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Original article

Screening of the *LIX1* gene in Japanese and Malaysian patients with SMA and/or SMA-like disorder

Teguh Haryo Sasongko [a,1], Gunadi [d,e,1], Surini Yusoff [d], Amin Baig Atif [a], Hayati Fatemeh [a], Abdulqawee Rani [a], Marzuki Marini [a], Che Badariah Ab Aziz [c], ZAMH Zabidi-Hussin [b], Hisahide Nishio [d], Bin Alwi Zilfalil [b,*]

[a] *Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia*
[b] *Department of Paediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia*
[c] *Department of Physiology, School of Medical Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia*
[d] *Department of Genetic Epidemiology, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan*
[e] *Laboratory for Neocortical Development, RIKEN Center for Developmental Biology, Kobe, Hyogo, Japan*

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Abstract

Background: The majority of spinal muscular atrophy (SMA) patients showed homozygous deletion or other mutations of *SMN1*. However, the genetic etiology of a significant number of SMA patients has not been clarified. Recently, mutation in the gene underlying cat SMA, *limb expression 1* (*LIX1*), has been reported. Similarity in clinical and pathological features of cat and human SMA may give an insight into possible similarity of the genetic etiology. *Patients and methods:* In this study, we screened for a mutation in *LIX1* using direct DNA sequencing in our SMA and/or SMA-like patients who retained *SMN1*. A total of 33 patients were enrolled in this study, of which 22 were Japanese and 11 were Malaysians. All these patients possessed at least two copies of *SMN1*. *Results:* We did not identify any pathogenic mutations in the coding regions or splice sites of *LIX1* in the patients. In addition, we described a polymorphism within *LIX1* intron 3, c.387 + 107A > T. We found that A-allele is significantly more frequent in SMA patients compared to normal individuals. *Conclusion:* Molecular genetic analysis of our SMA and/or SMA-like patients suggests that *LIX1* is not associated with the development of their disorders. However, the number of patients analyzed in this study was very limited, and a larger study with bigger sample size is needed to confirm this result.

Keywords: Spinal muscular atrophy; Non-deleted *SMN1*; *LIX1*; SNP; c.387+107A>T; Direct sequencing

1. Introduction

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by degeneration of motor neurons in the anterior horn of spinal cord, resulting in weakness of the proximal limb and trunk muscles. According to the disease severity, childhood-onset SMA is classified into three types: type I (severe form, unable to sit unsupported), type II (intermediate form, unable to stand or walk unsupported), and type III (mild form, able to stand or walk unsupported with gait disturbance) [1].

In humans, all three clinical subtypes were mapped to chromosome 5q11.2-13.3 [2,3]. The *SMN1*, *SMN2* and *NAIP* genes were subsequently identified in this SMA-related region [4,5]. *SMN1* and *SMN2* are almost identical genes that encode the same protein [4].

* Corresponding author. Address: Department of Pediatrics, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu, 16150 Kelantan, Malaysia. Tel.: +609 7663641; fax: +609 7647642.
E-mail address: zilfalil@kb.usm.my (B.A. Zilfalil).
[1] These two authors contributed equally to the work.

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SMN1 is now recognized as an SMA-causing gene since the majority of SMA patients showed a homozygous disruption of *SMN1* by deletion, rearrangement, or mutation [4,6,7]. Meanwhile, the *SMN2* and *NAIP* genes have been characterized as modifying factors of the clinical severity of SMA [8–12].

While the abnormality of *SMN1* is found in most SMA patients, the genetic etiology of a significant number of SMA patients has not been identified. Recently, Fyfe et al. [13] identified *limb expression 1* (*LIX1*) as a responsible gene for cat SMA. The cat SMA exhibited juvenile-onset skeletal muscle atrophy and weakness with onset at around 12 weeks of age, which are similar to human SMA type III. In addition, the inheritance pattern for cat SMA was also noted to be autosomal recessive [14].

The similarity of clinical and pathological features between cat and human SMA as well as similarity of *LIX1* and SMN proteins function and amino acid sequence may give insights into the possibility of *LIX1* involvement in human SMA. Furthermore, as is the case of SMN, the predicted secondary structure of *LIX1* is compatible with a role in RNA metabolism. Interestingly, unlike *SMN, LIX1* expression is largely restricted to the central nervous system, the fact of which may support *LIX1* involvement in SMA pathology [13,15]. However, there is so far no evidence to show the involvement of *LIX1,* which is located in chromosome 5q15, in human SMA. In this study we searched for a mutation of *LIX1* in Japanese and Malaysian patients with SMA and/or SMA-like disorder who retained *SMN1*.

2. Patients and methods

2.1. Patients

DNA samples were obtained from patients referred to the Department of Genetic Epidemiology Kobe University Graduate School of Medicine, Japan or Human Genome Center School of Medical Sciences Universiti Sains Malaysia, Malaysia for molecular genetic analysis of SMA. The diagnosis was based on the clinical criteria as setup by the 59th and 93rd ENMC International Workshop on SMA in 1998 and 2001 respectively [16,17]. We described a few patients with additional symptoms as SMA-like. The clinical information of the patients is shown in Table 1. All patients involved in this study have given their informed consent prior to blood taking.

2.2. DNA extraction, SMN1 and NAIP deletion test

DNA sample of Japanese patients was extracted from whole blood using a DNA extraction kit, SepaGene (Sanko Junyaku, Tokyo, Japan). DNA sample of Malaysian patients was extracted from whole blood using a DNA extraction kit, GeneAll® Exgene™ Blood SV (GeneAll Biotechnology Co. Ltd., Korea). The *SMN1* exon 7 and 8 were amplified with the primer sets designed by van der Steege et al. [18] and digested with DraI for exon 7 and DdeI for exon 8. The details of the analysis have been described in our previous report [19]. Exon 5 of the *NAIP* gene was detected using the published PCR method of Roy et al. [5].

2.3. Quantitative real-time PCR of the SMN1 copy numbers

The copy number of *SMN1* was determined by quantitative real-time PCR according to our previous method [11].

2.4. PCR and direct DNA sequencing of LIX1

PCR amplification of all the exons and splice-site junctions in *LIX1* were performed using the previously designed primers [13,15]. We directly sequenced all PCR products using a BigDye Terminator V3.0 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. The sequencing reaction product was electrophoresed in a genetic analyzer (ABI Prism 310; Applied Biosystems) and analyzed using DNA Sequencing Analysis Software (Applied Biosystems).

3. Results

3.1. Deletion and copy number analysis of SMN1, and deletion analysis of NAIP

All the patients in this study were diagnosed to have SMA and/or SMA-like disorders (Table 1). All patients were confirmed to have no deletion of *SMN1* gene by PCR-RFLP according to the method of van der Steege [18]. To rule out the possibility of compound heterozygous mutations within *SMN1*, we confirmed that all patients carry at least two copies of *SMN1* using quantitative real-time PCR according to our own protocols [11]. The *NAIP* gene was analyzed by PCR method described by Roy et al. [5]. All patients showed the presence of *NAIP* (data not shown).

3.2. Mutation analysis of LIX1

DNA samples were amplified using primers spanning each exon and adjacent introns of *LIX1*. Subsequently, the PCR products were sequenced. No obvious pathogenic changes were identified in either exons or splice sites of *LIX1* in the 33 patient samples.

3.3. A SNP within LIX1 intron 3

A SNP (c.387+107A>T) was found within *LIX1* intron 3 (Fig. 1). We compared the data of SMA