Genotype-Phenotype Correlation of *SMN1* and *NAIP* Deletions in Korean Patients with Spinal Muscular Atrophy

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Background and Purpose Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by progressive muscle weakness and atrophy. Most SMA patients have a homozygous deletion in survival of motor neuron 1 (*SMN1)* gene, and neuronal apoptosis inhibitory protein (*NAIP*) gene is considered a phenotype modifier. We investigated the genotype-phenotype correlation of *SMN1* and *NAIP* deletions in Korean SMA patients.

Methods Thirty-three patients (12 males and 21 females) treated at the Asan Medical Center between 1999 and 2013 were analyzed retrospectively. The polymerase chain reaction (PCR), restriction-fragment-length polymorphism analysis, and multiplex PCR were used to detect deletions in *SMN1* (exons 7 and 8) and *NAIP* (exons 4 and 5). We reviewed clinical presentations and outcomes and categorized the patients into three clinical types. *NAIP* deletion-driven differences between the two genotypes were analyzed.

Results Deletion analysis identified homozygous deletions of *SMN1* exons 7 and 8 in 30 patients (90.9%). Among these, compared with patients without an *NAIP* deletion, those with an *NAIP* deletion showed a significantly lower age at symptom onset (1.9 ± 1.7 months vs. 18.4 ± 20.4 months, mean$\pm$SD; $p=0.007$), more frequent type 1 phenotype (6/6 vs. 8/24, $p=0.005$), and worse outcomes, with early death or a requirement for ventilator support (4/4 vs. 2/12, $p=0.008$).

Conclusions Homozygous deletion in *SMN1* and a concurrent *NAIP* deletion were associated with an early onset, severe hypotonia, and worse outcome in SMA patients. Deletion analysis of *NAIP* and *SMN1* can help to accurately predict prognostic outcomes in SMA.

Key Words spinal muscular atrophy, *SMN1*, *NAIP*, genotype, phenotype.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by the degeneration of motor neurons in the spinal cord and brainstem, which results in progressive proximal muscle weakness and atrophy. SMA was first described by Werdnig and Hoffmann.[1] The estimated incidence of this disease is 1 in 6,000−10,000 births, with a heterozygous carrier frequency of 1 in 40−60.[2]

Patients with SMA are classified into the following five subtypes according to the age at onset and clinical severity:[3]

· Type 0 SMA is a prenatal-onset type that manifests as a very severe form.

· Type 1 SMA, which is called Werdnig-Hoffman disease, is an infantile type and constitutes 50% of cases. This type mostly presents as floppy infant syndrome, and the patients experience difficulties breathing and feeding.

· Type 2 SMA (Dubowitz disease) is an intermediate form showing less-severe symptoms.

· Type 3 SMA (Kugelberg-Welander disease) presents after 18 months, and the patients usually maintain the ability to sit and stand.

· Type 4 is an adult-onset type and is the mildest form.[4-6]

SMA is known to be caused by mutations in the survival of motor neuron (*SMN*) gene, which is located on chromosome 5q11.1−13. The telomeric copy of *SMN*, which is called *SMN1*, is the disease-determining gene, and about 95% of the affected patients have a homozygous deletion in *SMN1*.[7] In contrast, *SMN2*, a homologous centromeric copy of *SMN1*, is considered to be a disease-modifying gene because of the relationship between its copy number and disease severity.[8]

Another gene, the neuronal apoptosis inhibitory protein (*NAIP*) gene, which is adjacent to *SMN*, has been identified as being related to SMA. Previous studies have suggested that patients with a mutation in *NAIP* show more-severe symptoms, but the direct role of *NAIP* has not been fully investigated.

The prognosis of SMA is grave, with no curative treatment being available. However, the recent understanding of these representative genes associated with SMA suggests the role of phenotype modifiers and that there is potential for therapeutic intervention. Therefore, we aimed to describe the genotypes and clinical phenotypes of Korean pediatric SMA patients and determine the association between an *NAIP* deletion and clinical outcomes.

METHODS

A retrospective review of SMA patients treated from July 1999 to July 2013 was conducted at the Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea. Thirty-three patients who satisfied the diagnostic criteria defined by the International SMA Consortium[9] were included in this study. Demographic characteristics, including the sex, age at symptom onset, and familial history, were obtained from electronic medical records.

On the basis of age at onset and clinical severity, the patients were divided into types 1, 2, and 3. Clinical outcomes were assessed at an outpatient clinic, including the need for ventilator support or death. Laboratory data were obtained, including from electromyography, nerve conduction velocity, serum creatinine kinase, neuroimaging, and muscle biopsy.

Genetic studies to identify deletions in *SMN1* and *NAIP* were performed in all patients. The parents of each patient provided written informed consent. Genomic DNA was extracted from peripheral blood leukocytes using the Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA). The deletion in *SMN1* was analyzed according to a previously outlined method,[10] being detected by the restriction-fragment-length polymorphism analysis of polymerase chain reaction (PCR)-amplified fragments using the restriction enzymes *Dra*I for exon 7 and *Dde*I for exon 8. The deletion in the *NAIP^T* gene was detected using multiplex PCR of exons 4 and 5, with exon 12 as a PCR control.[11]

The clinical characteristics of patients with and without a deletion in *NAIP* were compared using Fisher's exact test or the Mann-Whitney test. A *p* value of <0.05 was considered statistically significant. All statistical analyses were performed using the SPSS software package (version 22, SPSS Inc., Chicago, IL, USA).

This study was approved by the Institutional Review Board of the Asan Medical Center (approval no. 2016-0322).

RESULTS

The demographic and clinical characteristics of the patients are listed in Table 1. The 33 patients comprised 12 males and 21 females with a mean age at symptom onset of 14.7 months. Fifteen patients were of type 1, 16 were of type 2, and 2 were of type 3, with mean ages at symptom onset of 3.9, 17.8, and 72 months, respectively. The most common reason for visiting the hospital was respiratory difficulty (49%), followed by hypotonia (30%), gait disturbance (15%), and developmental delay (6%).

The estimation of clinical outcomes was available for 19 patients in medical reviews of visits and calls. Seven of these patients had type 1 SMA, and the other 12 were type 2. Three

Table 1. Demographic and clinical characteristics of the SMA patients

Characteristic	Total (*n*=33)	Type 1 (*n*=15)	Type 2 (*n*=16)	Type 3 (*n*=2)
Sex, males:females	12:21	7:8	4:12	1:1
Age at symptom onset, mean (range), months	14.7 (0.1–96)	3.9 (0.1–24)	17.8 (3–36)	72 (48–96)
Initial presentation				
Respiratory difficulty	16 (49%)	3 (20%)	13 (82%)	0
Hypotonia	10 (30%)	9 (60%)	1 (6%)	0
Gait disturbance	5 (15%)	1 (7%)	2 (12%)	2 (100%)
Developmental delay	2 (6%)	2 (13%)	0	0

Except where indicated otherwise, data are *n* (%) values.
SMA: spinal muscular atrophy.

of the seven type 1 SMA patients died; the characteristics of these patients are listed in Table 2. The four survivors needed ventilator support, and this was started within a year after SMA symptom onset in three of them, and after 2 years in the fourth. Twelve patients with type 2 SMA had no respiratory problems, but all of them needed support from an electric wheelchair or walking aid.

Table 3 presents the results of genetic tests of the patients. Thirty patients (91%) had a homozygous deletion in *SMN1* located in exons 7 and 8. Two of the type 2 SMA patients had a deletion in *SMN1* in exon 7 only. One type 1 patient had a heterozygous deletion in *SMN1*, and DNA sequencing of *SMN1* showed a homozygous c.836G>T, p.R288M mutation, which was carried by the father of the patient. Six of the 30 patients with the homozygous *SMN1* deletion also had a deletion in *NAIP*.

Among the 30 patients with the homozygous *SMN1* deletion, clinical variables were compared between those with and without an *NAIP* deletion (Table 4). All six patients with an *NAIP* deletion had type 1 SMA. The mean age at symptom onset was lower in those with an *NAIP* deletion (1.9 months vs. 18.4 months, p=0.007). At the initial evaluation, the patients with an *NAIP* deletion had more-severe symptoms such as respiratory difficulty or hypotonia [relative risk (RR)=3.33, p=0.003].

Clinical follow-up information was available for 16 of the 30 patients: 4 patients had both *SMN1* and *NAIP* deletions and the other 12 had only an *SMN1* deletion. The proportion of patients who had died or received ventilator support was also higher in those with an *NAIP* deletion (RR=4.68, p=0.009). Among type 1 SMA patients, the presence or absence of an *NAIP* deletion did not differ significantly with the age at symptom onset (1.9 months vs. 5.5 months, p=0.114) or the initial symptoms (RR=3.00, p=0.198).

DISCUSSION

SMA has been established as the most common cause of death in infants with genetic susceptibility,[1,12] but current treatments are limited to providing supportive care for alleviating

Table 2. Clinical characteristics of the SMA patients who died

Patient no.	Gender	Age at onset, months	Clinical type	Initial presentation	Ventilator	Age at death, months
8	Male	1	1	Hypotonia	None	3
15	Male	2	1	Respiratory difficulty	Assisted/controlled mode from 7 months	89
23	Male	0.1	1	Hypotonia	None	2

SMA: spinal muscular atrophy.

Table 3. Genetic studies of the SMA patients

Mutation	Total (*n*=33)	Type 1 (*n*=15)	Type 2 (*n*=16)	Type 3 (*n*=2)
Exon 7 deletion	2 (6%)	0	2 (12.5%)	0
Exon 7/8 deletion, heterozygote	1 (3%)	1 (7%)	0	0
Exon 7/8 deletion, homozygote	24 (73%)	8 (53%)	14 (87.5%)	2 (100%)
Exon 7/8 deletion, homozygote+*NAIP* deletions	6 (18%)	6 (40%)	0	0

Data are *n* (%) values.
NAIP: neuronal apoptosis inhibitory protein, SMA: spinal muscular atrophy.

Table 4. Comparison of clinical characteristics in patients with *SMN1* deletions only and those with both *SMN1* and *NAIP* deletions

Characteristic	*SMN1* deletion (*n*=24)	*SMN1* and *NAIP* deletions (*n*=6)	*p*	RR (95% CI)
Males (*n*=11)	9	2	NS	
Age at symptom onset, mean±SD (range), months	18.4±20.4 (0.1–96.0)	1.9±1.7 (0.1–4.0)	0.007	
Clinical type (*n*=30)			0.005	
Type 1	8	6		
Types 2+3	16	0		
Initial presentation (*n*=30)			0.003	3.33 (1.53–7.27)
Respiratory difficulty and hypotonia	6	5		
Developmental delay and gait disturbance	18	1		
Clinical outcome (*n*=16)			0.009	4.68 (1.48–14.8)
Survivors without ventilator	10/12	0/4		
Died or on ventilator	2/12	4/4		

CI: confidence interval, *NAIP*: neuronal apoptosis inhibitory protein, NS: not significant, RR: relative risk, *SMN1*: survival of motor neuron 1.

the disease burden (e.g., respiratory support and physical therapy). Previous studies have shown that the most severe form (i.e., type 1 SMA) is also the most frequent. The results of the present study are consistent with these findings. Most type 1 SMA patients have poor outcomes with early death or respiratory failure.

Various disease-modifying strategies have recently been proposed following the elucidation of the essential aspects of the pathogenesis of SMA. The SMN protein is one of the key molecules involved in SMA pathology. The full-length SMN protein is considered to function as a chaperone in small nuclear ribonucleoprotein (snRNP) biogenesis and transport snRNPs to the nucleus for RNA splicing.[13] *SMN1* and *SMN2* are inversely duplicated on chromosome 5q13. The telomeric copy (*SMN1*) produces a full-length SMN protein, while the centromeric copy (*SMN2*) produces a truncated version of the SMN protein because of alternative splicing.[14,15] Future therapeutic strategies are aimed at increasing SMN protein levels, either by replacing *SMN1* or by splicing modulation or *SMN2* activation.[4] Although the relationship has not been fully elucidated, the related *NAIP* is also known to be associated with SMA disease severity.[16-18]

This study has revealed the clinical types and outcomes in Korean pediatric SMA patients and the genotype-phenotype correlation of *SMN1* and *NAIP* deletions. Most (*n*=30) of the 33 patients were confirmed to have a homozygous deletion in *SMN1*; the remaining 3 patients had a short heterozygous deletion or point mutation in *SMN1*. These results are consistent with previous studies finding that most SMA patients had a homozygous *SMN1* deletion.[7]

Several studies have shown that a deletion in *NAIP* is related to the disease type.[16-18] Shin et al.[19] conducted the first study of deletions in *SMN* and *NAIP* in Korean SMA patients; however, *NAIP* deletions were not detected in any of their five patients. A study of Korean SMA patients that analyzed deletions in candidate genes (i.e., *SMN1*, *NAIP*, and *p44*) did not find a genotype-phenotype correlation, but this could have due to the inclusion of only a small number of patients.[20] In a study of Chinese patients, Fang et al.[21] compared copy numbers of *SMN1*, *SMN2*, and *NAIP* in SMA patients and healthy controls and produced evidence of differences in copy numbers and gene structure between the SMA patients and controls. Qu et al.[22] further investigated the association of copy numbers of *SMN2* and *NAIP* with the disease course in SMA patients, and found that patients with fewer copies of *SMN2* and *NAIP* were characterized by a higher risk of death and a lower survival rate.

Our study included 33 SMA patients, 6 of whom had *NAIP* deletions and showed a more-severe phenotype of SMA. Moreover, the clinical outcomes of the patients were followed

up for a longer duration than in previous studies and showed a clear genotype-phenotype association with an *NAIP* deletion.

The limitations of our study include the inclusion of a relatively small sample and the lack of an analysis of the *SMN2* copy number. However, to the best of our knowledge, this is the first study to demonstrate a correlation between the clinical severity of SMA and *SMN1* and *NAIP* genotypes in Korean patients. Our results imply that an *NAIP* deletion may serve as an outcome predictor in SMA patients.

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