Phenotypes of SMA patients retaining SMN1 with intragenic mutation

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学 位 論 文 の 内 容 要 旨

Phenotypes of SMA patients retaining *SMN1* with intragenic mutation

SMN1 遺伝子内変異を有する脊髄性筋萎縮症患者の臨床像

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SUMMARY

Background:

Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by degeneration of anterior horn cells in the human spinal cord and subsequent loss of motor neuron. SMA is clinically divided into five groups; type 0 (the most severe form with onset in the prenatal period; severe respiratory problems after birth), type 1 (Werdnig-Hoffman disease; a severe form with onset before 6 months of age; unable to sit unsupported), type 2 (Dubowitz disease; an intermediate form with onset before 18 months of age; able to sit unaided, but unable to stand or walk), type 3 (Kugelberg-Welander disease; a mild form with onset after 18 months of age; able to stand and walk unaided) and type 4 (the mildest form with age of onset from adolescence to adulthood).

Variations in the survival of motor neuron (*SMN*) genes, survival motor neuron 1 (*SMN1*) and survival motor neuron 2 (*SMN2*), are related to the development of SMA and had been mapped on chromosome 5q13.2. *SMN1* is now considered the disease-causing gene for SMA, because *SMN1* is deleted in more than 95% and is deleteriously mutated in the remaining patients. Whereas, *SMN2* was considered to be a modifying factor of the SMA phenotype, because higher copy number of *SMN2* may be related to milder SMA phenotypes.

This *SMN2* and clinical severity correlation, however, is well observed in homozygous *SMN1* deleted patients. It is more difficult to conclude the relationship between *SMN2* copy number and clinical severity in SMA patients with intragenic mutation.

Here, we reported an algorithm for comprehensive SMA diagnostic and then elucidated the relationship between clinical phenotypes and *SMN2* copy number. We had detected 241 SMA patients in our laboratory, and identified 13 patients with intragenic *SMN1* mutations with five novel mutations among them. We compared "patients with homozygous *SMN1* deletion" and "patients with intragenic mutation" to further evaluate the relationship between clinical phenotypes and *SMN2* copy number.

Methods:

A total of 515 Japanese patients with suspicion of SMA were referred to our laboratory from 1996 to 2019. All patients presented with SMA-like symptoms; delayed developmental milestone, respiratory problem, muscle weakness, etc. All patients underwent *SMN1* deletion test and copy number analysis. Sequencing was performed to those who retained at least 1 *SMN1* copy for mutation screening, followed by assignment of mutation location for any suspected mutation to *SMN1* or *SMN2* using either mRNA analysis or genomic DNA analysis. To evaluate the effect of mutation to SMN transcript or SMN protein, *in silico* modeling or transcript analysis was performed. Finally, parametric analysis was performed to investigate

the relationship between *SMN2* copy number and clinical subtypes.

Results and discussions:

Our diagnostic procedures for SMA were: (1) *SMN1* deletion test, (2) *SMN1* and *SMN2* copy number analysis, (3) *SMN1* and *SMN2* mutation screening, (4) assignment of the mutation to *SMN1* using genomic DNA, and/or (5) assignment of the mutation to *SMN1* using mRNA.

We analyzed 515 SMA suspicion patients referred to our facility using this algorithm and confirmed 228 cases as SMA patients with homozygous *SMN1* deletion. Whereas, the rest of them (287 cases), retained at least one *SMN1* copy. Subsequently, 33 out of 287 patients with SMA-like symptoms carried only one *SMN1* copy and only 13 out of 33 carried deleterious *SMN1* mutation causing SMA.

We identified 10 different mutations of *SMN1* in 13 SMA patients using genomic DNA and/or mRNA analysis according to our diagnostic algorithm. These mutations consisted of missense, nonsense, frameshift and splicing defect-causing mutations. Among them, we firstly reported five novel mutations of *SMN1* in six patients which consisted of two non-sense mutations in two unrelated patients, c.79C>T (p.Gln27*) and c.188C>A (p.Ser63*); one missense mutation in two related siblings, c.422T>C (p.Leu141Pro); and two splicing defect-causing mutations in two unrelated patients, c.835-2A>G and c.835-3C>A.

Our database of SMA patient with homozygous *SMN1* deletion followed the conventional observation where low *SMN2* copies resulted in severe phenotype and high copy number of *SMN2* may be related to milder SMA phenotypes. Patients with SMA type 1 usually carry only one or two copies of *SMN2*. SMA type 2 is usually associated with three copies. SMA type 3 patients have three to four copies and patients with SMA type 4 usually have four or more copies. High *SMN2* copy number may improve the survival outcomes and motor function.

However, among the SMA patients with an intragenic *SMN1* mutation, the relationship between clinical phenotype and *SMN2* copy number becomes ambiguous. We calculated mean values of *SMN2* copy number in each clinical subtype among the patients with *SMN1* mutation. The mean values were 2.40 in patients with SMA type 1 (n = 10), 1.00 in patients with SMA type 2 (n = 1), and 1.00 in patients with SMA type 3 (n = 2). Our data indicated that SMA type 1 with intragenic mutation tends to have a higher *SMN2* copy number.

We performed Kruscal-Wallis H test to compare *SMN2* copy numbers in SMA type 1, type 2 and type 3. The H statistic (degrees of freedom) and the P value were H (2) = 6.429 and $P<0.05$, suggesting the presence of significant differences among the three types. We also performed Mann-Whitney U test to compare *SMN2* copy numbers in SMA type 1 and non-SMA type 1 (type 2 and type 3). The U statistic (sample sizes) and the P value were U (10, 3) = 30.000 and $P<0.05$, suggesting the presence of significant differences between the two types. Our statistical analysis supported the idea that higher copy number might be related to severer phenotype among the SMA patients with intragenic *SMN1* mutation.

We observed contradictory relationship between *SMN1* deletion and *SMN1* mutation in respect to SMA clinical severity. Generally, *SMN1*-deleted patients with a single copy of *SMN2* show the severest phenotype, Type 0. However, some intragenic mutation patients with milder phenotype showed only a single copy of *SMN2*, and some others with severe phenotype showed two or three copies of *SMN2*. For instance, a patient with a c.830A>G in the retained *SMN1* showed phenotype type 2, who could sit unaided and stand while holding onto something (such as a wall or table) for support. More surprisingly, 2 patients with a c 5C>T mutation (p.Ala2Val) in *SMN1* showed phenotype type 3. There was also a report of similar cases with a different mutation at the same location, c.5C>G (p.Ala2Gly) in *SMN1*, who presented with phenotype type 3 in spite of carrying only a single copy of *SMN2*. These findings, including ours, suggested that some missense mutations, c.5C>T or c.5C>G, may produce SMN proteins with some function (hypomorphic mutation or leaky mutation).

This observation, however, could not be generalized to all types of mutations. According to the report of de Holanda Mendonça et al., patients with c.770_780dup and c.734_735insC had a clinical phenotype correlated with *SMN2* copy number. These frameshift mutations may produce truncated proteins, which may be related to complete loss of function. Effect of *SMN2* copy number may be apparent in the patients with complete-loss-of-function mutations of *SMN1*.

Conclusions

SMA diagnosis should not be limited to the detection of *SMN1* deletion. Our comprehensive diagnostic procedures have pinpointed intragenic *SMN1* mutations in 13 SMA patients retaining *SMN1*, including 5 novel mutations. Intragenic *SMN1* mutations may give rise to various clinical phenotypes irrespective of *SMN2* copy number. Now we think that locations and types of intragenic *SMN1* mutations may contribute to the clinical phenotype more significantly than *SMN2* copy number.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
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論文題目 Title of Dissertation	Phenotypes of SMA patients retaining *SMN1* with intragenic mutation SMN1 遺伝子内変異を有する脊髄性筋萎縮症患者の臨床像		
審査委員 Examiner	主 査 Chief Examiner 野津寛大 副 査 Vice-examiner 松本理器 副 査 Vice-examiner 永瀬裕朗		

（要旨は１，０００字～２，０００字程度）

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by homozygous deletion or intragenic mutation of the *SMN1* gene. It is well-known that high copy number of its homologous gene, *SMN2*, modifies the phenotype of *SMN1*-deleted patients. However, in the patients with intragenic *SMN1* mutation, the relationship between phenotype and *SMN2* copy number remains unclear.

The authors have analyzed a total of 515 Japanese patients with SMA-like symptoms (delayed developmental milestones, respiratory failures, muscle weakness etc.) from 1996 to 2019. *SMN1* and *SMN2* copy numbers were determined by quantitative polymerase chain reaction (PCR) method and/or multiplex ligation-dependent probe amplification (MLPA) method. Intragenic *SMN1* mutations were identified through DNA and RNA analysis of the fresh blood samples.

As a result, a total of 241 patients were diagnosed as having SMA. The majority of SMA patients showed complete loss of *SMN1* (n = 228, 95%), but some patients retained *SMN1* and carried an intragenic mutation in the retaining *SMN1* (n = 13, 5%). Ten different mutations were identified in these 13 patients, consisting of missense, nonsense, frameshift and splicing defect-causing mutations. It should be noted here that some patients with milder phenotype carried only a single *SMN2* copy, while other patients with severe phenotype carried 3 *SMN2* copies.

In conclusion, intragenic mutations in *SMN1* may contribute more significantly to clinical severity than *SMN2* copy numbers.

The candidate, having completed studies on SMA and having advanced the field of knowledge in the area of SMN2 copy number influences to the severity of SMA, is hereby recognized as having qualified for the degree of Ph.D..