

Analysis of the Survival Motor Neuron and Neuronal Apoptosis Inhibitory Protein Genes in Malay Patients with Spinal Muscular Atrophy

B A Zilfalil, (MMed)*, A M H Zabidi-Hussin, (FRCPC)*, M S Watihayati, (BSc), M Y Rozainah, (BSc)**, L Naing, (M.Med.Stat)***, R Sutomo (PhD)****, H Nishio, (PhD)****, M Y Narazah, (PhD)**, M Matsuo, (PhD)*******

*Department of Pediatrics, **Human Genome Centre, School of Medical Sciences and ***Biostatistician, School of Dental Sciences, Universiti Sains Malaysia, Malaysia, ****Department of Public Health and *****Department of Pediatrics, Kobe University School of Medicine, Japan

Summary

In Malaysia, Spinal Muscular Atrophy (SMA) is diagnosed based on clinical observation with or without muscle biopsy. Molecular analyses of the SMA-related genes have not been available so far. In this preliminary study, we searched for homozygous deletion of Survival Motor Neuron (SMN1) and Neuronal Apoptosis Inhibitory Protein (NAIP) genes in Malay patients with SMA and found homozygous deletion of SMN1 exon 7 and 8 in all the patients while homozygous deletion of NAIP exon 5 was detected in only our type 1 patients but not in the type 3 patient. To the best of our knowledge, these are the first SMA cases diagnosed at the molecular level in Malaysia.

Key Words: *Spinal Muscular Atrophy (SMA), Survival Motor Neuron (SMN) gene, Neuronal Apoptosis Inhibitory Protein (NAIP) gene*

Introduction

Spinal Muscular Atrophy (SMA) is a lethal disease. In Malaysia, SMA is diagnosed based on clinical observation with or without muscle biopsy as most parents refuse consent for the invasive procedure. Molecular analyses of the SMA-related genes, which require just a small amount of blood, have not been available so far.

The SMA related genes are the Survival Motor Neuron (SMN) 1, SMN2 and Neuronal Apoptosis Inhibitory Protein (NAIP) genes. Most SMA patients irrespective of clinical severity show homozygous deletion of the SMN1 gene^{1,2}. However, homozygous deletion of the NAIP gene was found to be associated with the clinical

severity³. In this preliminary study, we searched for homozygous deletion of SMN1 and NAIP genes in Malay patients with SMA.

Objective

To identify the homozygous deletion of SMN1 exons 7 and 8 and NAIP exon 5 in Malay patients with SMA.

Materials and Methods

The protocols for this study were approved by the Ethics Committee of Universiti Sains Malaysia. Three unrelated Malay patients who fulfilled the diagnostic criteria for SMA as defined by the International SMA Consortium were enrolled into the study. Patients 1

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Corresponding Author: B A Zilfalil, Department of Pediatrics, School of Medical Sciences, Universiti Sains Malaysia
16150 Kubang Kerian, Kota Bharu, Kelantan

and 2 had a severe form (SMA type 1) while Patient 3 had a mild form (SMA type 3). Genomic DNA was extracted from 3 ml of whole blood from the patients after obtaining their informed consent. The analysis for the SMN genes was performed according to the method of van der Steege et al⁴. PCR was done to amplify the gene using primer sets for SMN exon 7 and another primer set for exon 8. To discriminate between the SMN1 and SMN2 gene products, the PCR product was then subjected to Dra I and Dde 1 restriction enzyme treatment. Dra I restriction enzyme digests only the exon 7 of SMN2 gene products, and not the SMN1 gene products, while Dde1 restriction enzyme digests only the exon 8 of SMN 2 and not the SMN 1 gene products. The final products were electrophoresed in 3% agarose gel and visualized by ethidium bromide staining. PCR amplification of the NAIP gene was performed according to the method of Roy et al³. PCR was done

to amplify NAIP exon 5. The PCR products were electrophoresed in 3% agarose gels and visualized by ethidium bromide staining.

Results

In control samples, non-digested and digested products of SMN1 and SMN2 exons 7 and 8 were seen on the gel (Figures 1 and 2). However, in the three patients, non-digested products of SMN1 exons 7 and 8 were not seen, but only digested products of SMN2 exons 7 and 8 were seen on the gel (Figure 1 and 2). According to the findings, SMN1 exons 7 and 8 were completely absent, but SMN2 exons 7 and 8 were retained in these patients. The PCR products of NAIP exon 5 were seen on the gel for control samples and the Patient 3 but not for Patients 1 and 2 (Figure 3).

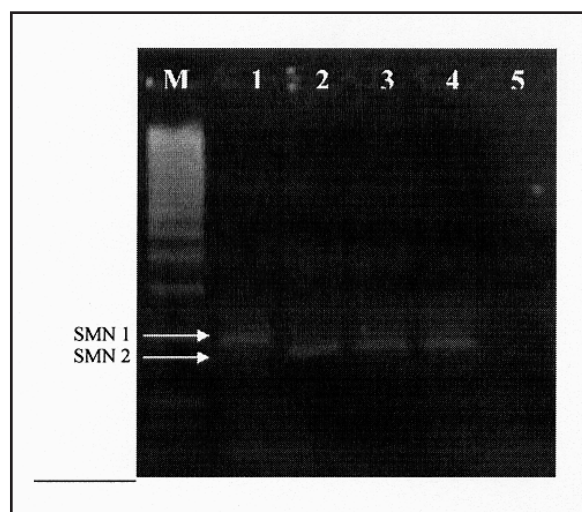


Fig. 1: SMN 1 and SMN 2 genes exon 7.

Deletion of exon 7 of SMN 1 gene is seen in Lane 2, 3 and 4.

Lane M: Ladder

Lane 1: Healthy control

Lane 2: Patient 1

Lane 3: Patient 2

Lane 4: Patient 3

Lane 5: PCR control (water)

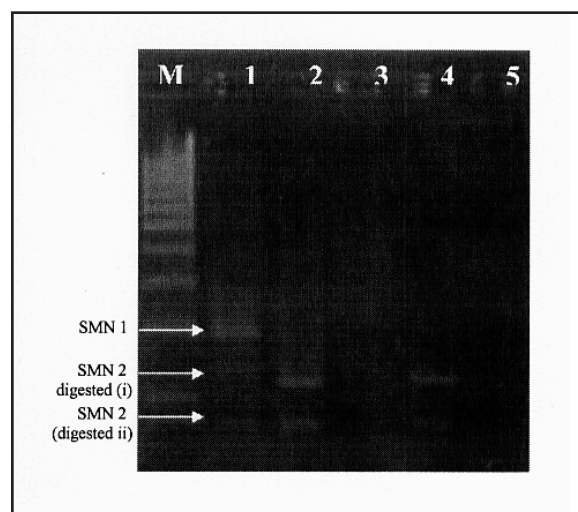


Fig. 2: SMN 1 and SMN 2 genes exon 8.

Deletion of exon 8 of SMN 1 gene is seen in Lane 2, 3 and 4.

Lane M: Ladder

Lane 1: Healthy control

Lane 2: Patient 1

Lane 3: Patient 2

Lane 4: Patient 3

Lane 5: PCR control (water)

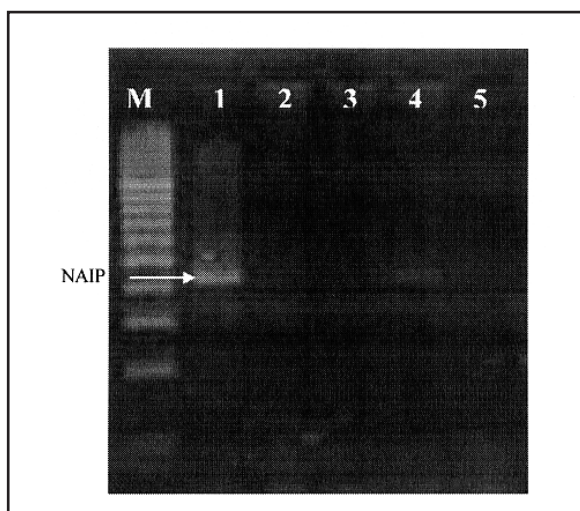


Fig. 3: NAIP gene exon 5.

Deletion of exon 5 of NAIP gene is seen in Lane 2 and 3.
 Lane M: Ladder
 Lane 1: Healthy control
 Lane 2: Patient 1
 Lane 3: Patient 2
 Lane 4: Patient 3
 Lane 5: PCR control (water)

Discussion

We identified homozygous deletion of SMN1 exon 7 and 8 in all our 3 SMA patients. These results correspond to the findings of Levebvre et al¹ and Erdem et al² who found more than 90% of their patients carried the homozygous deletion of SMN1 exon 7 and 8. Homozygous deletion of NAIP exon 5 is detected in only our type 1 patients but not in the type 3 patient. This finding is similar to Roy et al³ and Akutsu et al⁵ and who have demonstrated a correlation between deletion of the NAIP gene and the severity of SMA.

To the best of our knowledge, these are the first SMA cases diagnosed at the molecular level in Malaysia. However, further larger studies need to be done before we can make any firm conclusion regarding the SMN and NAIP genes in the Malay population.

Acknowledgements

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