) analysis. Genetic screening was performed using a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primers of each variant suitable for HRM were designed using Primer3 version 0.4.0 (http://bioinfo.ut.ee/primer3-0.4.0/) (primer sequences available on request). Melting files generated using Precision Melt Analysis™ Software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were then analyzed. To validate the variants obtained from additional HRM patterns, large amplicons containing additional patterns were subjected to further genetic screening by PCR and Sanger sequencing.

Genetic variant analysis

All identified variants were filtered for candidate mutations based on allele frequency in the 1000 Genomes Project database, functional mutation types, predictive software programs, and a review of the related published literature. To determine whether the identified genetic variants were novel mutations, the variants were also compared with published data, including Single Nucleotide Polymorphism Database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp/), the 1000 Genomes Project database, the GO-ESP database, and the inherited arrhythmias database (http://www.fsm.it/cardmoc/). To predict the deleterious effects of identified variants, three web-based in silico prediction programs were used in this study, namely MutationTaster (http://www.mutationtaster.org/), PROVEAN (http://provean.jcvi.org/index.php), and Human Splicing Finder (http://www.umd.be/HSF3/HSF.html).

Any variant identified in both SUNDS cases and controls was designated as a polymorphism. Polymorphisms with a minor allele frequency (MAF) exceeding 0.01 among the controls were classified as common polymorphisms. If the MAF was less than 0.01 among the controls, the polymorphism was regarded as rare polymorphism. Any variants observed only in SUNDS cases were designated as mutations. All nonsense, frameshift, and splice site variants were considered to be candidate pathogenic mutations, unless identified as a polymorphism.

Statistical analysis

All statistical analyses (unless otherwise specified) were performed using PASW Statistics for Windows, Version 18 (SPSS, Inc., Chicago, IL, USA). Continuous variables are shown as the mean ± standard deviation. The allele frequencies for each SNP site and genotype frequency for each polymorphism were calculated using the web-based SNPStat program (http://bioinfo.iconcologia.net/snpstats/start.htm). The genotype frequencies of the detected coding region polymorphisms in the SUNDS and control groups were tested for deviation from Hardy–Weinberg equilibrium using Pearson's chi-square goodness-of-fit test (de Finetti) and Fisher's exact test (SNPStat), respectively. Differences in genotype and allele frequencies between the SUNDS and control groups were also analyzed using Pearson's chi-square goodness-of-fit test (de Finetti) and Fisher's exact test (SNPStat), respectively. The association between multiple detected variants and LD was calculated using online SNPStat. To identify the detected variants that were inherited together, SNPStat was used to construct and estimate the haplotype frequency. The association between haplotypes and SUNDS was analyzed via logistic regression. The association between the detected polymorphisms and SUNDS risk was estimated by calculating the odds ratio (OR) and 95% confidence interval (95% CI) using

Pearson's chi-square test analysis. p values < 0.05 were considered statistically significant.

Results

A total of 12 SUNDS decedents (all males) were included in this study. Most subjects (83%) resided in Northeastern Thailand. The age of the subjects ranged 20–45 years (mean, 32.9 ± 6.4 years). All cases had a normal heart structure. Half of the cases died during the summer (April–June). Of the 12 SUNDS cases, only one case had a family history of SUNDS. A total of 151 unrelated healthy control cases were also included in the study, including 127 males and 24 females. The control cases ranged in age from 15 to 59 years (mean, 32.9 ± 11.4 years). Control subjects were classified into DR (80 males and 19 females; age range, 15–59 years; mean age, 31.9 ± 11.8 years), NE (25 males and 3 females; age range, 15–38 years; mean age, 28.3 ± 7.5 years), and NE^+ (22 males and 2 females; age range, 41–59 years; mean age, 45.3 ± 3.8 years).

The sequencing analysis revealed a total of 12 variations (Table 1). All variants were polymorphisms, including one non-synonymous variant [rs1805124 (c.1673A>G, H558A) in exon 12], four synonymous variants [rs6599230 (c.87A>G, A29A) in exon 2, rs7430407 (c3183A>G, E1061E) in exon 17, and rs1805126 (c.5457 T>C, D1818D) and rs376826051 (c.5712G>A, S1903S) in exon 28], and seven variants in the3′UTR [rs7429945 (c.*123A>G), rs41310757 (c.*753C>T), rs4073797 (c.*962 T>A), rs4073796 (c.*963C>T), rs11414422 (c.*1453_*1454insA), rs41315485 (c.*1537 T>C), and rs45592631 (c.*2145_*2146insGAGAAGAGAGTGGAAAAA-GAGGG)]. Two variants (rs7430407, rs45592631) displayed only homozygous variants, and they appeared to be major alleles found in various populations (Table 2).

Of the seven variants in the3′UTR, rs45592631 had a MAF of less than 0.01 (0.0068). Another two variants (rs11414422, rs4592631) were unclassified and further evaluated for functional effects using three prediction programs, which predicted both variants to be polymorphisms. However, one synonymous variant in exon 28 (rs376826051) was unclassified, and the MAF was not available in 1000 genomes. The ExAC database revealed that MAF of this variant was less than 0.01 (0.0005879). Two prediction programs (MutationTaster and Human Splicing Finder 3.0) found this variant to be disease causing due to a splice site change, whereas PROVEAN/SIFT predicted it to be a neutral polymorphism (Table 3).

Based on the aforementioned results, only 10 variants were selected for the association study between *SCN5A* variants and Thai SUNDS decedents. The genotype and allele frequencies of these variants identified in SUNDS and control cases were analyzed as shown in Table 4.

To evaluate the association between each *SCN5A* variant with SUNDS, the variants that were in Hardy–Weinberg equilibrium were evaluated for differences in allele and genotype frequencies between the SUNDS and control groups including subgroups. The results revealed that only three variants had some associations with SUNDS: rs6599230, rs1805126, and rs7429945.

rs6599230 was linked to a statistically significant difference between the SUNDS and control groups regarding genotype frequency for the co-dominant [A/G, OR = 0.20 (0.04–0.98); $p = 0.037$] and over-dominant modes [A/G, OR = 0.18 (0.04–0.84); $p = 0.012$]. This variant was also linked to a statistically significant difference between the SUNDS and DR groups concerning genotype frequency for the over-dominant mode [A/G, OR = 0.20 (0.04–0.94); $p = 0.02$] but not for allele frequency. Additionally, the variant was associated with a statistically significant difference between the SUNDS and NE groups regarding genotype frequency for the co-dominant [A/G, OR = 0.09 (0.01–0.56); $p = 0.009$], dominant [A/G-A/A, OR = 0.19 (0.05–0.84); $p = 0.025$], and over-dominant modes [G/G-A/A, OR = 0.09 (0.02–0.53); $p = 0.002$] but not for allele frequency.

rs1805126 was linked to a statistically significant difference between the SUNDS and NE groups regarding both allele [OR = 3.25 (1.20–8.77); $p = 0.018$] and genotype frequencies for the co-dominant [T/T, OR = 13.00 (1.11–152.36); $p = 0.038$] and dominant modes [T/C-T/T, OR = 9.53 (1.08–84.14); $p = 0.012$].

rs7429945 was associated with a statistically significant difference between the SUNDS and NE groups concerning genotype frequency for the co-dominant [A/A, OR = 13.0 (1.11–152.36); $p = 0.038$] and dominant modes [A/G-A/A, OR = 9.53 (1.08–84.14); $p = 0.012$] but not for allele frequency.

To evaluate the associations among multiple variants obtained from SUNDS subjects, LD and haplotype analyses were performed. A total of four variants (rs1805126, rs7429945, rs4073796, and rs4073797) were found to be

Table 1 Characteristics of SUNDS cases and controls

Characteristics	Cases ($n = 12$)	Controls ($n = 151$)		
		DR ($n = 99$)	NE ($n = 28$)	NE^+ ($n = 24$)
Mean age ± SD (range)	32.9 ± 6.4 years (20–45)	31.9 ± 11.8 (15–38)	28.3 ± 7.5 (15–38)	45.3 ± 3.8 (41–59)
Gender (males/females)	12/0	80/19	25/3	22/2

DR Thai controls resided in different regions of Thailand with age matched, *NE* Thai controls resided in Northeastern region of Thailand with age matched, NE^+ Thai controls resided in Northeastern region of Thailand with age greater than 40 years

Table 2 Allele frequency of each variant identified in this study, using 151 control subjects compared with frequency from various population

Exon	dbSNP ID	This study		Population				Minor allele frequency		
		Cases	Controls	CDX	CHS	KHV	JPT	ExAC	1000 Genomes	GO-ESP
2	*rs6599230* c.87	T = 0.33 *C = 0.67*	T = 0.37 *C = 0.63*	T = 0.40 *C = 0.60*	T = 0.30 *C = 0.70*	T = 0.29 *C = 0.71*	T = 0.38 *C = 0.62*	T = 0.2263/27239	T = 0.2183/1093	T = 0.1640/2022
12	*rs1805124* c.1673	*T = 0.92* C = 0.08	*T = 0.84* C = 0.16	*T = 0.93* C = 0.07	*T = 0.93* C = 0.07	*T = 0.87* C = 0.13	*T = 0.92* C = 0.08	C = 0.2217/26713	C = 0.2304/1154	C = 0.2463/3108
17	*rs7430407* c.3183	T = 0.00 *C = 1.00*	NA	T = 0.01 *C = 0.99*	T = 0.00 *C = 1.00*	T = 0.01 *C = 0.99*	T = 0.00 *C = 1.00*	T = 0.0872/10512	T = 0.0769/385	T = 0.1150/1471
28	*rs1805126* c.5457	A = 0.37 *G = 0.63*	A = 0.43 *G = 0.57*	A = 0.39 *G = 0.61*	A = 0.42 *G = 0.58*	A = 0.35 *G = 0.65*	A = 0.50 G = 0.50	G = 0.3878/46827	G = 0.4924/2466	G = 0.4314/5544
28	*rs376826051* c.5712	*C = 0.96* T = 0.04	*C = 1.00* T = 0.00	NA	NA	NA	NA	T = 0.0006/71	NA	T = 8e-5 /1
3′UTR	*rs7429945* c.*123	T = 0.37 *C = 0.63*	T = 0.44 *C = 0.56*	T = 0.39 *C = 0.61*	T = 0.42 *C = 0.58*	*T = 0.51* C = 0.49	T = 0.35 *C = 0.65*	NA	C = 0.4944/2476	NA
3′UTR	*rs41310757* c.*753	*G = 0.88* A = 0.12	*G = 0.78* A = 0.22	*G = 0.74* A = 0.26	*G = 0.75* A = 0.25	*G = 0.74* A = 0.26	*G = 0.76* A = 0.24	NA	A = 0.1144/573	NA
3′UTR	*rs4073796* c.*962	G = 0.46 *A = 0.54*	G = 0.42 *A = 0.58*	G = 0.39 *A = 0.61*	G = 0.43 *A = 0.57*	G = 0.35 *A = 0.65*	*G = 0.51* A = 0.49	NA	A = 0.4940/2474	NA
3′UTR	*rs4073797* c.*963	A = 0.46 *T = 0.54*	A = 0.42 *T = 0.58*	A = 0.40 *T = 0.60*	A = 0.43 *T = 0.57*	A = 0.35 *T = 0.65*	*A = 0.51* T = 0.49	NA	T = 0.4918/2463	NA
3′UTR	*rs11414422* c.*1453_*1454	*C = 0.54* CT = 0.46	C = 0.44 *CT = 0.56*	*C = 0.84* CT = 0.16	*C = 0.51* CT = 0.49	C = 0.44 *CT = 0.56*	*C = 0.65* CT = 0.35	NA	T = 0.3169/1587	NA
3′UTR	*rs41315485* c.*1537	*A = 0.92* G = 0.08	*A = 0.87* G = 0.13	*A = 0.89* G = 0.11	*A = 0.91* G = 0.09	*A = 0.91* G = 0.09	*A = 0.85* G = 0.15	NA	G = 0.1795/899	NA
3′UTR	*rs45592631* c.*2145_*2146	- = 0.00 *ins = 1.00*	NA	- = 0.00 *ins = 1.00*	- = 0.00 *ins = 1.00*	- = 0.01 *ins = 0.99*	- = 0.00 *ins = 1.00*	NA	- = 0.0068/34	NA

CDX Chinese Dai in Xishuangbanna, China; *CHS* Southern Han Chinese, China; *KHV* Kinh in Ho Chi Minh City, Vietnam; *JPT* Japanese in Tokyo, Japan; *ExAC* Exome Aggregation Consortium; *1000 Genomes* 1000 Genomes Project; *GO-ESP* NHLBI GO Exome Sequencing Project; *NA* not available
Data presented in italics are major allele

significant in LD analysis, each having a haplotype frequency exceeding 1%.

Discussion

SUNDS is believed to be associated mainly with cardiac sodium channel diseases that involve *SCN5A* mutations. Previous studies demonstrated a linkage between SUNDS and *SCN5A* variations (Zheng et al. 2015; Vatta et al. 2002). More recent studies (Liu et al. 2013; Liu et al. 2014; Huang et al. 2014) also reported that the cause of some cases of SUNDS might be *SCN5A* mutations that can result in a reduction of I_{Na}, with some mutations exhibiting a dominant-negative effect on wild-type (WT) channels, leading to an even more prominent decrease in current amplitudes (Hoshi et al. 2014).

All SUNDS cases in this study involved young males, the majority of whom resided in Northeastern Thailand, the region with the highest incidence of SUNDS (Srettabunjong 2019; Tungsanga and Sriboonlue 1993). Although some studies reported that SUNDS can affect both genders, its prevalence in males is thought to be about 4–14-fold higher than that in females (Cheng et al. 2011; Vatta et al. 2002; Gervacio-Domingo et al. 2007; Chen et al. 2016). Most SUNDS cases in this study occurred during the summer season (April–June), consistent with previous studies (Cheng et al. 2011; Srettabunjong 2019; Tungsanga and Sriboonlue 1993), suggesting that the season may be a risk factor for SUNDS.

In this study, postmortem genetic analysis was performed on all 28 coding regions of *SCN5A* in all 12 Thai SUNDS cases with a total of 12 variations (all polymorphisms) of *SCN5A*. The first three variants detected in this study (A29A, H558A, E1061E) were the same as those detected in a recent study by Liu et al. (2014), who also discovered seven missense mutations (V95I, R121Q, R367H, R513H, D870H, V1764D, S1937F). By contrast, there were no matches in variations between our study and a study conducted by Vatta et al. (2002), who found only three mutations (R367H, A735V, R1193Q). The fourth variant detected in our study (D1819D) was the same variant as found in a previous study by Cheng et al. (2011) who found only two polymorphisms, D1819D and 3666+69G>C, in *SCN5A*, which have been suggested as SUNDS-causing variants in Southern Chinese populations. However, the

Table 3 Prediction results of 12 variants of *SCN5A* obtained from SUNDS by three online prediction programs (MutationTaster, PROVEAN/SIFT, Human Splicing Finder)

Exon	Variant	Position	Web-based program		
			MutationTaster	PROVEAN/SIFT	Human Splicing Finder
2	rs6599230	*c.87A>G* *g.21452A>G* *p.Ala29=*	Polymorphism	Neutral/tolerated	No significant splicing motif alteration detected. This mutation has probably no impact on splicing.
12	rs1805124	c.1674A>G g.50744A>G p.His558Arg	Polymorphism	Neutral/tolerated	No significant splicing motif alteration detected. This mutation has probably no impact on splicing.
17	rs7430407	c.3183A>G g.73697A>G p.Glu1061==	Polymorphism	Neutral/tolerated	- New acceptor site* - New ESS site** - ESE site broken***
28	rs1805126	c.5457 T>C g.103758 T>C p.Asp1819=	Polymorphism	Neutral/tolerated	No significant splicing motif alteration detected. This mutation has probably no impact on splicing.
28	rs376826051	c.5712G>A g.104013G>A p.Ser1903=	Disease causing - Protein features (might be) affected - Splice site changes	Neutral/tolerated	- New acceptor site* - New ESS site** - ESE site broken***
3′UTR	rs7429945	c.*123A>G g.104475A>G	Polymorphism	NA	NA
3′UTR	rs41310757	c.*753C>T g.105105C>T	Polymorphism	NA	NA
3′UTR	rs4073797	c.*962T>A g.105314 T>A	Polymorphism	NA	NA
3′UTR	rs4073796	c.*963C>T g.105315C>T	Polymorphism	NA	NA
3′UTR	rs11414422	c.*1453_ *1454insA g.105805_ 105806insA	Polymorphism	NA	NA
3′UTR	rs41315485	c.*1537T>C g.105889 T>C	Polymorphism	NA	NA
3′UTR	rs45592631	c.*2145_ *2146 insGAGA AGAGAG TAGGAA AAAGGA GGG g.106497_106,498 insGAGA AGAGAG TAGGAA AAAGGA GGG	Polymorphism	NA	NA

NA not available
*Activation of an exonic cryptic acceptor site, with presence of one or more cryptic branch point(s)
**Creation of an exonic splicing silencer site (ESS site)
***Alteration of an exonic splicing enhancer site (ESE site)

detection of mutations in different populations may vary significantly depending on ethnic and regional differences between cohorts, as well as differences in mutation calling (Cheng et al. 2011).

Interestingly, some of the variants in the present study were previously described as pathologic variants of cardiac conduction defects, even if they produced heterogeneous effects. A synonymous variant, rs6599230 (c.87A>G, A29A), was reported to be associated with PR interval shortening in people of European ancestry (Magnani et al. 2014). Mohanty et al. (2016) reported that the G allele of this variant is a protective allele that reduces the risk of non-paroxysmal atrial fibrillation by 43%. This finding was consistent with that of a study performed by Qureshi et al. (2015) who analyzed *SCN5A* mutations in patients with LQTS. Although the rs6599230 polymorphism found in

Table 4 Comparison between odds ratios of allele frequency from controls

dbSNP ID	Odds ratio, (95% CI), *p*			
	All controls	DR	NE	NE^+
rs6599230 (c.87A>G, p.A29=)	1.10 (0.55–2.21) 0.77	0.91 (0.37–2.24) 0.30	0.62 (0.23–1.68) 0.35	1.10 (0.39–3.07) 0.86
rs1805124 (c.1674A>G, p.H558R)	0.48 (0.11–2.11) 0.33	0.45 (0.10–2.02) 0.30	0.76 (0.14–4.05) 0.75	0.39 (0.08–1.99) 0.26
rs1805126 (c.5457 T>C, p.D1819=)	2.20 (0.92–5.12) 0.07	0.51 (0.21–1.20) 0.13	3.24 (1.20–8.77) 0.02*	0.47 (0.17–1.27) 0.13
rs376826051 (c.5712G>A, p.S1903=)	13.09 (0.79–216.10) 0.07	8.57 (0.52–141.60) 0.13	7.21 (0.28–183.55) 1.20	6.19 (0.24–157.83) 0.27
rs7429945 (c.*123A>G)	2.15 (0.91–5.06) 0.07	1.96 (0.82–4.69) 0.13	0.86 (0.32–2.31) 0.76	0.51 (0.19–1.38) 0.18
rs41310757 (c.*753C>T)	0.52 (0.15–1.80) 0.30	0.52 (0.15–1.81) 0.30	0.58 (0.15–2.32) 0.76	0.48 (0.12–1.92) 0.30
rs4073796 (c.*963C>T)	1.15 (0.50–2.65) 0.74	1.00 (0.43–2.33) 0.99	0.61 (0.23–1.61) 0.31	0.71 (0.26–1.91) 0.50
rs4073797 (c.*962T>A)	1.15 (0.50–2.65) 0.74	1.00 (0.43–2.33) 0.99	0.61 (0.23–1.61) 0.31	0.71 (0.26–1.91) 0.50
rs11414422 (c.*1453_*1454insA)	1.53 (0.65–3.62) 0.33	1.55 (0.60–3.99) 0.36	3.73 (1.35–10.30) 0.01*	0.46 (0.17–1.26) 0.13
rs41315485 (c.*1537T>C)	6.30 (0.14–2.80) 0.54	0.63 (0.14–2.84) 0.55	0.47 (0.09–2.38) 0.37	1.00 (0.17–5.89) 1.00

*p value < 0.05 was considered as statistical significance

this study was linked to a statistically significant difference in genotype frequency between the SUNDS and control groups including the DR and NE subgroups, its ORs were extremely small.

The non-synonymous variant rs1805124 (c.1674A>G, H558R) is located in the intracellular domain I-II linker of *SCN5A*, and it results in the missense exchange of a histidine residue to an arginine residue at amino acid position 558. This variant is found in 20% of the population (Viswanathan et al. 2003), and it has been reported to modify the expression of an arrhythmia-causing mutation (Ye et al. 2003) and found to be associated with BrS (Chen et al. 2004). Shinlapawittayatorn et al. (2011) reported that this polymorphism could reverse the gating defects acquired from the *SCN5A*–P2006A mutation located in the C-terminal region of the sodium channel and restore normal function. They also suggested that this variant might play a crucial role in normalization from fast inactivation of this channel, and it might also be a disease-modifying gene, as previously described by other authors (Viswanathan et al. 2003; Ye et al. 2003; Poelzing et al. 2006). This result is suggestive of an interaction between sodium channel alpha subunits, and it is similar to the interaction with the V1951L mutation associated with sudden infant death syndrome, which is also located in the C-terminal region of the sodium channel. Although the sodium channel alpha subunit alone is sufficient to form a functional conducting pore, these findings were consistent with other reports (Aldrich et al. 1983; Undrovinas et al. 1992; Keller et al. 2005), suggesting that sodium alpha subunits may interact with each other. This variant also interacts with a BrS-associated mutation (*SCN5A*–R282H), resulting in restoration of the trafficking defect of the mutation (Poelzing et al. 2006). In addition, this variant and two synonymous variants (rs1805126, c.5457 T>C, D1819D and rs6599230, c.87A>G, A29A) were previously reported to be associated with cardiac conduction by affecting PR and QRS intervals (Magnani et al. 2014). However, this polymorphism was not associated with a statistically significant difference between the SUNDS and control groups including the DE, NE, and NE^+ subgroups regarding either allele frequency or genotype frequency in this study. This finding was consistent with that of a recent study in a Southern Han Chinese population by Liu et al. (2014).

Gouas et al. (2005) reported that rs1805126 (c.5457 T>C, D1819D), a synonymous variant, was more frequently found in healthy subjects with shorter QTc intervals and that the C allele might be protective. However, the present study found that this variant was linked to significant differences between the SUNDS and NE groups regarding both allele and genotype frequencies with large ORs of approximately 3 and 10, respectively, suggesting that this variant might be associated with SUNDS in the Thai population. This finding was consistent with that of the previous study by Cheng et al. (2011). Thus, whether the rs1805126 variant is associated with SUNDS requires further investigation.

For untranslated variants located in the 3′UTR of *SCN5A*, recent studies reported that rs41310757, rs4073797, rs4073796, and rs41315485 affect gene expression by changing the binding site of miRNA (Zhao et al. 2015; Daimi et al. 2015).

The rs7429945 (c.*123A>G) variant was found in patients with BrS (Uzieblo-Zyczkowska et al. 2014), and no defects in splicing sites were demonstrated by bioinformatics analysis. However, this study found that this variant was associated with significant differences between the SUNDS and NE groups regarding genotype frequency with large ORs of approximately

10. As such, whether the rs7429945 variant is associated with SUNDS requires further investigation.

With respect to multiple variant association with SUNDS, this study revealed that rs1805126 and rs7429945 have strong linkage disequilibrium with each other, suggesting that they may co-segregate.

Conclusion

This study employed postmortem genetic testing to investigate the association between *SCN5A* and SUNDS in a Thai population. To our knowledge, this is the first report on *SCN5A* genetic screening in Thai SUNDS decedents. As a preliminary investigation, this study identified 12 variants of *SCN5A*. Although no new mutations were found in this study, two identified variants (rs1805126 and rs7429945) deserve further study. Furthermore, they have strong linkage disequilibrium with each other, suggesting that they may co-segregate. Given the preliminary nature of this study, further study may be needed with a larger number of SUNDS cases and controls to confirm these results and elucidate the association between these variants and SUNDS in the Thai population. Other investigation of the association between common polymorphisms in *SCN5A* and SUNDS, including electrophysiological investigations, may also be needed. Since the SUNDS cases evaluated in this study were not subjected to a scan of the entire *SCN5A* gene, the incidence of rare variants residing outside the coding exons (which might have had a putative role in the function of this gene) should also be investigated to ensure a comprehensive understanding of potential pathogenic variants. Information and conclusions based on these findings may be beneficial to the relatives of people who died of SUNDS, and who might also be at risk for this disorder.

Abbreviations

BrS: Brugada syndrome; CDX: Chinese Dai in Xishuangbanna (China); CHS: Southern Han Chinese (China); dbSNP: Single Nucleotide Polymorphism Database; DNA: Deoxyribonucleic acid; DR group: A group of subjects who resided in regions other than Northeastern Thailand; ExAC: Exome Aggregation Consortium; GO-ESP: GO Exome Sequencing Project; HRM: High-resolution melting; JPT: Japanese in Tokyo (Japan); KHV: Kinh in Ho Chi Minh City (Vietnam); LD: Linkage disequilibrium; LQTS: Long QT syndrome; MAF: Minor allele frequency; $Na_V1.5$: 5 α-subunit of the cardiac voltage-gated sodium channel protein; NE group: A group of age-matched subjects who resided in Northeastern Thailand; NE^+group: A group of subjects older than 40 years who resided in Northeastern Thailand; OR: Odds ratio; PCR: Polymerase chain reaction; S1–S6: Transmembrane segment 1–6; SCN5A: Sodium cardiac channel voltage-gated 5 α-subunit gene

Acknowledgements

The authors are sincerely thankful for all decedents whose blood samples were employed in this study.

The manuscript has not been submitted or published elsewhere. However, its preliminary information was presented in part at the 21st Triennial Meeting of the International Association of Forensic Sciences held on the 21–25 August 2017, Toronto, Canada, and the abstract part was published in its abstract book (Forensic Sci Int 2017; 277 (Sup 1): 168–9).

Authors' contributions

SS initiated the study, applied for funding, registered to the ethical approval committee, guided the study design, collected the data, guided the data analyses, wrote the manuscript, and submitted the manuscript. DE collected the data, performed some experiments, and analyzed the data. WT guided the study design, the experiments, and the data analyses. OS guided the study design, the experiments, and the data analyses. All authors read and approved the final manuscript.

Funding

This research project was supported by Siriraj Research fund, Faculty of Medicine Siriraj Hospital, Mahidol University, Grant Number (IO) R015933011. The funder had no involvement in the development of the manuscript or decisions related to the manuscript.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

The study protocol was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital. Mahidol University (024/2559(EC1)).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Wanglang Rd, Bangkoknoi District, Bangkok 10700, Thailand. [2]Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. [3]Institute of Molecular Biosciences, Mahidol University, Nakonpatom 73170, Thailand.

Received: 17 May 2019 Accepted: 26 June 2019
Published online: 05 July 2019

References

Aldrich RW, Corey DP, Stevens CF (1983) A reinterpretation of mammalian sodium channel gating based on single channel recording. Nature 306(5942):436–441

Amin AS, Asghari-Roodsari A, Tan HL (2010) Cardiac sodium channelopathies. Pflugers Arch 460(2):223–237

Antzelevitch C (2007) Genetic basis of Brugada syndrome. Heart Rhythm 4(6):756–757

Benito B et al (2008) Gender differences in clinical manifestations of Brugada syndrome. J Am Coll Cardiol 52(19):1567–1573

Brunklaus A, Ellis R, Reavey E, Semsarian C, Zuberi SM (2014) Genotype phenotype associations across the voltage-gated sodium channel family. J Med Genet 51(10):650–658

Chen JZ, Xie XD, Wang XX et al (2004) Single nucleotide polymorphisms of the *SCN5A* gene in Han Chinese and their relation with Brugada syndrome. Chin Med J 117(5):652–656

Chen Q et al (1998) Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 392(6673):293–296

Chen Z, Mu J, Chen X, Dong H (2016) Sudden unexplained nocturnal death syndrome in Central China (Hubei): a 16-year retrospective study of autopsy cases. Medicine (Baltimore) 95(9):e2882

Cheng J et al (2011) Sudden unexplained nocturnal death syndrome in southern China: an epidemiological survey and *SCN5A* gene screening. Am J Forensic Med Pathol 32(4):359–363

Daimi H, Khelil AH, Hamda KB, Aranega A, Chibani JB, Franco D (2015) Absence of family history and phenotype-genotype correlation in pediatric Brugada syndrome: more burden to bear in clinical and genetic diagnosis. Pediatr Cardiol 36(5):1090–1096

Elger BS, Michaud K, Fellmann F, Mangin P (2010) Sudden death: ethical and legal problems of post-mortem forensic genetic testing for hereditary cardiac diseases. Clin Genet 77(3):287–292

Gervacio-Domingo G, Punzalan FE, Amarillo ML, Dans A (2007) Sudden unexplained death during sleep occurred commonly in the general

population in the Philippines: a sub study of the National Nutrition and health survey. J Clin Epidemiol 60(1):567–571
Gotoh K (1976) A histopathological study on the conduction system of the so-called "Pokkuri disease" (sudden unexpected cardiac death of unknown origin in Japan). Jpn Circ J 40(7):753–768
Gouas L et al (2005) Association of *KCNQ1*, *KCNE1*, *KCNH2* and *SCN5A* polymorphisms with QTc interval length in a healthy population. Eur J Hum Genet 13(11):1213–1222
Hoshi M et al (2014) Brugada syndrome disease phenotype explained in apparently benign sodium channel mutations. Circ Cardiovasc Genet 7(2):123–131
Hu D, Ziv E (2008) Confounding in genetic association studies and its solutions. Methods Mol Biol 448:31–39
Huang L, Liu C, Tang S, Su T, Cheng J (2014) Postmortem genetic screening of SNPs in *RyR2* gene in sudden unexplained nocturnal death syndrome in the southern Chinese Han population. Forensic Sci Int 235:14–18
Huang L, Tang S, Peng L, Chen Y, Cheng J (2016) Molecular autopsy of Desmosomal protein Plakophilin-2 in sudden unexplained nocturnal death syndrome. J Forensic Sci 61(3):687–691
Keller DI et al (2005) Brugada syndrome and fever: genetic and molecular characterization of patients carrying *SCN5A* mutations. Cardiovasc Res 67(3):510–519
Liu C et al (2013) Postmortem molecular analysis of *KCNQ1*, *KCNH2*, *KCNE1* and *KCNE2* genes in sudden unexplained nocturnal death syndrome in the Chinese Han population. Forensic Sci Int 231(1–3):82–87
Liu C et al (2014) Is sudden unexplained nocturnal death syndrome in southern China a cardiac sodium channel dysfunction disorder? Forensic Sci Int 236:38–45
Magnani JW et al (2014) CHARGE Consortium; NHLBI Exome Sequencing Project (ESP); UK10K: sequencing of *SCN5A* identifies rare and common variants associated with cardiac conduction: cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium. Circ Cardiovasc Genet 7(3):365–373
Mohanty S et al (2016) Novel association of polymorphic genetic variants with predictors of outcome of catheter ablation in atrial fibrillation: new directions from a prospective study (DECAF). J Intervent Cardiol Electrophysiol 45(1):7–17
Munger RG, Booton EA (1998) Bangungut in Manila: sudden and unexplained death in sleep of adult Filipinos. Int J Epidemiol 27(4):677–684
Nademanee K et al (1997) Arrhythmogenic marker for the sudden unexplained death syndrome in Thai men. Circulation 96(8):2595–2600
Poelzing S et al (2006) *SCN5A* polymorphism restores trafficking of a Brugada syndrome mutation on a separate gene. Circulation 114(5):368–376
Qureshi SF et al (2015) Mutational analysis of *SCN5A* gene in long QT syndrome. Meta Gene 6:26–35
Sangwatanaroj S, Yanatasneejit P, Sunsaneewitayakul B, Sitthisook S (2002) Linkage analyses and *SCN5A* mutations screening in five sudden unexplained death syndrome (Lai-tai) families. J Med Assoc Thail 85(Suppl1):S54–S61
Shinlapawittayatorn K, Du XX, Liu H, Ficker E, Kaufman ES, Deschênes I (2011) A common *SCN5A* polymorphism modulates the biophysical defects of *SCN5A* mutations. Heart Rhythm 8(3):455–462
Srettabunjong S (2019) Sudden unexplained nocturnal death syndrome: epidemiological and morphological characteristics in Thai autopsy cases. J Forensic Sci 64(3):773–777
Tatsanavivat P et al (1992) Sudden and unexplained deaths in sleep (Laitai) of young men in rural northeastern Thailand. Int J Epidemiol 21(5):904–910
Tungsanga K, Sriboonlue P (1993) Sudden unexplained death syndrome in north-East Thailand. Int J Epidemiol 22(1):81–87
Undrovinas AI, Fleidervish IA, Makielski JC (1992) Inward sodium current at resting potentials in single cardiac myocytes induced by the ischemic metabolite lysophosphatidylcholine. Circ Res 71(5):1231–1241
Uzieblo-Zyczkowska B, Gielerak G, Siedlecki P, Pająk B (2014) Genetic diversity of *SCN5A* gene and its possible association with the concealed form of Brugada syndrome development in polish group of patients. Biomed Res Int 2014:462609
van den Berg MP et al (2002) A large family characterised by nocturnal sudden death. Neth Heart J 10(7–8):304–312
Vatta M et al (2002) Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. Hum Mol Genet 11(3):337–345
Viswanathan PC, Benson DW, Balser JR (2003) A common SCN5A polymorphism modulates the biophysical effects of an SCN5A mutation. J Clin Invest 111(3):341–346
Watanabe H, Minamino T (2016) Genetics of Brugada syndrome. J Hum Genet 61(1):57–60
Ye B, Valdivia CR, Ackerman MJ, Makielski JC (2003) A common human *SCN5A* polymorphism modifies expression of an arrhythmia causing mutation. Physiol Genomics 12(3):187–193
Zhao Y et al (2015) Post-transcriptional regulation of cardiac sodium channel gene SCN5A expression and function by miR-192-5p. Biochim Biophys Acta 1852(10 Pt A):2024–2034
Zheng J et al (2015) A case-control study of sudden unexplained nocturnal death syndrome in the southern Chinese Han population. Am J Forensic Med Pathol 36(1):39–43

Publisher's Note