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Deletion of the *SMN1* and *NAIP* Genes in Vietnamese Patients with Spinal Muscular Atrophy

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The *SMN1* and *NAIP* genes are related to the development of spinal muscular atrophy (SMA), which is characterized by degeneration of motor neurons leading to progressive muscular weakness and atrophy. The *SMN1* gene is homozygously deleted in most SMA patients, and now recognized as a responsible gene for SMA. The NAIP gene is often deleted in the SMA patients with the severest form of SMA, and now considered to be a modifying factor of the severity of SMA. Our previous study of five Vietnamese SMA patients showed that the *SMN1* gene deletion was detected in one patient, although the NAIP gene deletion was not detected in any patients. In this study, we analyzed 12 Vietnamese SMA patients who were not enrolled in the previous study. The *SMN1* gene was homozygously deleted in six out of 12 patients, and the NAIP gene deletion was detected in five patients. Taken together with our previous data, the *SMN1* gene deletion was detected in seven out of 17 Vietnamese SMA patients and the NAIP gene deletion in five out of 17 Vietnamese SMA patients. These studies suggest that the SMN1 and NAIP gene deletions are not rare in Vietnamese SMA patients. Thus, the confirmation of SMA-related gene deletion will also be a useful tool for the diagnosis of SMA in Vietnam.

Spinal muscular atrophy (SMA) is one of the most common neuromuscular disorders, characterized by degeneration of motor neurons in the spinal cord leading to muscle atrophy. SMA is clinically classified into three subtypes based on the onset age and severity (6): type I (the severe form, also called “Werdnig-Hoffmann disease”), type II (the intermediate form) and type III (the mild form, also called “Kugelberg-Welander disease”). Genetic linkage studies mapped all three clinical subtypes of SMA to chromosome 5q13 (1,2,5), with two SMA-related genes in the SMA critical region, the *SMN*1 gene (4) and the *NAIP* gene (8).

The *SMN1* gene has been recognized to be responsible for SMA because *SMN1* is homozygously deleted in more than 90% of SMA patients, irrespective of the clinical severity, and intragenic mutations of *SMN1* are found in the remainders (4,7,11). The NAIP gene is often deleted in the SMA patients with the severest form of SMA (8), and now

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considered to be a modifying factor of the severity of SMA. However, the NAIP deletion has been found in controls with no phenotypic evidence of SMA (8).

We detected the *SMN1* gene deletion in one out of five Vietnamese SMA patients enrolled in the previous study (3). However, the *NAIP* gene deletion was not detected in the study (3). In the present study, we analyzed the molecular genetic features of other twelve Vietnamese SMA patients.

METHODS

After obtaining informed consent, we analyzed the *SMN1* and *NAIP* genes of twelve Vietnamese SMA patients (Table I). The diagnosis of SMA was carried out based on the clinical observation. These twelve patients were unrelated with the patients examined in the previous study (3). Genomic DNA was extracted from 3 ml of whole blood using a DNA extraction kit, SepaGene® (Sanko Junyaku Co., Ltd, Tokyo, Japan). PCR-enzyme digestion analysis of *SMN1* exons 7 and 8 was performed according to the method of van der Steege et al. (10). *SMN1* exons 7 and 8 have *SMN1*-specific sequences, but other *SMN1* exons share the same sequences with the corresponding *SMN2* exons. Thus, *SMN1* deletion was judged from the lack of *SMN1* exon 7 or *SMN1* exon 8. PCR amplification of the *NAIP*-specific sequence, exon 5, was performed according to the method of Roy et al. (8).

Table I: Clinical subtypes and deletion patterns of the Vietnamese SMA patients

No	Patient	Clinical Subtypes	SMN1 exon 7	SMN1 exon 8	NAIP exon 5	Reference
1	TVK-1	type 3	non-del	non-del	non-del	(3)
2	TVK-2	type 2	non-del	non-del	non-del	(3)
3	TVK-3	type 3	non-del	non-del	non-del	(3)
4	TVK-4	type 2	non-del	non-del	non-del	(3)
5	TVK-5	type 1-2	del	del	non-del	(3)
6	NDB-3	type 2	non-del	non-del	non-del	this study
7	NDB-5	type 1	non-del	non-del	del	this study
8	NDB-7	type 1	del	del	non-del	this study
9	NDB-8	type 2	del	del	non-del	this study
10	NDB-9	type 1	del	del	del	this study
11	NDB-11	type 3	del	del	del	this study
12	NDB-19	type 3	non-del	non-del	del	this study
13	NDB-20	type 2	non-del	non-del	non-del	this study
14	NDB-21	type 1	del	del	del	this study
15	NDB-22	type 3	del	non-del	non-del	this study
16	NDB-23	type 3	non-del	non-del	non-del	this study
17	NDB-24	type 3	non-del	non-del	non-del	this study

del and non-del: deletion and non-deletion, respectively

RESULTS AND DISCUSSION

We identified homozygous deletion of *SMN1* exon 7 in six out of twelve Vietnamese SMA patients (Fig. 1A). Interestingly, Patient 22 showed that exon 7 is deleted but exon 8 is not deleted, suggesting a difference between the deletion ranges of the two SMN1 genes or a hybrid SMN gene formation (*SMN2* exon 7 and *SMN1* exon 8 are fused) (Figs. 1A and 1B). *NAIP* exon 5 was homozygously deleted in five out of twelve Vietnamese SMA patients (Fig. 1C).

Taken together with our previous data (Table I), deletion of *SMN1* exon 7 was detected in seven out of 17 Vietnamese SMA patients enrolled in the two successive studies. As to the

NAIP gene, deletion of exon 5 was detected in five out of 17 Vietnamese SMA patients. Although the number of the patients examined in the two successive studies is still too small to expect the deletion frequency of the genes in Vietnamese SMA patients, our data strongly suggest that deletion of the genes is not rare in Vietnamese SMA patients.

In Vietnam, the diagnosis of SMA is usually carried out just based on the clinical observation. However, diagnosis of SMA is sometimes very difficult, even if electromyography (EMG) and muscle biopsy are available, because there are non-typical SMA patients. When the technique of PCR and enzyme-digestion to detect deletion of the SMA-related genes is introduced, the precision of diagnosis of SMA will be much more improved in Vietnam. This idea is supported by a report from Europe that the confirmation of SMN1 deletion is very useful in cases with diagnostic doubt (9).

In conclusion, our studies suggest that the *SMN*1 and *NAIP* gene deletions are not rare in Vietnamese SMA patients. Thus, the confirmation of SMA-related gene deletion will also be a useful tool for the diagnosis of SMA in Vietnam.

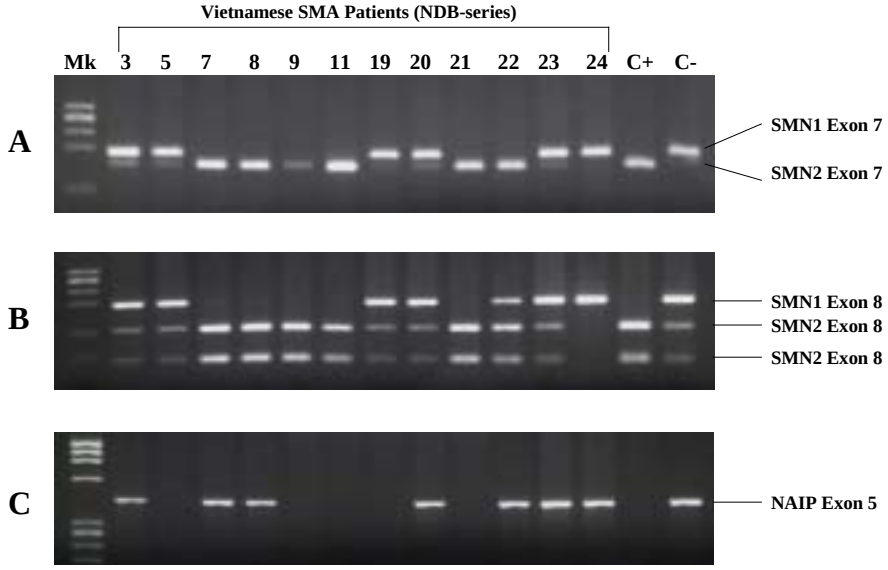

Figure 1. Deletion Analysis of *SMN1* and *NAIP* in Vietnamese Patients with SMA. Marker Lane (Mk) is Hae III-digested PhiX175 DNA fragment. C+ and C- denote disease and healthy controls, respectively.

（A） *SMN1* exon 7 deletion test. Six among 12 patients showed homozygous deletion of *SMN1* exon 7.

（B） *SMN1* exon 8 deletion test. Five among 12 patients showed homozygous deletion of *SMN1* exon 8. For patient 22, exon 7 is deleted but exon 8 is retained. The two designation show the two bands generated after enzyme-digestion of the *SMN2* PCR-products.

（C） NAIP exon 5 deletion test. Five among 12 patients showed homozygous deletion of *NAIP* exon 5.

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