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BRIEF COMMUNICATION

Carrier frequency of spinal muscular atrophy in Thailand

Donniphat Dejsuphong[1] · Aruchalean Taweewongsounton[2] · Pollawat Khemthong[3] · Sermsiri Chitphuk[2] · Wasana Stitchantrakul[2] · Piyamitr Sritara[3] · Atchara Tunteeratum[3] · Thanyachai Sura[3]

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Abstract

Spinal muscular atrophy (SMA) is one of the leading causes of death in infants and young children from heritable diseases. Patients diagnosed with SMA develop symmetrical progressive muscle weakness and atrophy from degeneration of alpha motor neurons. Approximately 95% of patients have a homozygous deletion of survival motor neuron 1 (*SMN1*) gene in exon 7 and inherited in autosomal recessive pattern. Considering the high prevalence of SMA carrier in many population, it is possible that SMA is one of the most common autosomal recessive disorders in Thailand and Southeast Asia. In this study, we analyzed DNA from peripheral blood of 505 healthy Thai adults using quantitative PCR-based for *SMN1* gene exon 7 copy number analysis. Individual samples with heterozygous deletion of *SMN1* gene were confirmed with MLPA. The result identified 9 samples (1.78%) with heterozygous deletion and 39 samples as more than 2 copies of *SMN1*. No homozygous deletion was detected in the samples. In conclusion, we established carrier frequency of SMA in selected Thai population at 1.8% from 505 participants. The prevalence coincides with prevalence in East Asia and Caucasian population. The result could be implemented for SMA carrier screening in couples at risk in the region.

Keywords Spinal muscular atrophy · SMN1 · Thailand

Introduction

Spinal muscular atrophy (SMA), a prevalent childhood onset neurogenetic disorder, is one of the major causes of death in infants and young children from heritable diseases. Patients diagnosed with SMA usually develop symmetrical progressive muscle weakness, and atrophy from degeneration of alpha motor neurons results in poor feeding, failure to thrive, respiratory failure, and death before reaching adulthood. Clinically, SMA is classified into four types ranging from type I (onset 0–6 months with life expectancy of less than 2 years) to type IV (adult onset weakness with longer life expectancy) [1]. At molecular level, SMA causes by mutation in survival motor neuron 1 (*SMN1*) gene on chromosome 5q13.2 with approximately 95% of patients have homozygous exon 7 deletion while the other 5% carry compound heterozygous mutation for exon 7 deletion and point mutation, producing non-functional protein. Most of the patients inherited the mutation from their parents while about 2% of the affected patients have de novo mutation. *SMN2*, a nearly identical gene to *SMN1*, locates on centromeric region of the same chromosome, and differs from *SMN1* in only five base pairs. Although it is a *SMN1* homolog, *SMN2* produces less functional protein due to difference in exon 7 splicing. Several studies showed that in the absence of *SMN1*, increase in *SMN2* copy numbers could partially alleviate the phenotype.

Carrier frequency of *SMN1* deletion has been reported between 1/40 and 1/60 in diverse ethnic groups including many East Asian populations but it is not yet established in Thailand and Southeast Asia [2, 3]. Carrier screening for *SMN1* exon 7 deletion could help establish carrier prevalence and develop national screening program for SMA in Thailand.

✉ Donniphat Dejsuphong
donniphat.dej@mahidol.ac.th

✉ Thanyachai Sura
thanyachai.sur@mahidol.ac.th

[1] Section for Translational Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[2] Research Center, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

[3] Department of Internal Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

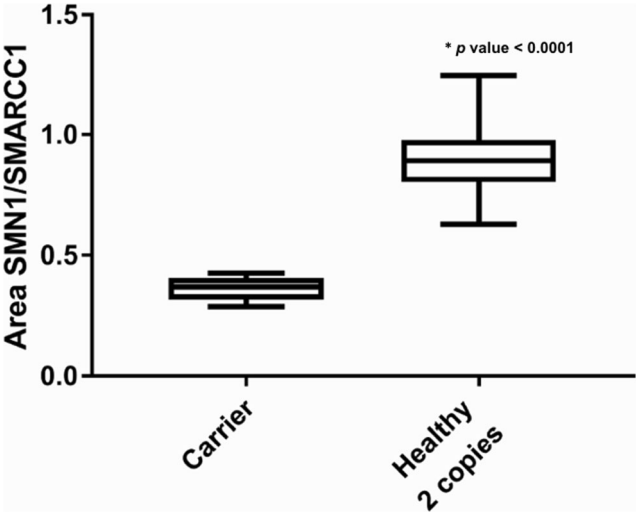

Fig. 1 Copy number analysis by real-time PCR of 505 individual samples (separate file)

Methods

Samples were collected from peripheral blood of employees of the Electricity Generating Authority of Thailand (EGAT, a large Thai state enterprise), with written informed consent from participants. None of the participant was diagnosed of SMA or childhood onset muscle weakness. The study was conducted at Faculty of Medicine Ramathibodi Hospital, Mahidol University in Bangkok, Thailand and approved by the ethics committee of the institute.

Analysis of *SMN1* copy number assay was done using quantitative real-time PCR as described in Sugarman et al. [4]. Each reaction contains *SMN1* primers and gene-specific hydrolysis probe with *SMARCC1* primers and hydrolysis probe as a reference gene performed using the BioRad CFX96 Real-Time PCR system (BioRad Laboratories, Hercules, USA). Samples were run in triplicate using the comparative threshold cycle (C_t) method. To differentiate *SMN2* from *SMN1*, a competitive, non-fluorescent, non-hydrolysable *SMN2* probe was added during the PCR reaction to prevent *SMN2* amplification. Obligated carrier and affected individual samples from Ramathibodi Hospital were used for standardization. Samples with heterozygous deletion or ambiguous result from real-time PCR were reanalyzed with multiplex ligation-dependent probe amplification (MLPA) using kit number P060-B2 containing 4 specific *SMN1/SMN2* probes according to manufacturer's protocol (MRC-Holland, Amsterdam, The Netherlands). The MLPA DNA fragments were analyzed on ABI3130XL (Thermo Fisher Scientific, Waltham, USA) with Coffalyser.net software (MRC-Holland, Amsterdam, The Netherlands).

Results

A total of 505 DNA samples from participants were analyzed with real-time PCR for *SMN1* copy number according to previously published work [4]. Using Taqman probes and *SMN2* inhibitor probe, the result would yield a *SMN1*-specific copy numbers without amplification of *SMN2* due to inhibitor probe. As a result, nine samples were identified as heterozygous deletion of *SMN1* exon 7 (Fig. 1). The range of *SMN1/SMARCC1* ratio in heterozygous carriers was between 0.29 and 0.43 (mean 0.36) and between 0.62 and 1.25 (mean 0.90) for two copies of specimen. These samples were reanalyzed with MLPA to confirm single copy of *SMN1* gene in individual sample, and the result was in agreement with real-time PCR data. The carrier frequency of 9/505 (approximately 1.78%) is comparable with other Asian population in previous reports; these include Korean and Chinese in Asia and Asian ethnics in the USA (1.6–2.1%, Table 1). Among 505 samples, 30 subjects (5.94%) carried three copies of *SMN1* and 9 subjects (1.78%) carried four or more copies (Table 2). No zero copy of *SMN1* was identified in the sample population.

Carrier screening using copy number analysis of *SMN1* gene with quantitative PCR could detect *SMN1* copy in the genome; however, this technique could not differentiate cis configuration of *SMN1* where two alleles of *SMN1* gene located on the same chromosome. According to the previous study, a cis configuration of *SMN1* prevalence is higher in Black American [4] and could affect carrier

Table 1 Carrier frequency with *SMN1* heterozygous deletion in Asian population

Population	Percent carrier	Total samples	Method	Reference
Asian [5] (SA)	1.80%	1009	Real-time qPCR	Hendrickson et al. 2009
Asian [4] (SA)	1.57%	4647	Real-time qPCR	Sugarman et al. 2012
Korean [6]	2.00%	100	MLPA	Yoon et al. 2010
Chinese [7] (mainland)	1.90%	212	MLPA	Fang et al. 2015
Chinese [8] (Hong Kong)	1.60%	569	Real-time qPCR	Chan et al. 2004
Chinese [9] (Taiwan)	2.10%	107,611	DHPLC	Su et al. 2011
Thai	1.78%	505	Real-time qPCR	(This study)

Table 2 Genotype of SMA carrier in Thai EGAT population

Copy number of *SMN1* per genome	Number of samples
0 copy	0
1 copy	9 (1.78%)
2 copies	457 (90.50%)
3 copies	30 (5.94%)
4 copies or more	9 (1.78%)

status interpretation where two copies subject could be either 1 + 1 or 2 + 0 allele configurations. Additionally, an approximate 5% of affected patients are compound of point mutation on one allele and deletion on the other [1]. Both cis-allele configuration of *SMN1* and point mutation could not be detected with quantitative PCR technique, and this should be taken into account for evaluation of test limitation.

Discussion

Severe form of SMA is one of the leading causes of infant mortality from genetic diseases. Milder form of SMA affects quality of life for both patients and their caregivers. Published data, together with our work, show that carrier frequency of Southeast and East Asian populations (1.6–2.1%) are similar to Caucasian (2.2%), whereas the prevalence of single *SMN1* allele is considerably lower in black (1%) and Hispanic (1.3%) ethnic groups [3]. In Thailand, thalassemia screening is the only prenatal genetic test offered to couples during antenatal care. Carrier screening of thalassemia effectively decreases newborn with severe thalassemia in the country over two decades. Since the data of SMA prevalence is limited, it is important to establish prevalence in the population. Planning to offer SMA testing during antenatal visit could greatly benefit SMA prevention in the country.

Considering severity of the disease and relatively high frequency of carrier in general population, the American College of Obstetricians and Gynecologists and American College of Medical Genetics and Genomics recommend prenatal carrier screening of SMA in couples regardless of race or ethnicity [10]. Screening of SMA carrier during early antenatal care or before conception will be able to recognize the risk of the baby born with SMA, allow carriers to make informed decision to prevent morbidity and mortality of the disease in the newborn. As cost of genetic screening becoming more affordable, expanded genetic screening program will improve planning for healthcare resources especially in the country with universal healthcare coverage such as Thailand. Our study establishes prevalence of SMA in the selected Thai population. The result could be implemented for SMA prevention in Thailand and Southeast Asia region in which combined population are more than 600 millions.

Authors' contributions DD and TS planned and designed the experiments. PK, ATu, and PS collected and provided the samples. DD, ATa, SC, and WS performed the experiments. DD and Ata analyzed the data. DD and TS wrote the paper. All authors read and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance and approved with the ethical standards of the institutional research committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (ID-05-51-19 V), and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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