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Original article

Relationships between long-term observations of motor milestones and genotype analysis results in childhood-onset Japanese spinal muscular atrophy patients

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Abstract

Aim: To clarify the long-term natural history of SMA in Japanese patients by investigating the peak motor milestones of cases 7 months through 57 years of age, in efforts to contribute to evaluating outcomes of new therapeutic interventions.

Methods: We sub-classified 112 SMA type I-III cases into type Ia, type Ib, type IIa, type IIb, type IIIa and type IIIb, according to peak motor milestone achieved, and analyzed the *SMN1, SMN2* and *NAIP* genes in relation to clinical subtypes.

Results: In type I cases, there was a significant difference ($p < 0.0001$), depending on whether or not head control was obtained, in the time of ventilation support being required. In type II cases as well, the time at which the ability to maintain the sitting position independently was lost also differed significantly ($p < 0.01$) between those acquiring the ability to sit unaided within eight months after birth and those acquiring this ability after eight months of age. In type III cases, being able versus unable to climb stairs was associated with a significant difference ($p = 0.02$) in the median time until loss of walking independently. Positive correlations were also seen between copy numbers and the clinical severity of SMA.

Conclusion: Our long-term results show peak motor milestone evaluations distinguishing between subtypes to be useful not only as outcome measures for assessing treatment efficacy in clinical trials but also for predicting the clinical courses of Japanese SMA patients.

Keywords: Spinal muscular atrophy; Motor milestones; Gene copy number; Clinical severity; *SMN2*; *NAIP*

1. Introduction

Spinal muscular atrophy (SMA), an autosomal recessive disorder characterized by degeneration and deficits of motor neurons in anterior horn cells of the spinal cord, shows progressive muscular atrophy and weakness of affected proximal muscles [1].

SMA is classified into four types on the basis of age at onset and the achievement of motor milestones. Type I (Werdnig-Hoffmann disease; OMIM 253300) has an onset before the age of six months and floppiness is evident in affected infants. It is impossible for these children to sit without support, and difficulty with nursing and inability to swallow, aspiration, respiratory failure, and tongue fasciculation are seen. These children require

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feeding support measures such as nasogastric tube feeding. The average life expectancy is 8 months, and mortality is 75–95% within 24 months in the absence of respiratory support [2]. Type II (Dubowitz disease; OMIM 253550) cases also have an onset in infancy but are able to maintain a sitting position, though they never gain the ability to stand or to walk without support, and tongue fasciculation is seen. Arthrogryposis and scoliosis become increasingly prominent with growth, and respiratory failure is likely to develop after an airway infection. Type III (Kugelberg-Welander disease; OMIM 253400), with childhood onset, is characterized by the ability to walk independently at first, with gradual deterioration, as individuals fall easily, lose the abilities to walk and get up, and elevating the arms becomes more difficult. Individuals with type IV (OMIM 271150), a slowly progressive lower motor neuron disorder, have their onset in adulthood [2–4]. Muscle weakness and atrophy are seen in all SMA types, along with diminution and, ultimately, disappearance of deep tendon reflexes [2,3]. The incidence of childhood SMA is 1–2 in 10,000 live births and the number of patients in Japan is presumed to be approximately 1000 [5,6].

SMA is caused by homozygous deletion, point mutation or gene conversion of the survival motor neuron 1 (*SMN1*) gene located on chromosome 5q13 [5,7,8]. A highly homologous gene named *SMN2* is present in this region, containing five discrepancies allowing *SMN1* to be distinguished from *SMN2*. The single base pair difference, a C to T transition in *SMN2* exon7, is responsible for alternative splicing of exon 7 and producing a high percentage of truncated *SMN2* transcripts lacking exon 7, and thereby in a lower abundance of the full-length transcript. Thus, this difference causes individuals with SMA to have low levels of both the full-length transcript and the functional SMN protein [9]. The neuronal apoptosis inhibitory protein (*NAIP*) gene potentially exerts an influence on the SMA phenotype. The number of copies of the *SMN2* gene and the existence of the *NAIP* gene are thought to be related to the clinical severity of SMA symptoms [10–12].

A major goal of disease-modifying therapy is to increase SMN protein levels. Fundamental therapeutic strategies have not yet been established for SMA. There has, however, been remarkable progress in the development of new therapeutic approaches for SMA. Examples include acceleration of the *SMN2* gene transcript by histone deacetylase (HDAC) inhibitor [13–18] and splicing modification of the *SMN2* gene by antisense oligonucleotides, which have shown promising efficacy in clinical trials [19,20]. These therapeutic trials are ongoing in Japan and several other countries.

As outcome measures for these clinical trials, it is important to document the clinical courses, in terms of motor milestones, of children with SMA. We investigated and analyzed the acquisition and loss of peak motor milestones, changes in respiratory function, and feeding support requirements, over the long-term clinical course, according to a sub-classification of SMA types. Our aim was to clarify the natural histories of the different SMA types in affected Japanese patients. We also examined the relationships of copy numbers of *SMN2* gene exons and the *NAIP* gene with phenotypic features of Japanese SMA cases.

2. Subjects and methods

2.1. Subjects

One-hundred and ninety-six individuals, ranging in age from 7 months to 57 years, with SMA were enrolled by questionnaire. One hundred and eighty subjects were from the Spinal Muscular Atrophy Research & Treatment: SMART Consortium, http://www.sma-rt.org while 16 were outpatients at the Institute of Medical Genetics, Tokyo Women's Medical University. Participants were enrolled from July 2014 through July 2016. One-hundred and fifty-one (77.0%) cases agreed to participate and 112 (57.1%) completed the questionnaire. Thirty-nine cases were excluded for the following reasons: no *SMN1* deletion or mutation, onset age over 20, administered valproic acid, and/or motor milestone information was insufficient.

Table 1
Characteristics of subjects.

Type	Maximum motor function	Subtypes		Number of subjects			Age at entry: median (range)
				M	F	Total	
I	Never sit independently	I a	Head control (−)	19	19	38	56.0m (0y7m-16y9m)
		I b	Head control (+)	4	5	9	32.0m (1y7m-35y7m)
			Total	23	24	47	
II	Never stand independently	II a	Sit independently >8mo	6	4	10	71.0m (2y5m-39y10m)
		II b	Sit independently ≦8mo	14	18	32	89.0m (1y9m-44y)
			Total	20	22	42	
III	Stand & walk independently	III a	Climb stairs (−)	6	4	10	198.5m (5y2m-52y11m)
		III b	Climb stairs (+)	8	5	13	185.0m (4y9m-57y6m)
			Total	14	9	23	