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Molecular Genetic Analyses of Five Vietnamese Patients with Spinal Muscular Atrophy

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Most patients with spinal muscular atrophy (SMA) have been reported to show homozygous deletion of the gene responsible for SMA, *SMN1*. However, whether SMA patients with homozygous deletion of the gene exist in Southeast Asian countries, including Vietnam, remains to be determined, because molecular genetic analyses of SMA patients from these countries have not been reported. In this preliminary study, we analyzed five Vietnamese SMA patients and found that *SMN1* gene exons 7 and 8 were completely absent in one of them, a 6-month-old girl with hypotonic muscles. Thus, homozygous deletion of the gene can be a cause of SMA in Vietnam, although other genetic abnormalities should be considered as etiological factors in many cases. In conclusion, we identified a homozygous deletion of the *SMN1* gene in a Vietnamese SMA patient. Since the number of the patients analyzed in this study was very limited, it is too early to determine whether homozygous deletion of the gene is not a main cause of SMA in Vietnam.

Spinal muscular atrophy (SMA) is one of the most common neuromuscular disorders resulting from the degeneration of anterior horn cells of the spinal cord. SMA is clinically classified into three subtypes based on the age at onset and severity: type I (severe form with onset before the age of 6 months, unable to sit without support, also called "Werdnig-Hoffmann disease"); type II (intermediate form with onset before the age of 18 months, unable to stand or walk without aid) and type III (mild form with onset after the age of 18 months, able to stand and walk, also called "Kugelberg-Welander disease") [17].

Genetic linkage studies have mapped all three subtypes of SMA to chromosome 5q13 [3,11,16] and, so far, two major SMA-related genes have been identified in this region: the neuronal apoptosis inhibitory protein gene (*NAIP*) [21] and the survival motor neuron gene (*SMN*) [14]. However, the functional role of the *NAIP* gene in the pathogenesis of SMA is not clear, because *NAIP* deletion has been seen in some control individuals with no phenotypic evidence of SMA [21].

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Two highly homologous copies of the *SMN* gene, *SMN1* and *SMN2*, are present within the 5q13 region, [14]. According to the previous reports, the *SMN1* gene is homozygously deleted in more than 90% of SMA patients [1,6,9,10,12,14,20,22,23,27,30] and deleteriously mutated in the remainder [2, 4,5,8,13,14,15,18,19,24,25,28,29], providing strong evidence that the *SMN1* gene is a gene responsible for SMA. In control subjects, the *SMN1* gene is not absent, whereas the *SMN2* gene is absent in about 4% with no pathological consequences [14].

Most Japanese and Chinese patients with SMA are homozygous for *SMN1* deletion [1,6,22]. However, whether homozygous deletion of the *SMN1* gene is also a cause of SMA in Southeast Asian countries including Vietnam remains to be elucidated, because molecular genetic analyses of SMA patients from these countries have not been reported. In order to determine whether homozygous *SMN1* deletion may be a cause of SMA in Vietnam, we used a polymerase chain reaction (PCR) and enzyme digestion method to analyze the molecular genetic features of five Vietnamese patients.

PATIENTS AND METHODS

Patients. After obtaining informed consent, we analyzed the molecular genetic features of five unrelated Vietnamese patients (Patients 1, 2, 3, 4 and 5). Neither electromyography nor muscle biopsy was performed in any patients. However, each patient fulfilled the diagnostic criteria for SMA defined by the International SMA Consortium [17].

Patient 1 was a 15-year-old Vietnamese boy, diagnosed as having type III SMA. He had had difficulty in walking since he was 4 years old. His limbs showed proximal muscular atrophy and weakness, although his intelligence was normal.

Patient 2 was a 3-year-old Vietnamese boy, diagnosed as having type II SMA. He was able to sit without support, but had never been able to crawl, stand or walk. His limbs showed proximal muscular atrophy and weakness, although his mental development was normal.

Patient 3 was a 5-year-old Vietnamese girl, diagnosed as having type III SMA. She had had difficulty in walking since she was 4 years old. Her limbs showed proximal muscular atrophy and weakness, although her intelligence was normal.

Patient 4 was a 4-year-old Vietnamese girl, diagnosed as having type II SMA. She was able to sit without support, but had never been able to crawl, stand or walk. Her limbs showed proximal muscular atrophy and weakness, although her mental development was normal.

Patient 5 was a 6-month-old Vietnamese girl, suspected of having type I-II SMA. Her muscle hypotonus was noticed at 3 months old.

PCR and enzyme-digestion analysis of *SMN1* exons 7 and 8. Genomic DNA was extracted from 3 ml of whole blood using a DNA extraction kit, SepaGene® (Sanko Junyaku Co., Ltd, Tokyo, Japan). PCR amplification was performed according to the method of van der Steege et al. [26]. The oligonucleotide primers for exons 7 of the *SMN1* and *SMN2* genes were R111 [14] and X7-Dra [26] and those for exons 8 of the *SMN1* and *SMN2* genes were 541C960 [14] and 541C1120 [14]. To discriminate between *SMN1* and *SMN2* gene products, the PCR products were digested with *Dra I* (Takara Biomedicals, Shiga, Japan) for exon 7 and *Dde I* (Takara Biomedicals) for exon 8 and the digested products were electrophoresed in 3% (W/V) agarose gels and visualized by ethidium bromide staining. To make sure of complete digestion, genomic DNA from a Japanese patient with SMA (Patient JP) was simultaneously analyzed. Patient JP was Case 6 in Table I of Akutsu et al. [1], and he lacked *SMN1* exons 7 and 8, and *NAIP* exon 5.

PCR amplification of *NAIP* exon 5. PCR amplification of the *NAIP* exon 5 was performed according to the method of Roy et al. [21]. Here, we have used the term "exon 5" as a widely accepted exon number, although this exon has also been called "exon 4" by Chen et al [7]. The PCR products were electrophoresed in 3% (W/V) agarose gels and visualized by ethidium bromide staining.

RESULTS

Deletion analysis of *SMN1* exons 7 and 8. The SMN genes, *SMN1* and *SMN2*, were analyzed by the PCR and enzyme-digestion method described by van der Steege et al. [26]. Restriction enzyme *Dra I* cleave the PCR-amplified fragments of *SMN 2* exon 7 and restriction enzyme *Dde I* cleave the PCR-amplified fragments of *SMN 2* exon 8. On the contrary, *Dra I* does not cleave the PCR-amplified fragments of *SMN 1* exon 7 or *Dde I* does not cleave the PCR-amplified fragments of *SMN 1* exon 8. Thus, PCR-amplified fragments of *SMN1* exons 7 and 8 can be separated from those of *SMN2* exons 7 and 8 after the restriction enzyme-digestion procedures.

We identified homozygous deletion of *SMN1* exons 7 and 8 in a Vietnamese patient (Patient 5) (Figs. 1A and 1B). Neither *SMN1* exon 7 deletion nor *SMN1* exon 8 deletion was detected in any of the other patients, suggesting that they retained at least one copy of the *SMN1* gene.

Deletion analysis of *NAIP* exon 5. The *NAIP* gene was analyzed by the PCR method described by Roy et al. [21]. All of the Vietnamese patients showed the presence of *NAIP* exon 5(Fig. 1C).

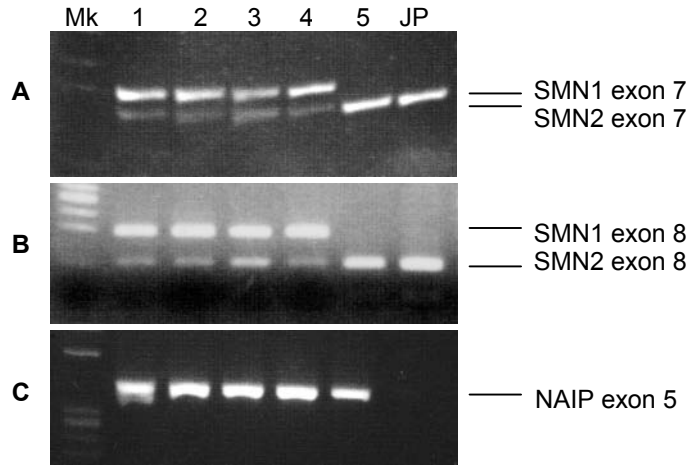

FIG. 1. Deletion analyses of the *SMN1* and *NAIP* genes.

(A) *SMN1* exon 7. Patient 5 and a Japanese SMA patient (JP; a disease control) showed the complete absence (homozygous deletion) of *SMN1* exon 7, whereas other patients showed the presence of *SMN1* exon 7. The marker lane (Mk) contains Hae III-digested PhiX174 DNA fragments.

(B) *SMN1* exon 8. Patient 5 and a Japanese SMA patient (JP) showed the complete absence (homozygous deletion) of *SMN1* exon 8, whereas other patients showed the presence of *SMN1* exon 8. The marker (Mk) is the same as that shown in (A).

(C) *NAIP* exon 5. Only a Japanese SMA patient (JP) showed the complete absence (homozygous deletion) of *NAIP* exon 5, whereas other patients, including Patient 5, showed the presence of *NAIP* exon 5. The marker (Mk) is the same as that shown in (A).

DISCUSSION

Patient 5 in this study is, to the best of our knowledge, the first Vietnamese case of SMA with homozygous deletion of the *SMN1* gene to be reported. SMA was suspected at her first medical examination, but she was so young that the diagnosis could not be confirmed clinically. Our results confirmed the diagnosis of SMA and indicate that *SMN1* deletion can be a cause of SMA in Vietnam.

According to our results, homozygous deletion of the *SMN1* gene was found in only one of five Vietnamese patients with SMA (20%). Compared with the *SMN1* deletion frequency in SMA patients reported from other countries (87-95%) [1,6,9,10,12,14,20,22,23,27,30], the *SMN1* deletion frequency is extremely low in Vietnamese SMA patients. It suggests that other genetic abnormalities than *SMN1* deletion should be considered in Vietnamese SMA patients. However, it is too early to determine whether *SMN1* deletion is not a main cause of SMA in Vietnam, because the number of SMA patients was very limited in this study.

None of our Vietnamese SMA patients showed homozygous deletion of the *NAIP* gene. The functional role of the *NAIP* gene in the development of SMA has not been elucidated, although some researchers have demonstrated a correlation between deletion of the *NAIP* gene and the severity of SMA [1,21,27,30]. However, *NAIP* deletion has been found in control subjects with no phenotypic evidence of SMA [21]. The presence of the *NAIP* gene may be independent of the clinical severity of SMA in Vietnam.

In conclusion, we identified a homozygous deletion of the *SMN1* gene in a Vietnamese SMA patient. Since the number of the patients analyzed in this study was very limited, we could not conclude that homozygous *SMN1* deletion is not a main cause of SMA in Vietnam.

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