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NAIP-Deletion Analysis in Malaysian Patients with Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease, which is characterized by degeneration of the anterior horn cells of the spinal cord. SMA is classified into 3 clinical subtypes, type I (severe), type II (intermediate), and type III (mild). Two genes, *SMN1* and *NAIP*, have been identified as SMA-related genes. The *SMN1* gene is now recognized as a responsible gene for the disease because it is deleted or mutated in most SMA patients. However, the role of the *NAIP* gene in SMA has not been fully clarified. To clarify the contribution of *NAIP* to the disease severity of SMA, we studied the relationship between NAIP-deletion and clinical phenotype in Malaysian patients. A total of 39 patients lacking *SMN1* (12 type I, 19 type II, and 8 type III patients) were enrolled into this study. Seven out of 12 patients with type I SMA (~60%) showed *NAIP* deletion. On the contrary, only 2 out of 20 type II patients and none of type III patients showed *NAIP* deletion. There was a statistically significant difference in *NAIP*-deletion frequency among the clinical subtypes (Fisher's exact probability test, p value = 0.014). In conclusion, according to our data that *NAIP* deletion was more frequent in type I SMA than in type II-III SMA, the *NAIP* gene may be a modifying factor for disease severity of SMA.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disease is characterized by degeneration of anterior horn cells in the spinal cord. SMA has been classified into three clinical subtypes, type I, type II and type III, based on the age of onset and severity. Type I SMA is a severe form, also known as Werdnig-Hoffmann disease, with the onset at birth or before 6 months of age. Type I patients are never able to sit without support and usually die before the age of 2 years. Type II SMA is an intermediate form with the onset after 6 months. Type II patients are unable to stand or walk without aid, and died after the age of 2 years. Type III is a mild form, also known as Kugelberg-Welander disease, with onset after 18months. Type III patients can stand and walk and they usually spend their life until adult.

In 1995, two genes, *SMN1* and *NAIP*, were identified as SMA-related genes (6,8). The *NAIP* gene lies adjacent to the *SMN1* gene. The *SMN1* gene is now recognized as a responsible gene for the disease because it is deleted or mutated in most SMA patients. On the contrary, the role of *NAIP* in SMA has not been fully clarified. Quite a few studies found that *NAIP* is frequently deleted in the patients with severer type of SMA (2,4). However, this gene was also found to be deleted in normal individuals without any SMA phenotype.

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To clarify the contribution of *NAIP* to the disease severity of SMA, we conducted a study to analyze the deletion frequency of the *NAIP* gene among SMA patients in Malaysia and to correlate it with the disease severity.

PATIENTS AND METHODS

This study was approved by the Research and Ethical Committee, School of Medical Sciences, Health Campus, Universiti Sains Malaysia.

Patients and DNA extraction. A total of 39 patients (12 type I, 19 type II, and 8 type III patients) who fulfilled the diagnostic criteria defined by International SMA Consortium (14) were enrolled into this study. Blood was obtained after getting the informed consent. Genomic DNA was extracted from the blood using GeneAll® DNA Blood Mini kit (Seoul, Korea).

***SMN* deletion analysis.** *SMN1* deletion was determined by the method described by van der Steege *et al* (1995). PCR was done using primers R111 (5'- AGA CTA TCA ACT TAA TTT CTG ATC A - 3') and X7-Dra (5' - CCT TCC TTC TTT TTG ATT TTG TTT- 3') to amplify exon 7, while primers 541C960 (5'- GTA ATA ACC AAA TGC AAT GTG AA - 3') and 541C1120 (5'- CTA CAA CAC CCT TCT CAC AG - 3') to amplify exon 8 of the *SMN* genes (*SMN1* and *SMN2*). The PCR products were then digested with restriction enzyme for 1 hour to differentiate between *SMN1* and *SMN*2. DraI was used for digestion of exon 7 while DdeI was used for exon 8. The digestion products were stained with SYBR® Green 1 and were then electrophoresed in 2.5% agarose gel.

***NAIP* deletion analysis.** *NAIP* deletion was determined by the method described by Roy *et al* (1995) (8). The PCR of *NAIP* exon 5 was performed using primers (1863 and 1864) (8). The amplification products were stained with SYBR® Green 1 and were electrophoresed in 2% agarose gel.

Statistics. To compare the frequencies of the *NAIP*-deletion in all genotypes, we used Fisher's exact probability test. $P < 0.05$ was considered as significantly different.

RESULTS

The deletion analysis of the *SMN1* and *NAIP* gene of Malaysian SMA patients were summarized in Table 1.

All the patients who were enrolled in this study showed deletion of the *SMN1* gene, which has helped us to eliminate the clinical bias and to look only at the frequency of *NAIP* deletion among these SMA patients lacking *SMN1*. After confirmation of *SMN1* deletion for these patients, their samples were then analyzed for *NAIP* deletion.

Among these samples, *NAIP* deletion was found in 7 out of 12 type I patients (58%) and 2 out of 19 type II patients (11%). *NAIP* deletion was not detected in any of the 8 type III patients (Table 1). There was a statistically significant difference in *NAIP*-deletion frequency among the clinical subtypes (Fisher's exact probability test, p value = 0.014) (Table 2).

Six out of 12 type I patients died from 3 to 15 months and 4 of the 6 patients deleted the *NAIP* gene (Table 1), indicating that type I patients with *NAIP* deletion may have poor prognosis with extremely short life span.

Table 1. Genotypes of Malaysian SMA patients.

Patients	Type of SMA	Sex	Status	Deletion Analysis		
				SMN1		*NAIP*
				Exon 7	Exon 8	Exon 5
Patient 1	I	M	Died at 3 mo	Del	Del	Del
Patient 2	I	M	Died at 3 mo	Del	Del	Del
Patient 3	I	F	Died at 13 mo	Del	Del	Del
Patient 4	I	F	Died at 15 mo	Del	Del	Del
Patient 5	I	M	Alive	Del	Del	Del
Patient 6	I	M	NA	Del	Del	Del
Patient 7	I	F	NA	Del	Del	Del
Patient 8	I	F	Died at 7 mo	Del	Del	Non-del
Patient 9	I	M	Died at 13 mo	Del	Del	Non-del
Patient 10	I	F	Alive	Del	Del	Non-del
Patient 11	I	F	NA	Del	Del	Non-del
Patient 12	I	F	NA	Del	Del	Non-del
Patient 13	II	F	NA	Del	Del	Del
Patient 14	II	F	NA	Del	Del	Del
Patient 15	II	M	NA	Del	Del	Non-del
Patient 16	II	M	NA	Del	Del	Non-del
Patient 17	II	F	NA	Del	Del	Non-del
Patient 18	II	F	NA	Del	Del	Non-del
Patient 19	II	M	NA	Del	Del	Non-del
Patient 20	II	M	NA	Del	Del	Non-del
Patient 21	II	M	NA	Del	Del	Non-del
Patient 22	II	F	NA	Del	Del	Non-del
Patient 23	II	F	NA	Del	Del	Non-del
Patient 24	II	M	NA	Del	Del	Non-del
Patient 25	II	M	NA	Del	Del	Non-del
Patient 26	II	F	NA	Del	Del	Non-del
Patient 27	II	F	NA	Del	Del	Non-del
Patient 28	II	F	NA	Del	Del	Non-del
Patient 29	II	F	NA	Del	Del	Non-del
Patient 30	II	M	NA	Del	Del	Non-del
Patient 31	II	M	NA	Del	Del	Non-del
Patient 32	III	M	NA	Del	Del	Non-del
Patient 33	III	M	NA	Del	Del	Non-del
Patient 34	III	F	NA	Del	Del	Non-del
Patient 35	III	M	NA	Del	Del	Non-del
Patient 36	III	M	NA	Del	Del	Non-del
Patient 37	III	F	NA	Del	Del	Non-del
Patient 38	III	F	NA	Del	Del	Non-del
Patient 39	III	F	NA	Del	Del	Non-del

NA=Information not available

Table 2. Deletion frequency of exon 7 and 8 of *SMN1* gene and the *NAIP* gene.

SMN 1		*NAIP*	CLINICAL SUBTYPE		
Exon 7	Exon 8	Exon 5	TYPE 1	TYPE 2	TYPE 3
Del	Del	Del	7 (58%)	2 (11%)	0 (0%)
Del	Del	Non-del	5 (22%)	17 (89%)	8 (100%)
	Total		12 (100%)	19 (100%)	8 (100%)

(Fisher's exact probability test, p value = 0.014)

DISCUSSION

We found in this study that the frequency of *NAIP* deletion is significantly higher in type I patients than type II-III patients, which supported our earlier study on the frequency of the *SMN1* and *NAIP* deletion in Malaysian SMA (13). Our results are also compatible with the previous studies from other countries (1,2,12). According to a report from Japan, most of the patients with severe phenotype have both *SMN1* and *NAIP* gene deleted (9). These findings indicate that the *NAIP* gene is a modifying factor for the disease severity or clinical phenotype of SMA.

In addition, the *NAIP* gene is highly expressed in anterior horn and motor cortex neurons of normal brain (7). Gotz *et al.* showed a mutation in *NAIP* gene causes the unregulation of cellular differentiation that can affect motor neuron dysfunction (3). Such reports also suggested the possibility of *NAIP* involvement in the development of SMA.

However, it also should be noted in our study that the life span of the patients seems to be different among those type I patients who had deletion of both *SMN1* and *NAIP*. One of them (*patient 5*) was still alive after the age of 3 years, although in terms of best motor achievement he is unable to sit unsupported. On the other hand, there were severely affected patients without *NAIP* deletion who died at an early age (*patient 8 and patient 9*). Although quite a few studies showed a significant correlation between the absence and presence of *NAIP* gene with clinical severity, a study has been reported from United Kingdom, showing no significant differences between *NAIP* deletion and the survival rate in type I patients (10). According to these observations, other modifying factors than the *NAIP* gene may be predicted.

A candidate of such modifying factors may be the copy number of the *SMN2* gene, a homologous gene of *SMN1*. An SMA-model mouse showed that the amount of the *SMN2* transgene was related with the clinical severity (5). This finding strongly suggested that the disease severity is correlated with *SMN2* copy number. Our group is currently studying the *SMN2* gene to find out the correlation between *SMN2* copy number and the disease severity of SMA.

Our finding on Malaysian patients with SMA showed that *NAIP* gene may be a modifying gene for this disease and could be used as a prognostic indicator of the disease.

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