Forensic Science International 236 (2014) 38–45

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Is sudden unexplained nocturnal death syndrome in Southern China a cardiac sodium channel dysfunction disorder?

Chao Liu [a,1], David J. Tester [b,1], Yiding Hou [c,1], Wen Wang [c], Guoli Lv [a], Michael J. Ackerman [b], Jonathan C. Makielski [d], Jianding Cheng [c,*]

[a] Guangzhou Institute of Forensic Science, Guangzhou 510030, China
[b] Departments of Medicine, Pediatrics, and Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, MN 55905, United States
[c] Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China
[d] Division of Cardiovascular Medicine, Department of Medicine, University of Wisconsin, Madison, WI 53792, United States

ARTICLE INFO

Article history:
Received 1 September 2013
Received in revised form 27 November 2013
Accepted 27 December 2013
Available online 7 January 2014

Keywords:
Sudden unexplained nocturnal death syndrome (SUNDS)
SCN5A gene
Cardiac sodium channel β subunits
MOG1 gene
GPD1-L gene

ABSTRACT

Sudden unexplained nocturnal death syndrome (SUNDS) remains an enigma to both forensic pathologists and physicians. Previous epidemiological, clinical, and pilot genetic studies have implicated that SUNDS is most likely a disease allelic to Brugada syndrome (BrS). We have performed postmortem genetic testing to address the spectrum and role of genetic abnormalities in the *SCN5A*-encoded cardiac sodium channel and its several associated proteins in SUNDS victims from Southern China. Genomic DNA extracted from the blood samples of 123 medico-legal autopsy-negative SUNDS cases and 104 sex-, age- and ethnic-matched controls from Southern China underwent comprehensive amino acid coding region mutational analysis for the BrS associated genes *SCN5A*, *SCN1B*, *SCN2B*, *SCN3B*, *SCN4B*, *MOG1*, and *GPD1-L* using PCR and direct sequencing. We identified a total of 7 unique (4 novel) putative pathogenic mutations (all in SCN5A; V95I, R121Q [2 cases], R367H, R513H, D870H, V1764D, and S1937F) in 8/123 (6.5%) SUNDS cases. Three SCN5A mutations (V95I, R121Q, and R367H) have been previously implicated in BrS. An additional 8 cases hosted rare variants of uncertain clinical significance (SCN5A: V1098L, V1202M, R1512W; SCN1B: V138I [3 cases], T189M [2 cases]; SCN3B: A195T). There were no non-synonymous mutations found in *SCN2B*, *SCN4B*, *MOG1*, or *GPD1-L*. This first comprehensive genotyping for SCN5A and related genes in the Chinese Han population with SUNDS discovered 13 mutations, 4 of them novel, in 16 cases, which suggests cardiac sodium channel dysfunction might account for the pathogenesis of 7–13% of SUNDS in Southern China.

1. Introduction

As an ethnic and region specific natural death, sudden unexplained nocturnal death syndrome (SUNDS) or sudden unexplained death during sleep (SUDS), is a disorder that prevails predominantly in Southeast Asia [1] and has various synonyms in different countries such as the Philippines (bangungut) [2], Thailand (lai-tai) [1], Japan (pokkuri) [3], and China (sudden manhood death syndrome) [4]. The annual incidence of SUNDS has been reported to be as high as 43 per 100,000 people aged 20–40 years in the Philippines [5] and 38 per 100,000 people aged 20–49 years in Thailand [6]. In Southern China, the incidence is about 1 per 100,000 people [4]. These reported worldwide syndromes have an unusual clinical phenotype [1–4] in common: the vast majority of victims were apparently healthy young males between 20 and 50 years old; death mostly occurred at night during sleep with symptoms of moaning, tachypnea, and abrupt tic of limbs; gross autopsy and microscopic findings showed no morphological changes to elucidate the cause of death. Since its initial description in 1917 in the Philippines [2], SUNDS has remained an enigma to both forensic pathologists and clinicians.

The ECG characteristics, as well as clinical phenotype among SUNDS survivors [1,7], strongly suggested that SUNDS is similar to Brugada syndrome (BrS), which is associated with loss-of-function mutations in the *SCN5A*-encoded cardiac sodium channel α subunit [8,9]. The linkage of *SCN5A* gene variation and SUNDS was originally established by Vatta et al. with the identification of three *SCN5A* mutations in 3 out of 10 probands with clinical evidence of SUNDS [10]. This association was subsequently confirmed by us with the identification of a reported BrS associated genetic variant in a sporadic SUNDS victim from Southern China following a

* Corresponding author at: Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-sen University, No. 74, Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China. Tel.: +86 20 87330704; fax: +86 20 87334353.
E-mail address: chengjd@mail.sysu.edu.cn (J. Cheng).
[1] These authors contributed equally to this work.

0379-0738/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.forsciint.2013.12.033

targeted analysis of *SCN5A* [4]. Based on these studies, SUNDS and BrS are now considered to be most likely phenotypically, genetically, and functionally the same allelic disorder [10]. However, only limited genetic studies involving small SUNDS cohorts, single BrS susceptible candidate gene, and non-comprehensive "hot-spot" screening have been completed.

Here, we performed postmortem genetic testing for 123 SUNDS cases from Southern China to determine the spectrum and prevalence of genetic abnormalities of the cardiac sodium channel (Nav1.5) macromolecular complex that have been implicated in the pathogenesis of BrS, including the *SCN5A*-encoded cardiac sodium channel α subunit [8,9], the *SCN1B*, *SCN2B*, *SCN3B*, and *SCN4B*-encoded cardiac sodium channel β subunits [11], *MOG1*-encoded multicopy suppressor of *Gspl* protein [12], and *GPD1-L*-encoded glycerol-3-phosphate dehydrogenase like protein [13].

2. Materials and methods

2.1. Subjects

From 2005 to 2012, 123 sporadic SUNDS cases (mean age = 30.9 ± 7.6 years, range 18–52 years) diagnosed by the Department of Forensic Pathology, Zhongshan School of Medicine, Sun Yat-sen University were collected. The inclusion criteria for SUNDS were as follows: (1) a Chinese Han male ≥ 17 years of age that was (2) previously healthy without any significant disease, (3) prior to experiencing a sudden unexpected death during sleep (4) and had a negative autopsy, toxicology, histology, and death-scene investigation resulting in an unexplained death. Genomic DNA collected from 104 unrelated healthy males (mean age = 33.7 ± 8.2 years, range 20–57 years) from Southern China served as controls. None of the control subjects had a history of syncope or cardiovascular disease. All participants or agents gave informed consent and the principles outlined in the Declaration of Helsinki were followed. The project was approved for human study by the ethics committee of Sun Yat-sen University.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral blood samples using the DNA Blood Midi Kit (Bo kun, Changchun, China). Using previously reported primers (some of primers were modified or designed by Primer Premier 5.0), all coding region exons and relevant exon-intron boundaries for *SCN5A* [14] (GenBank NM_198056.1), *MOG1* (NM_016492.4), *GPD1-L* (NM_015141.3), *SCN1B* (NM_001037.4), *SCN2B* (NM_004588.4), *SCN3B* (NM_001040151.1), and *SCN4B* (NM_174934.3) were PCR amplified. The PCR products were then sequenced in both directions on an ABI 3730XL Automated DNA Sequencer (Applied Biosystems, Foster City, CA). The obtained sequence data were compared with the corresponding normal cDNA sequence.

To assess the allele frequency of each identified genetic variant, 104 unrelated ethnically matched and region specific healthy controls were also sequenced. In order to be considered a putative pathogenic mutation, the genetic variant needed to be non-synonymous and absent in all ethnically matched controls as well as absent in four publicly available exome databases including the 1000 Genome Project [15] (http://www.1000genomes.org/ensembl-browser, n = 1094 subjects; 381 Caucasian, 246 African-American, 286 Asians, and 181 Hispanics), the NHLBI GO Exome Sequencing Project [16] (http://evs.gs.washington.edu/EVS/, n = 6500 subjects; 4300 Caucasians and 2203 African-Americans), the Exome Chip Design [17] (http://genome.sphumich.edu/wiki/Exome_Chip_Design, n = 12,000 subjects), and the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) [18].

2.3. Statistical analysis

Statistical analyses were conducted using SPSS (version 19.0) and a P value <0.05 was considered to be significant. Allele frequencies were calculated for each SNP site by the allele counting method. SNP frequencies in both of the groups were tested for deviation from the Hardy–Weinberg equilibrium using a chi-square test. Differences in genotype frequency and allele frequency between SUNDS cases and healthy controls, or Chinese Han population, or Chinese BrS cases were tested by chi-square test, or Fisher's exact probability test if necessary.

3. Results

3.1. Putative pathogenic mutations in Chinese sporadic SUNDS cases

Overall, 7 unique (4 novel, Fig. 1) SCN5A missense mutations, absent in 104 ethnically matched controls and all publically available exome databases and the international HapMap, were identified in 8/123 (6.5%, average age = 32 ± 7 years) SUNDS cases (Table 1). Two of the SCN5A mutations (V95I and R121Q [seen in 2 cases]) localized to the intracellular N-terminus, three to the Na^+ conducting channel pore region (R367H, D1S5-D1S6; D870H, DIIS5-DIIS6; and V1764D, DIVS6), one within the DI-DII inter-domain linker (R513H), and one (S1937F) in the C-terminus of SCN5A (Fig. 2). The SCN5A mutations V95I and R121Q have been previously reported to cause BrS [19,20]. The R367H-SCN5A mutation was originally reported in a sporadic SUNDS case and revealed a complete loss of sodium current during *in vitro* heterologous expression and electrophysiological studies performed in *Xenopus* oocytes [10]. Parental DNA was unavailable to determine whether these genetic variants represent sporadic *de novo* or familial derived mutations. There were no non-synonymous mutations identified in *GPD1-L*, *MOG1*, *SCN2B* or *SCN4B*.

3.2. Rare non-synonymous variants of uncertain clinical significance in Chinese sporadic SUNDS cases

Besides the 8 cases hosting a putative pathogenic mutation, 8 additional SUNDS cases hosted a rare non-synonymous SCN5A (V1098L, V1202M, and R1512W), SCN1B (V138I and T189M), or SCN3B (A195T) genetic variant with uncertain clinical significance (Table 2 and Fig. 2). While none of these variants were detected in our 104 ethnically matched Han Chinese controls, they have all either been reported in the literature as seen in a healthy control individual [21], listed in one of the publically available exome databases, or present in the international HapMap.

Although absent in all publically available exome databases (>12,000 subjects), the V1098L-SCN5A variant identified in a 26-year-old SUNDS case, was previously seen in 1/131 ostensibly healthy Asian control subjects and the R1512W-SCN5A identified in a 38-year-old SUNDS case, was previously identified in 1/148 Hispanic controls [21]. Interestingly, R1512W-SCN5A was initially identified in a BrS patient by Rook [22] and was subsequently found in another BrS patient by Deschênes [23], and electrophysiological studies showed a slowing of both inactivation and recovery from inactivation in this mutant channel [23]. The SCN5A variant V1202M (Fig. 1) seen in a 32-year-old SUNDS case, was seen in 1/4300 European American exomes of the NHLBI exome sequencing project. The V138I-SCN1B variant was detected in 3/123 (2.4%) SUNDS cases but was also identified in 11/12,000 (0.09%) subjects belonging to the exome chip design and in 3/2203 (0.14%) African American subjects from the NHLBI exome sequencing project. The T189M-SCN1B variant was detected in 2/123 (1.6%) SUNDS cases but also identified in 12/869 (1.5%) unrelated individuals of diverse ethnicities from the international HapMap project. The A195T-SCN3B variant was detected in a