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Phenotype-Genotype Correlation in Japanese Spinal Muscular Atrophy Patients : Analysis of DNA and mRNA of the SMN Gene

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder, classified into three types. The SMN (survival motor neuron) gene has been identified as a SMA-determining gene, designated SMNt and SMNc. Although the etiology of SMA is regarded as deletion of SMNt, the severity of clinical symptom is different among three types, and they show the same deletion of SMNt. In order to elucidate the basis of clinical variation, we analyzed the DNA and mRNA of the SMN gene in the Japanese 29 SMA patients. A large gene deletion including SMN and other genes was recognized in severe clinical type. We detected a hybrid gene, the products of gene conversion from SMNt to SMNc. Hybrid genes were found by a total nine patients, only in type II and III. We also performed sequence analysis of the cloned RT-PCR products of SMN exons 6, 7 and 8. We obtained telomeric sequence of exon 7 of one patient. It suggests that sequence conversion replacing SMNc with SMNt may have occurred at mRNA level in this patient. In conclusion, deletion of the SMNt and the modifier genes made the phenotype more severe, and partial gene conversion event was the explanation for the milder phenotype.

Key words: SMA, SMN gene, gene conversion

Introduction

Spinal muscular atrophy (SMA) is a frequent autosomal recessive disorder characterised by degeneration of lower motor neurons in the anterior horn of the spinal cord, leading to symmetrical proximal muscle weakness and atrophy. Affected individuals are classified into three groups on the basis of age at onset and disease progression. The spectrum of severity is broad with classification depending on the ability to sit or stand[1)~3)]. Type I SMA (Werdnig-Hoffmann disease) is the most severe form, with clinical onset before the age of 6 months, and patients are never able to sit without support and rarely live beyond the age of 2 years, owing to respiratory muscle weakness. Type II SMA is intermediate in severity. The onset is before the age of 18 months, and patients can sit but are unable to stand. Type III SMA (Kugelberg-Welander disease) is a much milder form, with the onset after 18 months and patients are able to stand and walk.

The SMN (survival motor neuron) gene, which has been identified as a SMA-determining gene, is located in chromosome 5q13[4)]. This region is characterized by the presence of many duplicated or inverted sequences. There are two copies of the SMN gene, the telomeric copy, SMNt and the centromeric copy, SMNc. Both copies contain nine exons, which encode identical amino acid sequence and span 20 kb of the genome. The SMN transcript is 1.7 kb and codes for a protein consisting of 294 amino acids. SMNt is distinguished from SMNc by only five nucleotide differences: one in intron 6, one in exon 7, two in intron 7 and one in exon 8. The C-to-T transition in exon 7 is a translationally silent nucleotide exchange.

Homozygous deletions of exons 7 and 8 (or only exon 7) of SMNt were described in up to 95% of SMA patients[4)~11)]. In Japan the figure is lower with 87% of Japanese SMA patients lacking SMNt[12)13)]. In this region of 5q13, there are also other modifier genes for SMA, such as neuronal apoptosis inhibitory protein (NAIP) gene[14)], and small EDRK-rich factor 1 (SERF1) gene[15)]. Deletions involving these genes may modify the SMA phenotype.

Although the same deletion of SMNt is found in nearly all cases of SMA, there is great variation in the severity of clinical symptoms. It has been suggested that milder phenotypes would be due to a smaller gene deletion including modifier genes[16)~19)], or gene conversion between the two copies of SMN[4)20)~27)]. In order to elucidate the basis of clinical variation in SMA, we analyzed the SMN gene structure in Japanese SMA patients.

Subjects and Methods

Patients with spinal muscular atrophy

We examined 29 SMA patients (7 type I, 11 type II and 11 type III) and 10 healthy individuals. Informed consent was obtained. The diagnosis of SMA was made in agreement with the criteria established by the International SMA Consortium[28)]. We obtained approval from the Intramural Ethical Committee as "Genetic analysis of spinal muscular atrophy patients". Of the 29 SMA patients, 28 were Japanese, and one was Japanese-African (the mother is Japanese and the father is African) (patient 7). There were 12 males and 17 females. The clinical course and findings of the patients are shown in Table 1. The motor functional level was classified into ten levels, from level 0 to level 9; level 0 is designated as no head control, level 1 as feasible head control, level 2 as being able to sit, level 3 as being able to sit and turn on buttocks, level 4 as being able to shuffle on buttocks in sitting position, level 5 as standing with support, level 6 as walking with support, level 7 as walking unaided, level 8 as climbing up stairs without support and level 9 as being able to run. The maximum motor functional level is the highest motor function level the patient had ever reached, and the current motor functional abilities were the motor functional level at the time of molecular genetic examination.

Other clinical features were evaluated including the presence of hypotonia, deep tendon reflex, fasciculation and scoliosis. Serum creatine kinase (CK) levels were also measured.

Analysis of the SMN, NAIP and SERF1 genes

DNA was isolated from peripheral venous blood by the phenol-chloroform extraction method. The polymerase chain reaction (PCR) and restriction enzyme digestion were performed using methods previously described by van der Steege et al[29)] to detect the deletion of SMNt exons 7 and 8. PCR amplification of the NAIP gene exons 5 and 6 was performed as described[14)]. Using the multi-copy microsatellite marker C212[15)] haplotype analysis was performed. None or one C212 allele per chromosome indicated a deletion of SERF1.

RNA preparation and reverse transcription (RT)-PCR analysis of exons 6, 7 and 8 of the SMN gene

Peripheral lymphocyte RNA was extracted by acid guanidinium thiocyanate-phenol-chloroform method[30)]. One microgram of total RNA was denatured with random primer (9mer) and 4 μl of 2.5 mM dNTPs (TAKARA, Japan) at 70 ℃ for 3 min. Four microliter of 5× reverse transcriptase buffer, 2 μl of 0.1 M DTT, M-MLV (Moloney-murine leukemia virus) reverse transcriptase (GIBCO BRL, describe the state name in USA) and RNase inhibitor (GIBCO BRL) were added to give a total volume of 20 μl. The reaction was performed at 42 ℃ for 60 min. The single strand cDNAs were amplified with 180 ng each of the exon 6 forward primer, 541C618[4)] and the exon 8 reverse primer 541C1040[4)], dNTPs, Ex Taq Polymerase (Takara, Japan) and Ex Taq buffer. The reaction conditions consisted of an initial denaturation at 94 ℃ for 7 min, followed by 30 cycles of 94 ℃ for 1 min, 66 ℃ for 1 min, and 72 ℃ for 2 min. PCR products were analyzed by electrophoresis in 3% agarose gels.

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene[31)] was amplified as an internal control to allow analysis and comparison of the SMN transcripts between samples.

Table 1 Clinical course of SMA patients

Type	Patient	Onset age (y : m)	Current age (y : m)	Age at respirator use (y : m)	Deceased age (y : m)	Wheel chair bound (y : m)	motor functional level		Hypotonia	PTR	ATR	Tongue fasciculation	Scoliosis	CK (IU/l)
							Maximum	Current						
	1	0 : 0	3 : 9	0 : 6			0	0	+ +	−	−	+	−	216
	2	0 : 1	0 : 4		0 : 4		0	0	+ +	−	−	+	−	420
	3	0 : 1	0 : 7		0 : 7		0	0	+ +	−	−	+	−	na
	4	0 : 2	5 : 8	0 : 7			0	0	+ +	−	−	+	−	na
I	5	0 : 2	0 : 4		0 : 4		0	0	+ +	−	−	+	−	250
	6	0 : 4	4 : 0	0 : 11			0	0	+ +	−	−	+	−	59
	7	0 : 4	12	1 : 0	12		0	0	+ +	−	−	+	−	31
	8	0 : 3	8 : 1		11		1	0	+ +	−	−	+	+ +	91
	9	0 : 3	9 : 5				3	3	+	−	−	+	+	39
	10	0 : 4	15 : 11				3	2	+	−	−	+	+	nd
	11	0 : 5	1 : 0				2	2	+	+	+	+	−	215
	12	0 : 7	1 : 3				2	2	+	−	−	−	−	147
II	13	0 : 8	1 : 4				3	2	+	−	−	+	na	97
	14	0 : 11	4 : 8				6	3	+	−	−	+	+	72
	15	0 : 11	1 : 2				3	3	+	−	−	−	−	80
	16	1 : 0	7 : 8				3	2	+	−	−	+	na	76
	17	1 : 3	8 : 8				6	3	+	−	−	+	+ +	78
	18	1 : 3	14 : 2				5	3	+	−	−	+	+	97
	19	0 : 6	33			3 : 5	7	2	+	−	−	+	+ +	19
	20	1 : 0	6 : 0				9	9	+	na	na	−	−	140
	21	1 : 0	37			5	7	4	+	−	−	+	na	46
	22	1 : 6	26				8	4	+	−	−	+	na	566
	23	1 : 6	22			7 : 10	8	2	+	−	+	+	+	118
III	24	1 : 6	11 : 10				8	7	+	−	−	+	−	332
	25	1 : 7	9			3 : 9	7	7	+	−	+	+	+	95
	26	3	22			8	8	6	+	−	−	+	+	120
	27	3	24				9	8	+	−	−	−	−	1,344
	28	15	47				9	8	+	−	−	−	−	806
	29	40	49				9	8	+	−	−	−	−	634

The maximum motor functional level was the motor functional abilities the patient had ever reached and the current motor functional level was the motor functional abilities at the time of molecular genetic examination.

The motor functional level 0: designated as no head control, 1: feasible head control, 2: being able to sit, 3: being able to sit and turn on buttocks, 4: being able to shuffle on buttocks in sitting position, 5: standing with support, 6: walking with support, 7: walking unaided, 8: climbing up stairs without support, 9: being able to run.

PTR: patellar tendon reflex, ATR: ankle tendon reflex, CK: creatine kinase.

+ , + +: the clinical symptom was recognized, + +: means that the degree of the symptom was more severe, −: the symptom was not recognized, na: not available of the information.

Detection of a SMN hybrid gene

The presence of a hybrid gene, which results from gene conversion of exons 7 and 8 in between SMNt and SMNc, was analysed using PCR and restriction enzyme digestion. DNA was amplified with the primer for intron 6, RV6[22], and the primer for exon 8, 541C1120[4]. The PCR conditions have been described elsewhere[22]. PCR products were digested with *DdeI* (Roche, Germany) and *EcoRV* (Roche) overnight at 37 ℃ and were analyzed in 2.5% agarose gels.

The single stranded cDNAs were analyzed by PCR amplification and enzyme digestion, using *DdeI* and *XmnI* (Roche), by the method described[22] and were analyzed by electrophoresis in a 4% agarose gel.

Sequence analysis of the RT-PCR products and the cloned products of the SMN gene exons 6, 7 and 8

Direct sequencing analysis of the RT-PCR products of SMN exons 6, 7 and 8 was performed. RT-PCR amplification products were also subcloned into a TA cloning vector (Promega, describe the state name in USA) according to the manufacturer's instructions. Ten clones containing the SMN gene were cultured from each sample, and the DNA was purified with a plasmid miniprep kit (QIAGEN, describe the state name in USA). Cycle sequencing reactions were performed with the Big-Dye Terminator Reaction Kit (Applied Biosystems, describe the state name in USA). The products of cycle sequencing were purified through Centri-Sep columns (Applied Biosystems). An ABI Prism 310 machine (Applied Biosystems) was used for sequencing.

Statistical analysis of the correlation between clinical severity and the presence of the hybrid gene

The phenotype-genotype correlation was analyzed by comparing maximum motor functional level, onset age and the presence of the hybrid gene between type II and III SMA patients using t test without correspondence.

Results

Clinical course

The clinical course of SMA is markedly different among the three types (Table 1). Most type I patients die of acute respiratory failure. The maximum motor functional level and current motor function were level 0 in all type I patients in this study. In type II, the maximum motor functional level varied from 1 to 6, while the current motor function was 2 or 3. In type III, the maximum motor function was ranging from level 7 to 9, with the current motor function varied from level 2 to 9.

The average serum CK level ranged from 19 IU/*l* to 1,344 IU/*l*. There was no significant difference in serum CK value, among the three SMA types, using Student's t test without correspondence. Between types I and II, p value was 0.018. Between types II and III, p value was 0.508. Between types III and I, p value was 0.045. There was no statistical significant difference between the value of serum CK and the presence of the hybrid gene.

DNA deletion analysis

The results of DNA deletion analysis for 29 SMA patients are shown in Table 2.

In type I patients, the deletion of both SMNt exons 7 and 8 was found in all 7 patients. In type II patients, the deletion of only exon 7 was seen in one patient (patient 16), while the rest had deletion of both exons 7 and 8. In the milder form, type III, we found the deletion of only exon 7 in 4 patients (patients 19, 24, 27 and 28) and the other 7 patients had the deletion of both exons 7 and 8. Four patients of type I (patients 1, 3, 5 and 6) also showed the deletion of NAIP exons 5 and 6. In haplotype analysis using the multi-copy microsatellite marker C212, eight (four out of four type I, three out of five type II and one out of two type III) patients showed SERF1 deletion.

Detection of a SMN hybrid gene

Genomic DNA was analysed for the presence of SMN hybrid genes by PCR and restriction enzyme digestion. The primers RV6 and 541C1120 amplify the region from intron 6 to exon 8. Subsequent restriction enzyme analysis with *EcoRV* and *DdeI* allows the discrimination of SMNt and hybrid genes.

Table 2 Results of analyses of DNA and mRNA in SMA patients

Type	Patient	SERF 1	NAIP		SMN		Hybrid gene	
			exon 5	exon 6	exon 7	exon 8	DNA	mRNA
	1	nd	del	del	del	del	−	−
	2	nd	no-del	no-del	del	del	−	−
	3	+	del	del	del	del	−	−
I	4	+	no-del	no-del	del	del	−	−
	5	nd	del	del	del	del	−	−
	6	+	del	del	del	del	−	−
	7	+	no-del	no-del	del	del	−	−
	8	nd	no-del	no-del	del	del	−	−
	9	nd	no-del	no-del	del	del	−	−
	10	nd	no-del	no-del	del	del	−	+
	11	+	no-del	no-del	del	del	−	−
	12	−	no-del	no-del	del	del	−	−
II	13	−	no-del	no-del	del	del	−	+
	14	nd	no-del	no-del	del	del	−	−
	15	+	no-del	no-del	del	del	−	−
	16	nd	no-del	no-del	del	no-del	+	+
	17	nd	no-del	no-del	del	del	−	−
	18	+	no-del	no-del	del	del	−	−
	19	nd	no-del	no-del	del	no-del	+	−
	20	nd	no-del	no-del	del	del	−	+
	21	nd	no-del	no-del	del	del	−	−
	22	nd	no-del	no-del	del	del	−	−
	23	+	no-del	no-del	del	del	−	−
III	24	nd	no-del	no-del	del	no-del	+	−
	25	−	no-del	no-del	del	del	−	−
	26	nd	no-del	no-del	del	del	−	−
	27	nd	no-del	no-del	del	no-del	+	−
	28	nd	no-del	no-del	del	no-del	+	−
	29	nd	no-del	no-del	del	del	−	+

In type I, the deletion of both SMNt exons 7 and 8 were found in all 7 patients.
In type II, the deletion of only exon 7 was seen in one patient (patient 16), while the rest had the deletion of both exons 7 and 8. In type III, we found the deletion of only exon 7 in 4 patients (patient 19, 24, 27 and 28) and the other 7 patients had the deletion of both exons 7 and 8.
del: deletion, no-del: no-deletion, +: existent, −: not existent, nd: not done.

As described elsewhere[22], the size of SMNt was 960 bp, while that of SMNc was 780 bp. A hybrid gene resulting from the fusion of the 5′ part of the SMNc to the 3′ part of SMNt as a result of conversion event that effected exons 7 and 8 of SMNt would give a 900 bp fragment. An 840 bp fragment would result from the fusion of the 5′ part of the SMNt to the 3′ part of SMNc. In this study, the 840 bp fragment was not detected. Only in type II (9%) and III (36%) patients, hybrid genes (900 bp) were detected. The hybrid gene was identified from DNA in all 5 patients that had the deletion of only exon 7 (patients 16, 19, 24, 27 and 28).

In mRNA, RT-PCR and restriction enzyme analysis produced two fragments in control subjects: 330 bp and 303 bp for SMNt and SMNc, respectively[22]. A product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt results in a 351 bp product. This product of the fusion was detected in 5 patients (type II: 27%, type III: 18%, total: 17%); one patient who had the deletion of only exon 7 (patient 16), and the other four patients who showed the deletion of exons 7 and 8 (patients 10, 13, 20 and 29) (Figs. 1 and 2).

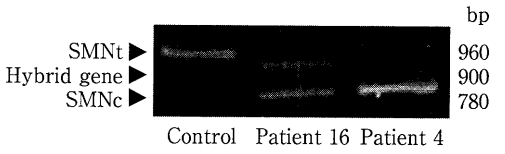
Sequence analysis of the cloned RT-PCR products of the SMN exons 6, 7 and 8

We performed the cloning of the SMN gene RT-PCR product in patients 2, 3, 4, 10, 13, 16, 19, 20, 22,

a

Type	Hybrid gene			
	DNA	(%)	mRNA	(%)
I	0/7	0	0/7	0
II	1/11	9	3/11	27
III	4/11	36	2/11	18
Total	5/29	17	5/29	17

b



c

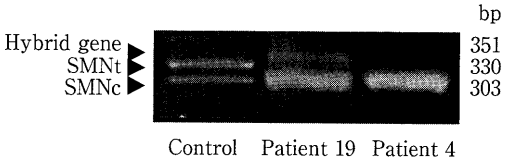


Fig. 1 Detection of the hybrid gene by PCR and enzyme-digestion analysis

a: The hybrid gene of DNA was identified in 5 patients (type II: 9%, type III: 36%, total: 17%). The product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt of mRNA was detected in 5 patients (type II: 27%, type III: 18%, total: 17%). The hybrid gene in both DNA and mRNA were found in one patient (patient 16).

b: Gel-electrophoresis of the enzyme-digestion products in DNA analysis. Control had SMNt (960 bp-band) and SMNc (780 bp). Five patients had the hybrid gene (900 bp).

c: Gel-electrophoresis of the enzyme-digestion products in mRNA. Control had SMNt (330 bp-band) and SMNc (303 bp). Five patients had the product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt (351 bp).

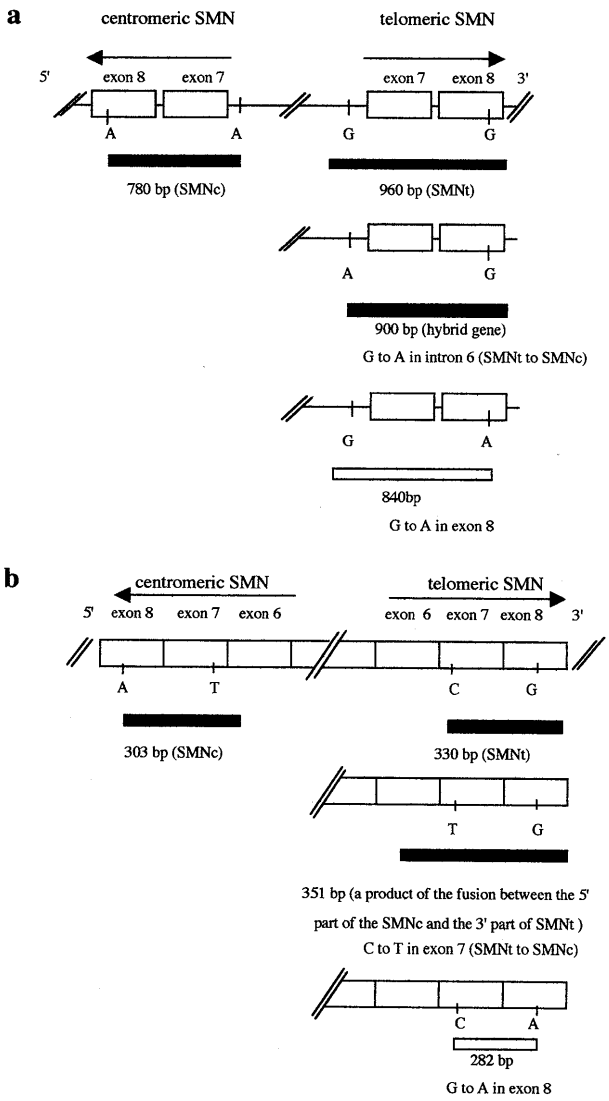


Fig. 2 Schematic presentation of PCR, RT-PCR and enzyme-digestion analysis of SMNt and SMNc

a: DNA analysis. The black bars represent the products after digestion. The 900 bp bar indicates the gene conversion resulting from the fusion of the 5′ part of the SMNc to the 3′ part of SMNt.

b: mRNA analysis. The black bars represent the products after digestion. The 351 bp bar indicates the gene conversion resulting from the fusion of the 5′ part of the SMNc to the 3′ part of SMNt.

24 and 26, because the result of the direct sequencing did not be enough for explaining of this complicated structure of SMN gene. Ten clones containing the SMN gene were cultured from each sample. By cloning of the SMN gene RT-PCR products followed by sequencing, the telomeric sequence of exon 7 was confirmed in the clone of patient 24 (Fig. 3).

Clinical severity and hybrid gene

The distribution of patients with type II or type III SMA according to maximum motor functional level by the presence of hybrid gene (a product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt) is shown in Fig. 4. The levels of the patients who had the hybrid gene tended to be higher, though there was no significant difference ($p = 0.1627$). Only patients with the hybrid gene reached the highest motor function level of 9; level 3 in three (33%), level 7 in one (11%), level 8 in one (11%) and level 9 in four (44%). Among patients who did not have a hybrid gene, seven patients were level 0, one patient was level 1, two patients were level 2 and there were no patients that reached more than level 8. In this study, the symptom of the patients who had a hybrid gene tended

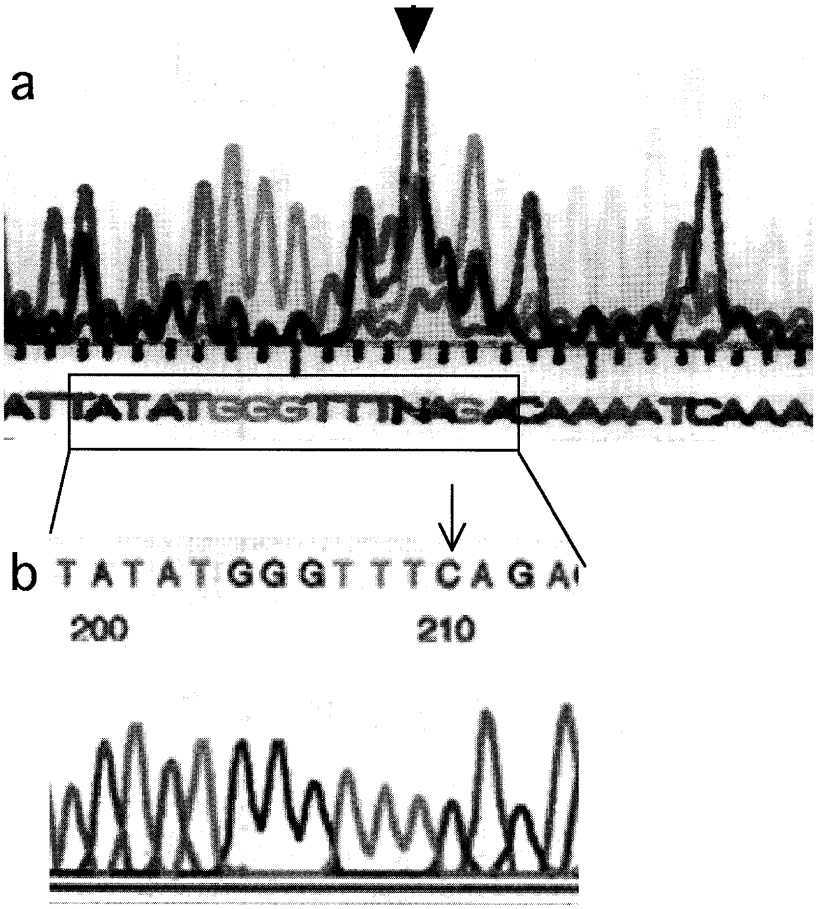


Fig. 3 Sequence analysis of RT-PCR products of exon 6, 7 and 8
a: By direct sequencing of RT-PCR products of exons 6, 7 and 8, the mixed sequence (arrow head) that contained SMNt and SMNc.
b: As a result of cloning of RT-PCR products of exons 6, 7 and 8, the clones that had the sequence of SMNt (arrow) were identified.

to be milder.

There was no correlation between the presence of the hybrid gene and current motor functional abilities ($p = 0.011$) or maximum motor function ($p = 0.036$). The distribution of patients according to age of onset by the presence of hybrid gene was shown in Fig. 5. The onset age was generally higher, in the patients who had the hybrid gene ($p = 0.1166$).

Discussion

The SMA region on chromosome 5q13 includes the SERF1, SMN and NAIP genes. This region contains a 500 kb duplication and inversion, which causes high gene instability and leads to deletions or gene conversions. It has been suggested that a large gene deletion including SMN and other modifier genes in the SMA region caused more severe clinical symptoms[16)~19)]. Our results support this hypothesis as the deletion of all 3 genes, SERF1, SMNt and NAIP was only seen in 3 patients with type 1 SMA (patients 3, 4 and 6). However a large deletion involving SERF1 and SMNt in the SMA gene region had variable clinical features. This deletion was found in patients with all 3 forms of SMA (patient 7 in type I, patients 11, 15 and 18 in type II and patient 23 in type III). In those patients, no SMN hybrid genes (the product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt) were detected which could have influenced the clinical outcome.

Two types of SMN gene conversion were seen (summarized in Tables 3, 4 and Fig. 6). In type A, homozygous conversion was present in exon 7 of SMNt, which led to four copies of exon 7 of SMNc. If three copies of exon 7 of SMNc were observed, one

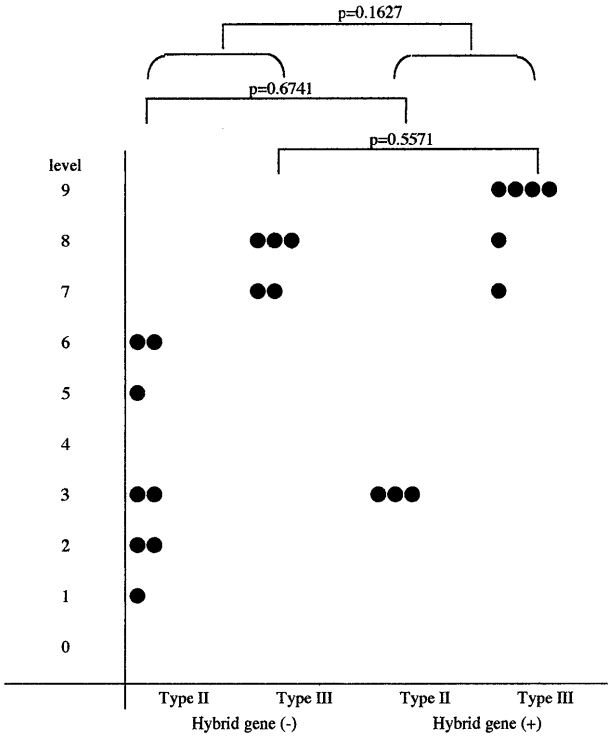


Fig. 4 The distribution of patients of type II or type III SMA according to maximum motor functional level by the presence of hybrid gene (the product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt)
The levels of patients who had hybrid gene showed a tendency to be higher, though there was no significant difference ($p = 0.1627$). There was no significant difference ($p = 0.6741$ in type II and $p = 0.5571$ in type III).

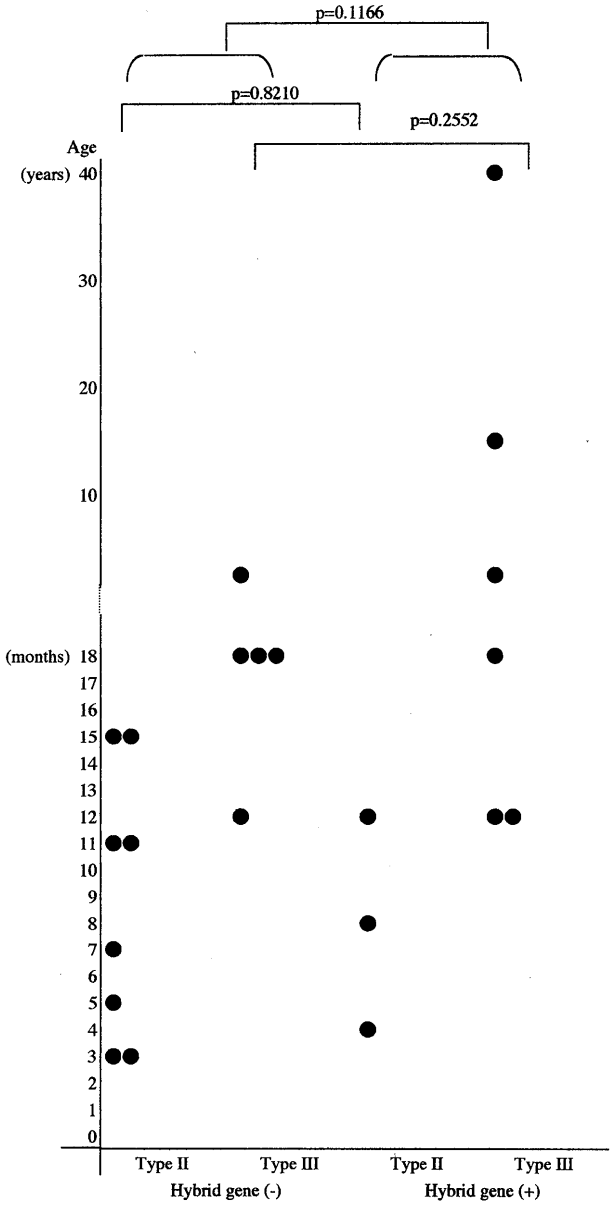


Fig. 5 The distribution of patients of type II and type III according to the age of onset by the presence of hybrid gene (the product of the fusion between the 5′ part of the SMNc and the 3′ part of SMNt)
The onset age showed a tendency to be higher, in the patients who had the hybrid gene ($p = 0.1166$). In each type, there was also no correlation in type II ($p = 0.8210$) and type III ($p = 0.2552$).

allele showed the conversion in exon 7 of SMNt, while the other allele showed the deletion of both exons 7 and 8 of SMNt (type A*). We concluded type A or A* conversion was present in patients 13, 16 and 28. In type B, the conversion was present in both exons 7 and 8 of SMNt in one allele and the conversion in exon 8 of SMNc with the deletion of both exons 7 and 8 of SMNt in the other allele. There is a possibility of the presence of type B* with the conversion in exons 7 and 8 of SMNc in addition to homozygous deletion of exons 7 and 8 of SMNt. We concluded type B or B* conversion was present in patients 10, 19, 20, 21, 24, 25, 27 and 29. Type A was seen in two patients in type II SMA and in one patient in type III SMA, while type B conversion was detected in one patient in type II and seven patients in type III SMA.

We examined that the correlation between the presence of hybrid gene and the motor functional level. About the maximum motor functional level, among 5 patients with hybrid gene, one was level 3, one level 7, one level 8 and two were level 9. All five patients (patients 16, 19, 24, 27 and 28) with the hybrid gene, also contained a homozygous deletion of

only SMNt exon 7. It has been proposed that the deletion of only SMN exon 7 meant the sequence conversion from SMNt to SMNc[22)23)]. The five patients with a deletion of only exon 7 of SMNt also contained the hybrid gene, suggesting that the gene conversion had resulted not deletion of SMNt exon 7. As the hybrid gene was only seen in type II and type III SMA patients, the gene conversion from SMNt to SMNc was associated with a milder phenotype. This is consistent with other reports[23)32)]. However the hybrid gene has also been found in type I patients, with almost equal distribution in the three

Table 3 DNA rearrangement of SMA patients, which was presumed by the presence of hybrid gene and the sequence of mRNA analyses

Type	Patient	Deletion or Conversion
	1	large deletion involving NAIP
	2	deletion of SMNt
	3	large deletion involving SERF1 and NAIP
I	4	large deletion involving SERF1 and NAIP
	5	large deletion involving NAIP
	6	large deletion involving SERF1 and NAIP
	7	large deletion involving SERF1
	8	deletion of SMNt
	9	deletion of SMNt
	10	Type B conversion
	11	large deletion involving SERF1
	12	deletion of SMNt
II	13	Type A conversion
	14	deletion of SMNt
	15	large deletion involving SERF1
	16	Type A conversion
	17	deletion of SMNt
	18	large deletion involving SERF1
	19	Type B conversion
	20	Type B conversion
	21	Type B conversion
	22	deletion of SMNt
	23	large deletion involving SERF1
III	24	Type B conversion
	25	Type B conversion
	26	deletion of SMNt
	27	Type B conversion
	28	Type A conversion
	29	Type B conversion

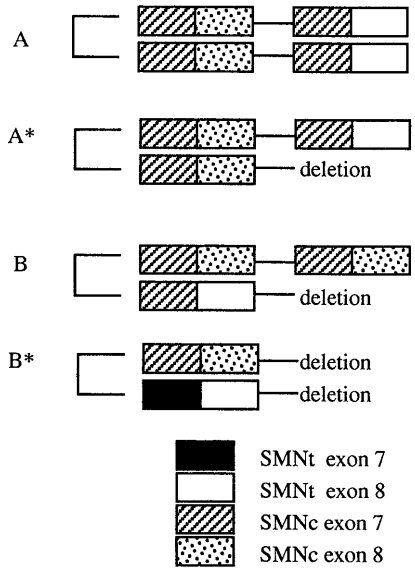


Fig. 6 Details of the rearrangement in two types of conversion
Type A: homozygous conversion in exon 7 of SMNt, which lead to four copies of exon 7 of SMNc.
Type A*: one allele showed conversion in exon 7 of SMNt, while the other allele showed deletion of both exons 7 and 8 of SMNt.
Type B: conversion in both exons 7 and 8 of SMNt in one allele and conversion in exon 8 of SMNc with deletion of both exons 7 and 8 of SMNt in the other allele.
Type B*: conversion in exons 7 and 8 of SMNc in addition to homozygous deletion of exons 7 and 8 of SMNt.

Table 4 Detail of rearrangement in two types of conversion

Conversion type	Rearrangement of allele 1				Rearrangement of allele 2			
	SMNc		SMNt		SMNc		SMNt	
	exon 7	exon 8	exon 7	exon 8	exon 7	exon 8	exon 7	exon 8
A	SMNc exon 7	SMNc exon 8	*SMNc exon 7*	SMNt exon 8	SMNc exon 7	SMNc exon 8	*SMNc exon 7*	SMNt exon 8
(A *)	SMNc exon 7	SMNc exon 8	*SMNc exon 7*	SMNt exon 8	SMNc exon 7	SMNc exon 8	deletion	deletion
B	SMNc exon 7	SMNc exon 8	*SMNc exon 7*	*SMNc exon 8*	SMNc exon 7	*SMNt exon 8*	deletion	deletion
(B *)	SMNc exon 7	SMNc exon 8	deletion	deletion	*SMNt exon 7*	*SMNt exon 8*	deletion	deletion

types[21)33)~35)]. There is no difference between the races. In this study, there was the mild correlation between the presence of the hybrid gene and current motor functional abilities ($p = 0.011$) or maximum motor functional level ($p = 0.036$). The age of onset tended to be higher in the patients who had the hybrid gene within the same subtypes. Comparing clinical features according to the presence of the hybrid gene in each patient suggested that there were a little difference with clinical course (Tables 1 and 2).

In cloning sequence analysis, we obtained clones, which had telomeric sequence of exon 7 in the sample of patient 24. Why was the clone of SMNt exon 7 found in sequence analysis? The possible explanation is that the sequence conversion replacing SMNc with SMNt had occurred at the mRNA level in this patient. In some previous reports, it was indicated that the gene conversion from SMNc to SMNt was found in unaffected individuals[21)22)36)37)]. Van der Steege et al[22)] reported that a hybrid gene having the 5′ part of SMNt fused to the 3′ part of the SMNc would be as functional as the normal SMN gene. It seems that the gene conversion of SMNc to SMNt may be also related to mild clinical severity. The mechanism of the gene conversion replacing SMNc with SMNt is not clear. We will continue the additional study to elucidate the mechanism in SMA patients, carriers and unaffected individuals.

In conclusion, the deletion of the SMN gene and modifier genes, NAIP and SERF1, tend to make the SMA phenotype more severe, and SMN gene conversion events would result in a milder phenotype.

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日本人の脊髄性筋萎縮症における表現型と遺伝型の関係
—SMN 遺伝子の DNA および mRNA 解析—

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脊髄性筋萎縮症（SMA）の原因遺伝子 survival motor neuron 遺伝子（SMNt）にはコピー遺伝子（SMNc）が存在し，患者では SMNt が欠失している．SMA の各臨床型（I，II および III 型）でその遺伝子の構築は異なる．我々は，SMN 遺伝子の構築と臨床的重症度との関係を検討するため，SMN 遺伝子を DNA，mRNA で解析した．当科での遺伝子診断を希望され，保護者または本人からインフォームドコンセントを得た SMA 患者 29 名を対象とし，末梢血リンパ球を用いた．遺伝子欠失範囲は，SMNt のエクソン 7, 8，修飾遺伝子の NAIP と SERF1 の解析により同定した．また，SMNt が SMNc へ変換したことを示す hybrid gene を検出した．SMN エクソン 7, 8 の RT-PCR 産物を直接塩基配列決定法で解析し，さらにクローニングを行った．DNA の分析では，I 型で主に SERF1 から NAIP までの広範囲の欠失を，II，III 型では SMNt のみの欠失を認め，欠失範囲が広いと重症となる傾向があった．Hybrid gene は 9 例で存在した．最高到達運動レベルは hybrid gene のある例がない例に比しやや高い傾向にあった．クローニング産物の塩基配列解析では 1 例で SMNt の配列を認めた．得られた結果より 29 例の DNA の構築を分類すると，重症の I 型では全例が真の欠失を示し，II, III 型では遺伝子変換を示すものを認めた．SMNt から SMNc への遺伝子変換により SMNc の量が増えた例と SMNc から SMNt への遺伝子変換により SMNt の欠失を代償している例が存在したが，これらは，真の欠失を示す例よりも臨床型は軽症になることが示唆された．遺伝子変換は軽症の II 型，III 型のみで認めたことから，SMN 遺伝子における遺伝子変換は臨床症状の軽減化と関係すると考えられた．