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CLINICAL FEATURES AND MOLECULAR GENETIC DIAGNOSIS OF PROXIMAL SPINAL MUSCULAR ATROPHY IN CHILDHOOD

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degenration of anterior horn cells in the spinal cord, leading to symmetrical proximal muscle weakness and atrophy. Affected individuals are classified into three types depending on the onset age, highest motor functional ability and clinical course. In 1990, the gene for SMA was mapped to chromosome region 5q13 for all three types of SMA. In 1995, the SMN (survival motor neuron) and NAIP (neuronal apoptosis inhibitory protein) genes were reported as candidate genes for SMA. In this study, we analyzed clinical features of SMA patients and deletions of these two candidate genes. The subjects included 39 SMA patients and two atypical SMA cases, one with mental retardation and the other with cardiomegaly. We classified 39 SMA cases based on the classification devised by the International SMA Consortium Meeting Report. Twenty cases satisfied all three criteria of this classification, and could clearly be classified as one of the three types. Our results of classifying cases according to each criterion suggested that clinical features of SMA constitute a broad spectrum encompassing the three types. Thus, it is most useful to classify patients according to their highest motor functional ability. Thirty-three cases of typical SMA (85% of all, 90% of type I, 100% of type II and 54% of type III) showed deletions of exons 7 and 8 or only exon 7 of the SMN gene. Two of type I (5% of all) showed deletions of exon 5 and 6 of the NAIP gene with deletions of the SMA genes. The other cases (2 of type I and 5 of type III) had no deletions of these genes. Involvement of the SMN and NAIP genes appears to be related to the severity of SMA. The one atypical case showed deletion of the SMN gene. The other one with mental retardation may be heterogeneous.

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of anterior horn cells in the spinal cord, leading to skeletal muscle denervation and thereby symmetrical proximal muscle weakness and atrophy[1)]. Affected individuals are classified into three groups depending on the age at onset and disease progression. Type I SMA (Werding-Hoffmann disease) is the most severe form of this disease. The onset is prior to 6 months (m) of age, patients are never able to sit without support, and rarely live

beyond 2 years (y) of age owing to respiratory muscle weakness. Type II SMA is of intermediate severity. The onset is before the age of 18 m, patients are never able to stand or walk without support, but generally live past age 2 y. Type III SMA (Kugelberg-Welander disease) is a much milder form. The onset of symptoms is after 18 m and patients are ambulatory.

In 1990, the gene for SMA was mapped to chromosome region 5q for all three types of SMA. Brzustowicz et al[2] performed a blind search with 115 microsatellite DNA markers for three families with chronic SMA (including types II and III), and mapped the gene locus of chronic SMA to 5q11.2-13.3. Melki et al[3] reported the SMA gene locus to be in the 5 q12-14 region, based on linkage analysis of 24 chronic SMA families. The locus of acute SMA was reported to be in the same region by Melki et al[4] and Gilliam et al[5]. These results suggest that different mutations in the same gene or continuous genes account for the three types of SMA. In 1995, French and Canadian groups independently reported candidate genes for SMA; SMN (survival motor neuron) and NAIP (neuronal apoptosis inhibitory protein)[6][7]. Deletions within the SMN gene were found in 226 of 229 SMA patients, whereas NAIP gene deletions were found in variable proportions of patients among the three types but the frequency was highest in the clinically severe types. Thus, two candidate genes were detected, but the cause of SMA including the responsible protein has not been determined. Analyses of the relationships between the phenotypes and the candidate genotypes are anticipated to facilitate understanding of the course of SMA.

In this study, we classified 41 SMA patients clinically, based on the age at onset, highest motor functional ability achieved and clinical course, as well as studying these individuals for deletions of the SMN and NAIP genes.

Subjects and Methods

1. Subjects

We analyzed DNA and clinical features in 39 SMA patients (nos. 3-41) from 37 unrelated families, all of whom had a childhood onset and had been diagnosed based on clinical features or muscle biopsy by pediatric neurologists at Tokyo Women's Medical College or by neurologists/pediatric neurologists at other hospitals. Of the 39 SMA patients, 35 were Japanese, two Korean (nos. 34, 36), one Japanese-African (the mother is Japanese and the father is African) (no. 12) and one Indian (no. 6). There were 18 males and 21 females. The age range at the time of the study was 4m to 38y1m (mean 9.8y), that of the onset age from 1m to 8y (mean 1.0 y).

We also studied two atypical SMA cases. One (no. 1), a Japanese boy whose onset was at birth, was mentally retarded. The other (no. 2), who had cardiomegaly, was a Chinese girl whose onset was at 1m.

For the DNA analysis, 26 normal controls and 26 healthy members (parents and siblings) of SMA families were also studied. All 41 patients, or their parents, and the normal subjects serving as controls provided informed consent to undergo DNA analysis for the SMN and NAIP genes.

2. Methods

1) Classification of SMA patients

We clinically classified our 39 patients into three SMA types based on the diagnostic criteria suggested in the report of the 1990 International SMA Collaboration Workshop[8] and the 1992 International SMA Consortium Meeting[9] (Table 1-A). This classification is comprised of three criteria: onset age, highest motor functional ability and clinical course. As to type I SMA, the onset is before 6m, these patients are never able to sit without support and die before 2y. Type II SMA manifests between 6 and 18m, patients cannot stand or walk alone, but survival exceeds 2y. In type III SMA the onset age is after 18m, these patients can stand or walk alone, and can survive until adulthood. To determine the importance of each criterion, we initially classified our patients according to onset age, highest motor functional ability and

Table 1-A Classification of SMA in the report of International SMA Consortium Meeting

	Age of onset	Course	
		Milestone	Course
I	from birth to 6m	never able to sit without support	death<2y
II	before 18m	unable to stand or walk without aid	death>2y
III	after 18m	stand and walk	death in adulthood

Table 1-B SMA classification criteria reviced for this study

Type	Onset age	Highest motor function	Clinical course
I	<6m	sit with support	death or respirator dependency before 2y
II	7-18m	stand or walk with support	death or respirator dependency after 2y to 18y
III	>18m	stand or walk without support	death beyond 18y

clinical course, each independently. The classifications based on onset age and highest motor functional ability were clear, while that based on clinical course was ambiguous. Thus, we revised the clinical course criterion of clinical course for the same of simplification (Table 1-B); in type I, patients die or become respirator dependent before 2y, while in type III they live beyond 18y (adulthood). Then, if there was a discrepancy among the criteria, the patient was tentatively classified according to the highest motor functional ability.

2) Analysis of the candidate genes

DNA specimens were extracted for peripheral blood lymphocytes (no. 1-7 and 10-41) or muscle tissues obtained by biopsy (no. 9) or autopsy (no. 8), according to standard techniques. We examined two candidate genes, the SMN gene and the NAIP gene, using these DNA samples, as described below.

The SMN gene was analyzed according to the method of van der Steege et al[10]. We used primers R111/X7-Dra for exon 7, and 541C960/541C1120 for exon 8. Primer sets for polymerase chain reaction (PCR) amplification were synthesized. Genomic DNA (20 μg) from each sample was amplified by PCR using these primers with 10× PCR reaction buffer, 2.5 mM $MgCl_2$, each d-NTP mix and 0.5 U Taq DNA polymerase. Samples were incubated in a DNA thermocycler (Perkin Elmer Cetus) for 35 cycles under the following conditions: 94°C for 1 min, 55°C for 1 min and 72°C for 1 min for exon 7, and 94°C for 1 min, 59°C for 1 min and 72°C for 1 min for exon 8. PCR products were digested with the enzyme Dra-I for exon 7 and Dde-I for exon 8 at 37°C for 3 hours, and then analyzed on 2.5% agarose gels.

The NAIP gene was analyzed as follows. We synthesized the primers for exons 5, 6 and 13 (Roy et al, 1995), as well as the primers for exon 4 (4F; 5′-CTCACTGCTTCCTACTAAAG-3′, 4R; 5′-CTGAGGTTTATCCACAGTTC-3′) based on the cDNA sequence of the NAIP gene reported by Roy et al[7]. Genomic DNA (20 μg) from each sample was amplified by PCR using these primers with 10× PCR reaction buffer, 2.5 mM $MgCl_2$, each d-NTP and 0.5 U Taq polymerase. Samples were incubated in a DNA thermocycler for 35 cycles under the following conditions: 94°C for 30 sec and 65°C for 4 min. PCR products were analyzed on 3% agarose gel.

3) Analysis of the correlation between clinical features and deletions of the SMN and NAIP genes

The phenotype-genotype correlation was analyzed selecting a limited number of clinical features (clinical SMA subtypes, onset age, highest motor functional ability, age at the

initiation of artificial ventilation, age at death or final follow-up, and by distinguishing is different gene deletion patterns).

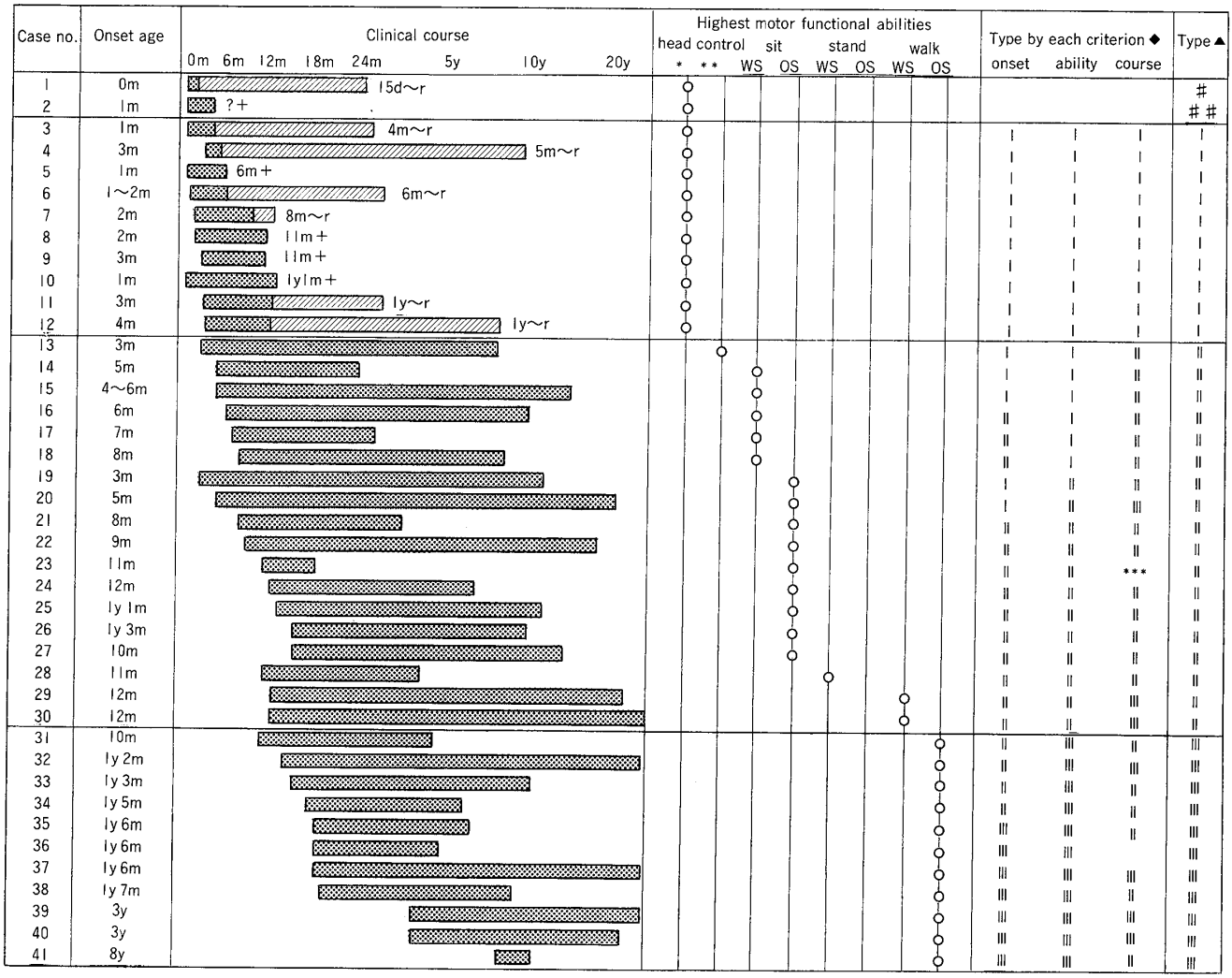
Results

1. Clinical evaluation of SMA patients in whom the SMN and NAIP genes were studied

The clinical features, i.e. onset age, highest motor functional ability and course, of the study subjects are summarized in Table 2. Five cases (nos. 2, 5, 8, 9, 10) died due to respiratory infection and dysfunction between 6m and 1y1m of age. Seven cases (nos. 1, 3, 4, 6, 7, 11, 12) were respirator dependent at the time of the current study. In these cases, it is highly likely that death due to respiratory insufficiency was aver-

Table 2 Clinical features of 41 SMA patients; onset age, clinical course and the highest motor functional ability

10 Cases (nos. 3-12): type I, 18 cases (nos. 13-30): type II, 11 cases (nos. 31-41): type III. No. 1 was an atypical SMA with mental retardation. No. 2 was an atypical SMA with cardiomegaly. no. 2: Chinese, no. 6: Indian, no. 12: Japanese-African (the mother is Japanese and the father is African), nos. 34 and 36: Korean, other 36 cases: Japanese. Cases 16 and 18, and 22 and 29, are pairs of siblings.



r : respirator, + : deceased, alive without respirator, alive with respirator, * : not acquired, ** : acquired,
ws : with support, os : without support
: neurogenic muscular atrophy with mental retardation, ## : atypical SMA with cardiomegaly,
*** : follow-up duration inadequate to allow evaluationof clinical course,
◆: type classification according to single criterion,
▲ : ltimate type classification according to global judgement.

ted by artificial ventilation. Cases 16 and 18, and 22 and 29, are pairs of siblings.

In 20 (nos. 3-12, 21, 22, 24-28, 37, 39, 40) of our patients, all three criteria were consistent with one type of SMA. Thus, only about half of our patients could be unequivocally classified as one of the three types of SMA.

The classification based solely upon the age of onset yielded the following results; 15 (nos. 3-15, 19, 20) were type I, 17 (nos. 16-18, 21-34) type II, and 7 (nos. 35-41) type III. Classification based on highest motor functional ability was as follows; 16 (nos. 3-18) type I, 12 (nos. 19-30) type II, and 11 (nos. 31-41) type III. Data concerning clinical course were sufficient for evaluation only in 38 cases; 10 cases (nos. 3-12) were classified as type I, all of whom were respirator dependent or had died before 2y, 21 (nos. 13-19, 21, 22, 24-28, 31, 33-36, 38, 41) as type II or III had all lived beyond 2y without requiring a respirator but had not yet reached 18y at the time of this study, and 7 (nos. 20, 29, 30, 32, 37, 39, 40) as type III, all of whom lived beyond 18 y.

Results of evaluating consistency among the three criteria were as follows; of the 15 type I cases classified according to onset age, 10 never acquired head control, one (no. 13) acquired head control, two (nos. 14, 15) were able to sit with support, while the remaining two (nos. 19, 20) could sit without support at some point in their clinical course, and could thus be classified as type II based on the highest motor functional ability. Of the 17 type II cases classified according to onset age, 3 were able to sit with support, i.e. they were type I according to highest motor functional ability, 7 without support, one was able to stand with support, 2 were able to walk with support, while the remaining 4 (nos. 31-34) walked unassisted, and could be classified as type III based on highest motor functional ability. All of the type III cases classfied according to onset age could walk unassisted, i.e. there was no inconsistency.

The results of tentatively classifying all 39 SMA patients according to maximum motor ability and clinical course were as follows; 10 cases (nos. 3-12) type I, 18 (nos. 13-30) type II, and 11 (nos. 31-41) type III. In case no. 13, the onset was before 6m, and the ability to sit was never attained, indicating a type I classification. However, he is alive at 9y9m without respirator assistance as in most type II cases. Cases nos. 14-18 were able to sit with support, i.e. type I according to highest motor functional ability, but lived beyond 2y and did not need a respirator at the time of this study. Therefore these cases were classified as type II. Cases nos. 28-30 were able to stand or walk, but support was necessary, and the duration of these peak motor functional abilities was only 3～4 months.

2. Analysis of the SMN and NAIP genes (Fig. 1, Table 3)

On SMN gene analysis, 30 of our 39 SMA cases (8 SMA type I, 17 type II and 5 type III) showed homozygous deletions of both exon 7 and exon 8. Three cases (nos. 10, 24, 39) showed deletions only of exon 7. Case no. 10 was classified as type I, no. 24 as type II and no. 39 as type III, employing all three criteria. Six cases (1 SMA type I and 5 type III) had no deletions of either exon 7 or exon 8. In short, 33 of 39 SMA cases (85%) were either missing both exon 7 and exon 8 or had a deletion of exon 7 alone of the SMN gene. According to the clinical classification using all three criteria, 9 of 10 cases (90%) with type I SMA showed deletions of exon 7 or 8 of the SMA gene, while all 19 (100%) type II cases and 5 of 10 (50%) type III cases had such deletions. The control subjects and healthy family members (parents and siblings) did not have homozygous deletions of the SMN gene.

Of the two atypical SMA patients, case no. 1 had no deletions of either exon 7 or exon 8, while case no. 2 had deletions of both exons.

The NAIP gene analysis revealed only two (nos. 5, 11) of the 39 cases (5%) to have homozygous deletions of exons 5 and 6 of this gene. Both cases were classified as SMA type I. The type II and III cases had no NAIP gene deletions. Similarly, none of the unaffected controls

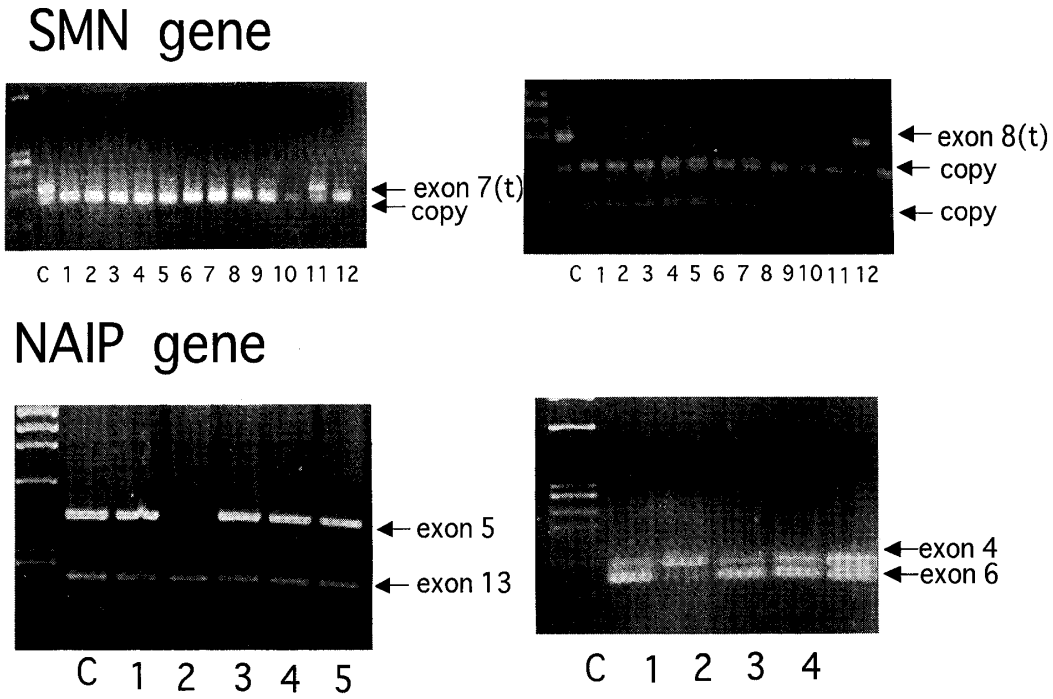


Fig. 1 Demonstration of deletions of the SMN and NAIP genes
Upper: PCR products of the SMN gene are shown for some of our SMA patients. C means normal control. The left picture shows the results for exon 7 of the SMN gene, the right for exon 8. Lanes 1-10 and 12 illustrate the absence of exons 7 and 8.
Lower: PCR products of the NAIP gene are shown. The left picture shows the results for exons 5 of the NAIP gene. Lane 2 shows the exon 5 deletion. The right picture shows deletion of exon 6 in lane 2.

or the parents and healthy siblings of SMA patients in this study had NAIP gene deletions.

In short, the results of anlayzing these two candidate genes were classifiable into four deletion patterns (A-D) including no deletions (Table 4-A, B). Pattern A, complete deletion of exons 7 and 8 of the SMN gene and of exons 5 and 6 of the NAIP gene, was observed in two cases (nos. 5, 11). Overall, 20% of type I cases and 5% of all SMA cases showed to this pattern. Pattern B, the deletion of exons 7 and 8 of the SMN gene but no deletion of exons 5 and 6 of the NAIP gene, was demonstrated in 60% of type I, 94% of type II, 45% of type III and 72% of all typical cases. Pattern C, the deletion of exon 7 of the SMN gene alone, was found in one case of each type: 10% of type I, 6% of type II and 9% of type III. Pattern D, i.e. no deletions of either exon 7 or 8 of the SMN gene or of exon 5 or 6 of the NAIP gene, was demonstrated in 10% (1/10) of type I, 45% (5/11) of type III and 15% (6/39) of all typical cases.

One atypical SMA case (no. 2) showed pattern B, the othe (no. 1) pattern D.

3. Clinical features and deletions of the SMN and NAIP genes

1) Inclusion and exclusion criteria for the diagnosis and deletions

Table 2 shows the inclusion and exclusion criteria for the diagnosis of SMA, as well as the clinical features of our cases. Cases no. 1 and 2 had exclusion criteria, and were thus regarded as having atypical SMA in this study. The typical cases with deletions accounted for 85% of our entire patient group, 90% of type I, 100% of type II and 50% of type III inclusive. Bilaterally symmetrical muscle weakness was noted in all but one case (no. 34, a type III case) in whom weakness was asymmetrical. Muscle fiber fasciculation involving the tongue was seen in 90% (9/10) type I, 89% (16/18) of type II, 45% (5/11) of type III cases and 77% (30/37) of all cases.

Table 3 Clinical details based on the criteria for SMA diagnosis and results of analysis of the SMN and NAIP genes

Types	Case no.	Gene deletion				Inclusion criteria							Exclusion criteria	Others
		SMN gene		NAIP gene		Muscle weakness			Fasciculation		Neurogenic change			
		exon7	exon8	exon5	exon6	symmetrical	proximal >distal	legs> arms	tongue	others	EMG	muscle biopsy		
*	2	+	+	−	−	+	+	U	+	−	ND	+	cardio-megaly	
I	4	+	+	−	−	+	+	+	+	NA	NA	NA	−	
	5	+	+	+	+	+	+	+	+	NA	+	+	−	
	6	+	+	−	−	+	+	+	+	−	ND	+	−	
	7	+	+	−	−	+	+	+	+	NA	ND	NA	−	
	8	+	+	−	−	+	U	+	+	NA	+	+	−	
	9	+	+	−	−	+	+	+	+	finger	+	+	−	
	10	+	−	−	−	NA	NA	+	+	finger	NA	NA	−	
	11	+	+	+	+	+	+	U	+	−	+	+	−	
	12	+	+	−	−	+	+	+	NA	NA	+	+	−	
II	13	+	+	−	−	+	+	+	+	−	+	+	−	intestinal perforation
	14	+	+	−	−	+	U	+	+	finger	normal	+	−	
	15	+	+	−	−	+	+	+	+	−	+	+	−	ovarian tumor
	16	+	+	−	−	+	+	+	+	−	+	+	−	
	17	+	+	−	−	+	+	+	−	−	+	+	−	
	18	+	+	−	−	+	+	+	+	−	ND	ND	−	
	19	+	+	−	−	+	+	+	+	−	+	+	−	
	20	+	+	−	−	+	+	+	+	−	+	NA	−	
	21	+	+	−	−	+	+	+	+	NA	ND	+	−	
	22	+	+	−	−	+	+	+	+	finger	+	+	−	
	23	+	+	−	−	+	+	+	−	−	ND	+	−	
	24	+	−	−	−	+	+	+	+	−	+	+	−	
	25	+	+	−	−	+	+	+	+	finger	+	+	−	
	26	+	+	−	−	+	+	+	+	finger	+	+	−	
	27	+	+	−	−	+	+	+	+	finger	+	+	−	
	28	+	+	−	−	+	+	+	+	finger	+	+	−	
	29	+	+	−	−	+	+	+	+	finger	+	+	−	
	30	+	+	−	−	+	+	U	+	face(rt)	ND	+	−	diabetes mellitus
III	33	+	+	−	−	+	+	=	+	finger	+	+	−	
	35	+	+	−	−	+	U	U	−	NA	NA	+	−	
	36	+	+	−	−	+	+	+	+	NA	+	+	−	
	37	+	+	−	−	+	+	+	+	finger	+	+	−	
	38	+	+	−	−	+	+	+	+	finger	+	+	−	visual disturbance
	39	+	−	−	−	+	+	+	+	NA	NA	NA	−	
*	1	−	−	−	−	+	+	+	NA	NA	NA	+	mental retardation	
I	3	−	−	−	−	+	+	+	+	−	+	ND	−	
III	31	−	−	−	−	+	+	U	−	NA	+	NA	−	
	32	−	−	−	−	+	+	U	−	−	+	+	−	pneumothorax
	34	−	−	−	−	rt>lt	+	+	−	−	NA	+	−	
	40	−	−	−	−	+	+	=	−	finger	+	+	−	EEG abnormality
	41	−	−	−	−	+	NA	NA	−	−	+	NA	−	

ND : not done, U : unknown, * : atypical SMA case, NA : information not avaliable.

Upper : 34 cases had deletions involving exons 7 or 8 of the SMN gene or of exons 5 or 6 of the NAIP gene. Lower : 7 cases had no deletions of either exons 7 and 8 of the SMN gene or of exons 5 and 6 of the NAIP gene.

Tongue fasciculation was recognized in 88% (29/33) of cases with deletions, i.e. patterns A, B and C, but in only 17% (1/6) of those with no deletions. Muscle fasciculation of the fingers or face was recognized in 50% (2/4) of type I, 39% (7/18) of type II, 50% (4/8) of type III and 43%

Table 4-A Deletion patterns involving the SMN and NAIP genes in each clinical type

Pattern	Gene deletion pattern			Clinical type				Gene deletion	
	SMN gene		NAIP gene	I	II	III	total	SMN gene	NAIP gene
	exon 7	exon 8	exon 5,6	n %	n %	n %	n %	n %	n %
A	deleted	deleted	deleted	2 20 [2 17]	0 0	0 0	2 5 [2 5]		
B	deleted	deleted	not deleted	6 60 [7 58]	17 94	5 45	28 72 [29 71]	33 85 [34 83]	2 5 [2 5]
C	deleted	not deleted	not deleted	1 10 [1 8]	1 6	1 9	3 8 [3 7]		
D	not deleted	not deleted	not deleted	1 10 [2 17]	0 0	5 45	6 15 [7 17]	6 15 [7 17]	37 95 [39 95]
total				10 100 [12 100]	18 100	11 100	39 100 [41 100]	39 100 [41 100]	39 100 [41 100]

[ ] : including two atypical SMA

Table 4-B Deletion patterns involving the SMN and NAIP genes and clinical types

Pattern	Gene deletion pattern			Clinical type			
	SMN gene		NAIP gene	I	II	III	total
	exon 7	exon 8	exon 5,6	n %	n %	n %	n %
A	deleted	deleted	deleted	2 100 [2 100]	0 0	0 0	2 100 [2 100]
B	deleted	deleted	not deleted	6 21 [7 24]	17 61 [17 60]	5 18 [5 17]	28 100 [29 100]
C	deleted	not deleted	not deleted	1 33 [1 33]	1 33 [1 33]	1 33 [1 33]	3 100 [3 100]
D	not deleted	not deleted	not deleted	1 17 [2 29]	0 0	5 83 [5 71]	6 100 [7 100]

[ ] : including two atypical SMA

(13/30) of all typical cases examined for this findings. Other findings of our subjects included intestinal perforation, ovarian tumor, diabetes mellitus, pneumothorax, visual disturbance and EEG abnormality in one case each.

2) Highest motor functional ability and deletion pattern (Table 5)

Among 10 cases who never attained head control, 2 (20%) showed pattern A, 6 (60%) pattern B, 1 (10%) pattern C and 1 (10%) pattern D. One who attained head control showed pattern B. Five cases who were able to sit with support showed pattern B. Among 9 cases who were able to sit without support, 8 showed pattern B and one pattern C. In short, those who were able to sit with or without support showed pattern B or C, not A or D. No. 38, who was able to stand with support, had pattern B. Among 13 cases capable of walking with or without support, 7 showed pattern B, one pattern C and 5 pattern D.

In other words, patients with pattern A had a highest motor functional ability of head control, while in pattern B peak functions were variable and included head control (27.5%), sitting with support (17%), sitting unaided (27.5%), standing with support (13.4%), walking with support (6.7%) and walking alone (17.2%). In pattern C, one case each attained head control, sitting unaided and walking alone. Five of the 7 pattern D cases, i.e. those with no known deletions, were able to walk unaided while the other two never attained head control.

3) Onset age and deletion pattern (Fig. 2)

Two pattern A cases both had an onset before 3m (mean 0.2y). In the pattern B, C and

Table 5 Number of cases by gene deletion pattern according to peak motor function

Highest motor functional ability	Gene deletion pattern			
	A	B	C	D
Walk alone		5	1	5
Stand without support				
Walk with support		2		
Stand with support		1		
Sit without support		8	1	
Sit with support		5		
Head control		1		
No head control	2	6(1)	1	1(1)

Deletion patterns
A : SMN gene(exons 7,8) and NAIP gene(exon 5) deletion
B : SMN gene(exons 7,8) deletion
C : SMN gene(exon 7) deletion
D : no deletion
( ) : atypical SMA

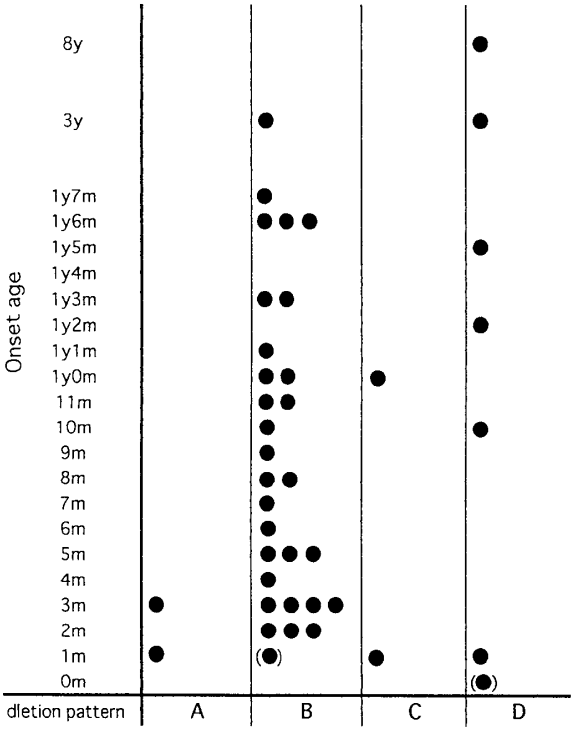


Fig. 2 Distribution of patients according to onset age by deletion pattern
A: SMN gene (exons 7, 8) and NAIP gene (exon 5) deletion, B: SMN gene (exons 7, 8) deletion, C: SMN gene (exons 7, 8) deletion, D: no deletion.
( ): typical SMA.

D cases the onset age ranged from 2m to 1y7m (mean 0.7y), 1m to 3y (mean 1.7y) and less than 1m to 8y (mean 2.4y), respectively. The latter pattern, i.e. pattern D, was associated with markedly variability in onset age.

4) Deletion pattern and ages at initiation of ventilatory assistance, death and final follow-up (Fig. 3)

Of the two pattern A patients, one (no. 5) died before 6m, while the other (no. 11) was respirator dependent at 1y. Among 28 cases with pattern B, 2 (no. 8, 9) died before 1y, and 4 (no. 4, 6, 7, 12) became respirator dependent before 1y. Four cases not requiring ventilatory assistance were younger than 2y at the time of this study. Eighteen cases lived beyond 2y, 4 of

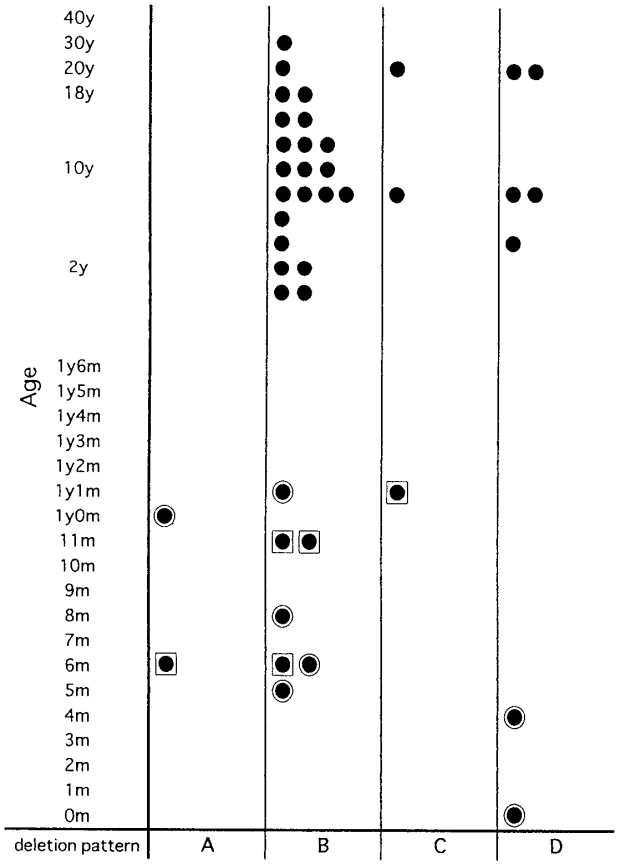


Fig. 3 Distribution of patients according to ages at initiation of ventilatory assistance, death and final follow-up by deletion pattern
A: SMN gene (exons 7, 8) and NAIP gene (exon 5) deletion, B: SMN gene (exons 7, 8) deletion, C: SMN gene (exon 7) deletion, D: nodelation.
( ): atypical SMA, ○: respirator dependent, □: deceased.

whom are still being followed and are presenting more than 18y. Among the 3 cases with pattern C, no. 10 died at 11m, no. 24 was followed until 8y2m and no. 39 was followed until age 23y4m. Among 6 cases with pattern D, one (no. 3) became respirator dependent, and 5 lived beyond 2y. Two of the 5 were followed-up beyond 18y.

Discussion

1. The problem of classification according to clinical features

SMA is well known as presenting diverse clinical phenotypes, but all types have in common the underlying pathology of degeneration of anterior horn cells in the spinal cord, resulting clinically in proximal dominant muscular atrophy and weakness. Clinical classification of this disease has long been debated. The currently used classification criteria have numerous drawbacks, but a satisfactory alternative has yet to be established. In general, the childhood onset form of this disease is broadly classified into three types according to onset age, highest motor functional ability and clinical course. According to the international classification system[9], type I is the most severe form of SMA, as described above, while type III is the mildest. Type II is intermediate between types I and III. Correlative assessment of each component among the three clinical criteria, in terms of its consistency with the other two, revealed that only 20 patients could be classified unequivocally based on all three criteria. In other words, patients classified accoridng to onset age often had a clinical course or peak motor functional ability not consistent with the type of SMA indicated by onset age. The existence of cases showing a borderline clinical picture is widely recognized. Ultimately, nearly half of our cases were assigned a conflicting SMA types based on classification using the three criteria.

As shown in Table 1, all 39 typical SMA were classified into one of the 3 types by first employing one of the three criteria and were then ultimately classified based on total impression, especially highest motor functional ability. Investigation of the onset ages of our cases revealed that all 10 cases ultimately classified as type I and most of those classified as type II satisfied the onset age criterion. Two of the 15 cases classified as type I according to onset age, however, showed peak motor functional abilities consistent with type II. Five of these cases lived beyond 2y without ventilatory assistance and one survived into adulthood. The onset age range for ultimate classification of type III cases was broad, 10m to 8y, with 4 cases (36%) having their onset before 18m. With a later onset, i.e. after 1y, transient walking or standing is more common. The onset age overlapped extensively between cases ultimately classified as type II and those designated type III. Furthermore, four of 17 cases classified as type II based on the onset age achieved highest motor functional abilities consistent with type III. Thus, onset age tends to suggest a more severe prognosis than the outcome most patients actually experience.

Conversely, clinical course, which is markedly influenced by a variety of factors including both parental and medical care as well as concomitant illnesses, often overestimates or underestimates the severity of SMA. The clinical course in type is known to be closely related to respiratory muscle dysfunction. In our view, the severity of respiratory muscle involvement constitutes a useful marker for distinguishing between type I and type II, and thus it is a useful criterion for classification, as exemplified by case no 13. It should also be pointed out that the life span of type I cases is nowadays greatly prolonged by the use of artificial ventilation (e.g. nos. 3, 4, 6, 7, 11, 12), such that age at death has lost its significance as an index of clinical course in SMA patients in recent years. We also assessed highest motor functional ability as the sole criterion for classifying SMA patients clinically. Typical SMA type I cases can not sit unaided and never attain head control. We consider cases who can main-

tain a sitting posture with or without support to have typical type II SMA, and those who can walk without support to have type III. This criterion was apparently the most useful. However, the use of this criterion has inherent disadvantages. The duration of peak motor functional ability, e.g. walking in type III, was often quite brief, whereas lower abilities, e.g. sitting unaided, were generally maintained for the greater part of the patient's life. Thus, a patient who walked even briefly would ultimately be classified as type III despite having a clinical course basically compatible with type II. The marked variability in the durations of various motor functional abilities and the exsistence of borderline cases, such as nos. 13, 28, 29 and 30, makes SMA classification extremely difficult. These results underscore the difficulties encountered in attempting to classify SMA patients using all three criteria. Dubowitz[11] proposed a flexible classification for the full spectrum of SMA. This classification includes types 1.1-1.9, as represented by type I herein, types 2.1-2.9 as type II and types 3.1-3.9 as type III ("Type 4.0"=normal). Type 1.1 is the most severe form of type I, while type 1.9 is the mildest and is similar to type II (Types 2.1-2.9 and types 3.1-3.9 show a similar gradation). This system is, however, very difficult to apply in the clinical setting.

Given all of the above considerations and our experience, classification according to highest motor functional ability appears to yield the fewest inconsistencies and most accurately reflects the severity of SMA. We therefore relied on this criterion in correlating genotype with phenotype, in this study.

2. Consideration of the results of DNA analysis

As a result of the current analytic study of the SMN and NAIP genes (Table 3 and 4), two type I cases were revealed to have deletions of exons 7 and 8 of the SMN gene and of exons 5 and 6 of the NAIP gene. There were no type II or III cases with this deletion pattern. Deletion of both exon 7 and 8 of the SMN gene with no the NAIP gene deletions (pattern B), was demonstrated in 28 cases including 6 type I, 17 type II and 5 type III cases. Deletion pattern B was most commonly found in type II (type II 94%>I 60%>III 45%). One case of each type had a deletion of exon 7 of the SMN gene alone. In the remaining 6 cases (15%), one of type I and 5 of type III, no deletions of either exons 7 and 8 of the SMN gene or exons 5 and 6 of the NAIP gene were found. Deletions in type I cases tended to be larger than those in type II; in type I, two cases had deletions of both SMN and NAIP genes, and six had deletions of exons 7 and 8 and one has a deletion of only exon 7 of the SMN gene, while in type II, the vast majority of cases (94%) showed deletions of exons 7 and 8, but non had deletions of both the SMN and NAIP genes. Nearly half of type III cases had deletions of exons 7 and 8 or only exon 7 of the SMN gene, while the remainder had no known deletions. These results simply that the size of the SMA gene deletion determines disease severity and to correlate with clinical type. Case no. 3, who had no deletions, however, was a typical type I SMA case clinically.

Lefebvre et al[6] reported cases with a point mutation or short deletions in the consensus splice sites of introns 6 and 7. Our case no. 3 may have had such a short deletion or point mutation, which would not have been detected by the methods employed in this study. Although difficult, classifying SMA is important for determining the prognosis of patients with this untreatable disease. The aforementioned difficulties encountered in classifying individual SMA patietns into one of the three clinical subtypes are quite understandable given that the gene responsible for SMA is the same for all three types. A relationship between deletion size and clinical severity has been suggested, though the precise mechanism responsible for the variable severity has yet to be elucidated.

3. The DNA analysis of atypical cases

Case no. 1 had mental retardation, one of the exclusion criteria for the diagnosis of SMA,

and did not have tongue fasciculation. Rudnik-Schöneborn et al[13] reported that cases with SMN gene deletions could be classified into three types: diaphragmatic SMA, SMA with olivopontocerebellar atrophy and SMA with congenital arthrogryposis and bone fractures as well as SMA plus variant. Our case no. 1 did not conform to any of these types. This case may be genetically heterogeneous without liknage to 5 q13. Case no. 2 showed deletions of the SMN gene, suggesting that some atypical cases with exclusion criteria may nonetheless have SMA.

4. Review of the literature on DNA analysis of SMA

We compared the frequencies of deletions of the SMN and NAIP genes among reports in the international literature (Table 6). The frequency of deletions in Japanese cases, i.e. our present patients, was 86% (30/35) for the SMN gene and 6% (2/35) for the NAIP gene. The NAIP gene deletion frequency was 23% in type I and 0% in types II and III. Reports from other countries cited in Table 6 all showed the SMN deletion rate to be over 90%, markedly higher than in our study. Although Ishikawa et al[22] reported a 91% deletion rate for the SMN gene in a small number of Japanese patients, SMN and NAIP gene deletions appear to be less common in Japan than in other countries, in general. The reasons for these discrepancies are unknown, but racial differences may be contributory. It is possible that the deletion is smaller in Japanese than in other races.Examination of materials submitted to our laboratory from other racial groups, 1 Korean, 1 Japanese-African, 1 Indian and 1 Chinese, revealed all to have deletions of exons 7 and 8 of the SMN gene. Another Korean case had no deletions of either gene. The incidence of NAIP gene deletions tended to be higher in the severe type than in milder forms of SMA. Roy et al[7] reported comparable results. These observations suggest that the deletion size is related to SMA types.

The NAIP gene is homologous with the apoptosis inhibitor proteins from baculovirus. Roy et al[7] suggested that a failure of normal inhibition of motor neuron apoptosis due to mutation of the NAIP gene may be the pathogenetic mechanism causing this disease at the molecular level. Recently, Lefebvre et al[12] reported that the protein product of the SMN gene is located within a novel nuclear structure and interacts with RNA-binding proteins.

5. Relationship between the type of DNA

Table 6 Frequencies of deletions of SMN and NAIP genes in reports from several countries

Country	n	SMN gene deletion(%)	NAIP gene deletion (%)*	References
France	229	98.6		Lefebvre et al, 1995[6]
Canada	110		I : 45, II, III : 18	Roy et al, 1995[7]
UK	140	97.8		Rodrigues et al, 1995[14]
Switzerland	62	90.4	46.8	Spiegel et al, 1996[15]
Belgium	23	91.3		Goemans et al, 1996[16]
Netherlands	103	93	37	Cobben et al, 1995[17]
Bulgaria		91		Joadainova et al, 1996[18]
Turkey	69	91	I : 70, II : 15	Erdem et al, 1996[19]
Kuwait	13	100	0	Moosa et al, 1996[20]
Taiwan	89	98.9	21.3	Chang et al, 1995[21]
Japan	11	91	0	Ishikawa et al, 1996[22]
Japan	35	86	I : 23, II, III : 0	Shiraiwa et al : this study 1997

* I, II, III : SMA type, I, II, III.

The figure shown here represents the gene deletion frequency in Japanese SMA patients, i.e. those in the present study. Calculations were made excluding four non Japanese patients (cases nos. 6, 12, 34 and 36) from the study population.

deletion and clinical features

There have been a limited number of DNA studies of SMA, including prenatal diagnosis[23)24)], but there are no reports precisely describing the clinical features of patients in relation to their deletion patterns. It is important to provide information about the quality of life of patients showing specific deletions, as part of the genetic counseling offerd in association with prenatal diagnosis. It is also essential for the family to be aware of their child's prognosis and certain difficult decisions, most notably whether or not one should initiate ventilatory assistance. This prognostic information allows parents to make appropriate plans for the child' s future, i.e. education and social adaptation.

Although the number of patients in our study is limited, our study demonstrated that patients with large deletions including the NAIP gene never attain head control while those with the type B deletion pattern show a broad range of motor abilities. Furthermore, 17% of the latter patients were able to walk and 13% lived beyond 18y. As there were only three patients with the type C deletion pattern, the data are insufficient to speculate as to the significance of deletion of exon 7 only. Even among the casas with no deletions, i.e. pattern D, one case was atypical and may thus have a different mutation, while the other had a clinical picture compatible with typical SMA type I as confirmed by one of the authors (Fukuyama). Further study of more cases is necessary before definitive conclusions can be drawn regarding the clinical relevance of the SMN and NAIP gene deletions examined in the present study.

The three types of SMA described herein have in common a constellation of clinical characteristics, supporting the assumption that they represent a single genetic disease. Nonetheless, SMA constitutes a broad and continuous spectrum, clinically, perhaps based on different levels of SMA gene expression. Our hope is that the SMA gene will be identified, allowing elucidation of the molecular parthogenetic mechanism underlying this disease, and eventually leading to effective treatment of patients and prevention of this devastating disease.

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小児期の近位型脊髄性筋萎縮症の臨床像と分子遺伝学的診断

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脊髄性筋萎縮症（SMA）は脊髄の前角細胞の変性，脱落により，筋萎縮や筋力低下を呈する常染色体性劣性の遺伝病である．本疾患は発症年齢，最高到達運動能力，経過により3つの型に分類される．分子遺伝学的には，1990年に3型ともに5q13に存在することが証明され，さらに1995年SMAの候補遺伝子としてSMN（survival motor neuron）遺伝子とNAIP（neuronal apoptosis inhibitory protein）遺伝子が報告された．本研究ではSMA患者を臨床的に分類し，SMN遺伝子，NAIP遺伝子について解析した．また，臨床型と遺伝子学的解析結果の関係を検討した．対象はSMA患者39名と精神遅滞，心肥大を各々伴ったatypical SMA 2名である．1990年のInternational SMA Collaboration Workshop Reportの臨床型分類をもとに39名を分類した．この分類における発症年齢，最高到達運動能力，経過の3つの基準のすべてを満たし，臨床型に分類できたのは39例中20例であった．発症年齢，運動能力，経過のそれぞれについて分類した結果，発症年齢，経過では幅拡い臨床像を示し，臨床的に分類するのには最高到達運動能力および経過が有用であった．また，遺伝子解析の結果，全体の85%（33/39：I型90%，II型100%，III型54%）においてSMN遺伝子のexon 7のみ，またはexon 7および8のホモ接合性の欠失を認めた．また，I型の2名（5% 2/39）においてSMN遺伝子の欠失と同時にNAIP遺伝子のexon 5および欠失が認められた．両遺伝子の認められた2例ともにI型の重症例であったが，その他のSMN遺伝子のみの欠失した症例は臨床症状にかなりの幅が認めれていた．いずれの欠失も認められなかった7例のうち5例はIII型であった．以上より臨床的重症度と遺伝子の欠失サイズの相関が示唆された．atypical SMAのうち1例にSMN遺伝子の欠失が認められた．また，精神遅滞をともなった1例は欠失が認められず，異なる疾患の可能性も考えられた．