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Centromeric SMN Deletions in Various Congenital Muscular Dystrophies

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Abstract: We studied centromeric SMN gene deletions in various forms of congenital muscular dystrophies. Our study cohort consisted of 48 patients (43 families): 24 with merosin-positive CMD, 18 with merosin-deficient CMD and 6 with muscle-eye-brain (MEB) disease. None of the patients showed deletions of the telomeric gene; however, the deletion frequency of the centromeric gene was 27%. In a multiplex family, the mildly affected sibling, who is ambulant, had a

preserved centromeric copy of the SMN gene, whereas in the severely affected sibling the copy was missing. The centromeric copy of the SMN gene may have a contributing role in the phenotype of patients with CMD, as well as other modifying genes or environmental factors.

Key Words: Congenital muscular dystrophies, survival motor neuron gene, SMN, deletion, SMA

Introduction

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, which results in loss of motor neurons in the spinal cord. Affected individuals are classified into 3 groups that have been mapped to chromosome 5q11.2-13.3 (1). The survival motor neuron gene (SMN) is one of the candidate genes of SMA. It is present in two highly homologous copies within the SMA region. They are called telomeric SMN (SMN1) and centromeric SMN (SMN2). These two copies differ by two single nucleotides; one in exon 7, which does not alter the amino acid composition, and one in exon 8, which is an untranslated exon. Therefore, both genes predict identical proteins. Exon 7 of the telomeric copy is disrupted by deletion or gene conversion in more than 95% of patients with SMA of all grades of severity (2-4). SMN encodes a protein located within a novel nuclear structure and interacts with RNA binding proteins. A 38 kDa SMN protein is expressed from the telomeric and centromeric SMN genes and is found in the cytoplasm and nucleus. SMN is expressed at high levels in the brain, kidney and liver, at moderate levels in skeletal and cardiac muscle, and at low levels in fibroblasts and lymphocytes (5,6).

There may be some clinical overlaps between SMA and other congenital myopathies, especially when the case does not present with fasciculations or tremors. Since the age of onset in SMA matches that in congenital muscular

dystrophies (CMD), it may be difficult to differentiate them clinically in the absence of typical stigmata of the individual conditions.

CMD is a clinically and genetically heterogeneous group of muscle disorders with autosomal recessive inheritance. CMD has been classified into several subgroups. One subgroup of patients lacks the expression of the laminin α 2 chain (merosin-deficient) in the skeletal muscle basement membrane. Linkage analysis localized the defective gene to the region of the LAMA2 merosin locus on chromosome 6q2 (7). The LAMA2 gene is very large, with more than 64 exons and a transcript of more than 10 kb. Another subgroup of patients with classical CMD has normal levels of laminin α 2 chain (merosin-positive) and basic protein defects are unknown. This latter group is also highly heterogeneous (8).

There are other forms of CMD with structural brain involvement. A reduced expression of laminin α 2 chain has been reported in Fukuyama's congenital muscular dystrophy (FCMD), a condition almost exclusive to Japan with typical findings of severe mental retardation and cortical abnormalities of the brain (9). However, this represents a secondary phenomenon, because the gene for FCMD is on chromosome 9. Another form of CMD, the muscle-eye-brain (MEB) disease, which is characterized by severe mental retardation, ocular and brain abnormalities, has recently been mapped to chromosome 1p32-43 (10). There is evidence that the

laminin α 2 chain is also reduced in MEB (11). The most severe condition of the spectrum is the Walker-Warburg syndrome (WWS), and recent data shows that WWS is also heterogeneous.

Patients with WWS and severe lissencephaly usually expire before age 1; nevertheless, milder forms survive and may run a course clinically similar to MEB (12). Finally, one peculiar form of CMD, rigid spine syndrome with early respiratory failure, has recently been mapped to chromosome 1p35-36 (13).

In the present study, we analyzed SMN gene deletions in various congenital muscular dystrophies.

Materials and Methods

We analyzed 48 patients from 43 CMD families. Of these patients, 24 were classified as having merosin-positive CMD, 18 merosin-deficient CMD and 6 MEB disease. All patients fulfilled the necessary diagnostic criteria for CMD and plus syndromes set by European Neuromuscular Center (ENMC) sponsored CMD consortium. There were 5 families with 2 siblings affected in our patient group. Two hundred nine SMA patients with 40 parents and 34 healthy control individuals had previously been examined for the deletions. Informed consent was obtained from each patient.

DNAs were extracted from white blood cells (19 patients) and paraffin-embedded muscle tissue sections (29 patients) by standard methods (14). The methods of

the PCR used to amplify exons 7 and exons 8 of the SMN genes were similar to those used by Van der Steege et al. (4). To distinguish centromeric and telomeric exons 7 and 8, each PCR product was digested with Dral and Ddel restriction enzymes, respectively. Digestion products were run on 2.5% agarose gel containing ethidium bromide and visualized under UV fluorescence (Figure 1).

Results

Although we did not detect any deletion of the centromeric copy of the SMN exon 7 and 8 in either SMA patients (n: 209) and parents (n: 40) or the healthy control group (n: 34), the overall centromeric deletion frequency rate was 27% in CMD patients. Table 1 shows the centromeric deletion frequencies of CMD subgroups and Table 2 shows the centromeric deletions of the families with 2 affected siblings. Family I had two siblings with discordant phenotype; the 18-year-old sister has never walked, whereas the 14-year-old brother originally walked at the age of 3 years and is still ambulant. In this family, only the severely affected sibling had the deletion

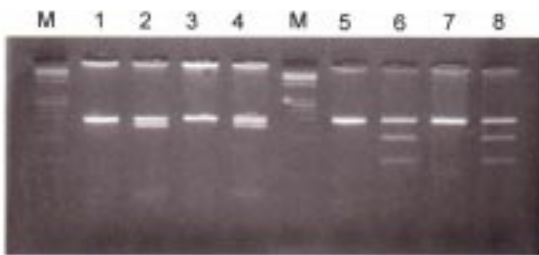


Figure 1. Restriction enzyme assay of the SMN gene. (The PCR products of exon 7 were digested with Dral and exon 8 with Ddel).

- M: Molecular weight marker (ϕ X174)
- Lane 1,5: Undigested PCR products
- Lane 2,4: Healthy individuals for exon 7
- Lane 3: Deletion of centromeric exon 7 in the index case
- Lane 6,8: Healthy individuals for exon 8
- Lane 7: Deletion of centromeric exon 8 in the index case

Table 1. Centromeric deletion frequencies of the CMD subgroup.

Patients	Allele frequency of centromeric exon 7 deletion	Allele frequency of centromeric exon 8 deletion
Merosin-positive CMD	14/48 (29%)	12/48 (25%)
Merosin-deficient CMD	4/36 (11%)	2/36 (6%)
MEB	8/12 (67%)	8/12 (67%)

Table 2. Centromeric deletions of the families with 2 affected siblings.

Family		Centromeric exon 7 deletion	Centromeric exon 8 deletion
Family I (Merosin-positive CMD)	Sibling 1	-	-
	Sibling 2	+	+
Family II (Merosin-positive CMD)	Sibling 1	+	+
	Sibling 2	+	+
Family III (Merosin-deficient CMD)	Sibling 1	-	-
	Sibling 2	-	-
Family IV (Merosin-deficient CMD)	Sibling 1	-	-
	Sibling 2	-	-
Family V	Sibling 1	+	+
	Sibling 2	+	+
MEB	Sibling 2	+	+

of the centromeric copy of the SMN gene. In the other 4 families there was no discrepancy. None of the CMD patients showed telomeric SMN exon 7 and 8 deletion.

Discussion

In our study, centromeric SMN gene deletion frequency was 27%. Twenty-four merosin-positive CMD, 18 merosin-deficient CMD and 6 MEB patients showed 29%, 11%, 67% deletions of the centromeric copy of SMN exon 7, respectively. Previous reports have given an incidence of 4-5% centromeric SMN deletion in normal populations (15,16). Another study on the Turkish population reports centromeric SMN deletion of exon 7 and 8 with an incidence of 9% (17). Even though our number of control subjects was only 34, our results of deletion incidence in CMD are still higher than these control results. Two patients (1 merosin-positive, 1 merosin-deficient) showed centromeric exon 7 deletion but not exon 8.

In SMA patients lacking the telomeric copy, the SMN protein was detected. This showed that not only the telomeric copy but also the centromeric copy produced the SMN protein. The protein encoded by the centromeric SMN gene is probably functionally active, as its level is directly correlated to the clinical severity of the SMA (5,18). The 1.5 kb SMN transcript encodes a protein of 294 amino acids. The protein is expressed in humans, is conserved through mammalian species and shows no homology with any known protein (3).

SMN monoclonal antibodies show several intense dots. These structures are similar in number (2-6) and size (0.1-1.0 μ) to coiled bodies, and are found near or

associated with coiled bodies. These prominent nuclear structures are termed gems, for Gemini of coiled bodies.

The gems appear to interact directly with the coiled bodies that are believed to have a role in mRNA processing. The number of gems as detected by SMN antibodies correlates with the phenotype of the SMA patients (19,20).

In searching for hnRNP-interacting proteins, Liu et al. found that SMN interacts with the RGG box region of hnRNP U, with itself, with fibrillarin and with several novel proteins (6). Gall et al. postulate that coiled bodies function in the assembly and sorting of snRNP complexes for 3 RNA processing pathways: pre-mRNA splicing, RNA processing and histone pre-mRNA 3' end formation (21,22).

All forms of CMD are usually severe. However, not all CMD cases had missing centromeric copies of the SMN gene denoting the complexity of the interactions. Nevertheless, considering the phenotypic difference of the two siblings in one of our multiplex families, we can speculate that the presence of this copy may have a potentiating role in modifying the clinical picture. In conclusion, since RNA processing is a common biological pathway, its disruption may have an additional contribution to several phenotypes such as we have demonstrated here.

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